Impact of Mindfulness Meditation on Brain Function, Connectivity, and Structure

By

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Abstract

Mindfulness meditation provides promise for stress relief and well-being. However, most non-clinical studies suffer major limitations. We addressed many of the limitations through larger samples, a design combining a randomized active-controlled trial (RCT) with cross-sectional analysis of long-term training, and complementary brain and behavioral measures. Mindfulness meditation involves paying attention to the present moment, with a non-reactive stance toward experience. This dissertation rigorously tested the impact of mindfulness meditation training on the neural correlates of emotional nonreactivity, resting state functional connectivity (RSFC) in brain networks associated with attention and mind-wandering, and structural neuroplasticity. We examined the impact of short-term training with Mindfulness-Based Stress Reduction (MBSR), which is widely used, extensively tested and has demonstrated clinical efficacy.

In Chapter 1, we examined amygdala reactivity and functional connectivity with ventromedial prefrontal cortex (VMPFC) during emotional stimuli, which have been implicated in emotion regulation. Mindfulness meditation was associated with reduced amygdala reactivity and increased functional connectivity with VMPFC. Increased amygdala-VMPFC functional connectivity was associated with decreased amygdala reactivity from pre- to post-intervention. In Chapter 2, we replicated and extended prior work showing increased RSFC between posterior cingulate cortex (PCC) and lateral prefrontal cortex (LPFC), which are implicated in mind-wandering and attention, respectively. Increased PCC–LPFC RSFC is hypothesized to reflect better attentional control of mind-wandering. In support of this hypothesis, we found that increased self-reported attention was associated with stronger PCC–LPFC RSFC from pre- to post-intervention. In Chapter 3, we attempted to replicate and extend research showing changes in regional gray matter volume (GMV) associated with mindfulness meditation training. However, there were no significant differences in GMV between meditators and controls. Yet, time practicing mindfulness meditation was associated with differences in amygdala and hippocampus GMV.

This dissertation provides evidence for tangible neural changes associated with mindfulness meditation training, which are consistent with hypothesized benefits in emotional nonreactivity and attention. The lack of replication of group differences in brain structure highlights the need for publishing null results, and for rigorous RCTs of MBSR in community samples. Finally, effects of practice time were evident across measures, underscoring the importance of sustained meditation practice.

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Mindfulness meditation is often touted as a practice to improve well-being and as a remedy for the stress and anxiety that large swaths of the population suffer at sub-clinical levels – symptoms that contribute to worse health (Chida et al., 2008; DeLongis et al., 1988; Haller et al., 2014; Kelly and Ismail, 2015; Klitzman et al., 1990). An accessible intervention for reducing stress is critically needed, since a 79% of Americans who were polled in 2018 reported experiencing stress every day, which is higher than at any point in the prior decade (Inc, 2019). Given the popularity of meditation – over 14% of Americans reported practicing in a 2017 survey, and the fact that meditation is one of the fastest-growing complementary health trends in the United States (as assessed from 2012 to 2017) (Clarke et al., 2018), rigorous examination of its effects and replication are critical. While there is a wealth of literature on the efficacy of mindfulness meditation practices for improving emotion regulation and symptoms of anxiety, depression, and pain, among other clinical syndromes (Alberts et al., 2012; Garland et al., 2012; Wielgosz et al., 2019), evidence for neural and affective changes in healthy adults remains scarce and/or plagued by low sample sizes and weak methodological design (Chiesa and Serretti, 2009; Davidson and Kaszniak, 2015).

Measurement is also an area for improvement, given the shortcomings of using self-report questionnaires, including issues of demand characteristics (Morrison and Hunt, 1996), and the fact that the ability to reflect on one’s subjective state is expected to change with mindfulness training (Davidson and Kaszniak, 2015). Brain measures provide another way to probe mindfulness-related change that may not be evident in behavioral measures – brain changes may precede behavioral changes, as in the case of Alzheimer’s Disease, in which the degeneration of gray matter volume in basal forebrain predicted later cognitive decline (Schmitz and Spreng, 2016). Additionally, brain measures can provide a means to assess changes that are subtle or for which we have not yet developed sensitive behavioral measures, as in the case of mild traumatic brain injury (Bigler, 2013).

The practice and known benefits of mindfulness meditation

Mindfulness encompasses many styles of meditation, and can be considered a cognitive state realized by any one of a family of meditation practices (Davidson, 2010; Wielgosz et al., 2019). While definitions of mindfulness meditation may vary, the core features include a practice of keeping attention focused on present moment experience with a “non-judgmental” stance (Dahl et al., 2015; Kabat-Zinn, 2003; Lutz et al., 2015). The non-judgmental nature of mindfulness meditation can range from refraining from making a value judgment of thoughts and feelings (e.g., that a given response or feeling is “good” or “bad”), to halting conceptual elaboration or discursive thinking in certain forms of practice (Lutz et al., 2015; Santorelli, 2014). Through the process of maintaining focus on the present moment, mindfulness mediation also involves observing the transitory nature of experience (Lutz et al., 2015) – thoughts and feelings arise and, sooner or later, pass away.

Prior research provides evidence that mindfulness meditation may have cognitive and affective benefits. Cognitive changes include: improved working memory (Zeidan et al., 2010), decreased rumination and distraction (Jain et al., 2007), and decreased mind-wandering (Mrazek et al., 2013). However, results of recent meta-analyses are mixed for mindfulness meditation training-related changes in inhibition, selective attention, and executive function (Chiesa et al., 2011; Gallant, 2016; Lao et al., 2016; Wielgosz et al., 2019). Affective changes include: reduced emotional interference (Ortner et al., 2007), decreased negative mood (Jha et al., 2010), improved emotion regulation and increased positive mood (Goyal et al., 2014; Hofmann et al., 2010; Jain et al., 2007). These benefits are relevant for clinical populations, as well as for healthy individuals.

Mindfulness-Based Stress Reduction (MBSR) is a secular, manualized mindfulness intervention that was developed at the University of Massachusetts Medical School, initially for patients with chronic pain conditions (Kabat-Zinn, 1990, 1982). MBSR entails an 8-week series of classes led by a trained instructor in a group setting. Classes include breath awareness meditation, body scan meditation, yoga and walking meditation. Participants are assigned daily "homework" to practice meditation based on exercises taught in the classes, which build in length over the course to reach 20 to 30 minute meditation sessions (Kabat-Zinn, 1990; Santorelli, 2014). MBSR has been shown to reduce negative experience (Goldin and Gross, 2010; Kaviani et al., 2011), improve recovery from a negative challenge (Britton et al., 2012; Raes et al., 2009), ameliorate symptoms of multiple psychopathologies (Wielgosz et al., 2019), and reduce stress (Chiesa and Serretti, 2009). A recent meta-analysis of MBSR in healthy, non-clinical adults found evidence for large effects on stress reduction and medium effects on reducing anxiety, depression and distress, and improving quality of life – based entirely on self-report questionnaires, with the exception of a single behavioral measure in one study. However, only one of the 29 studies (published as of 2014) included an active control group, and less than half (13 of 29) were randomized (Khoury et al., 2015). Therefore, while there is a growing body of evidence indicating beneficial effects for MBSR in healthy, nonclinical populations, further research is needed that employs the rigorous methodology of a well-powered randomized controlled trial with behavioral and brain measures, in addition to self-report questionnaires.

Cognitive mechanisms

Mindfulness meditation practices aim to increase **meta-awareness**, which is one potential mechanism underlying cognitive and affective benefits of mindfulness meditation training such as MBSR (Dahl et al., 2015; Lutz et al., 2015; Wielgosz et al., 2019). Meta-awareness is the ability to monitor the contents of consciousness – "thinking about thinking" (Schooler et al., 2011). Phasic meta-awareness can be illustrated by the instance of asking "where is my attention right now?" Thus, noticing when one's mind has wandered is an example of momentary meta-awareness (Schooler, 2002), which facilitates re-orienting attention back to the present moment (in the case of mindfulness meditation) or to another relevant task (e.g., reading the text of a book, engaging in conversation, etc.) (Schooler et al., 2011). Another form of meta-awareness involves a more sustained, "background" process of noticing features of an experience without losing focus on the central task with which one is engaged (Lutz et al., 2015). For example, while hiking through a forest one might be simultaneously aware of the sounds of birds, the smell of the trees, an emotional tone (perhaps serenity or awe), as well as a central focus on following the path ahead, avoiding rocks and other trip hazards. In these ways, the capacity for meta-awareness enables attentional focus through monitoring, and noticing the emotional tone or reactions as they arise in the periphery of a given task. Mindfulness meditation has been associated with reduced mind-wandering, a behavioral target of meta-awareness, as well as improved meta-awareness of distraction (Mrazek et al., 2014).

Dereification is another core process that mindfulness meditation training is intended to foster, which is the perception of thoughts as transient interpretations of reality (e.g., mental objects), rather than as inherent reflections of reality holding some ultimate truth. (Lutz et al., 2015; Wielgosz et al., 2019). The inverse of dereification is experiential fusion – when thoughts are experienced as inseparable from the objects that they represent, as in the case of being so immersed in a movie that it seems real, and, e.g., one flinches in response to the images (Dahl et al., 2015). In other words, thoughts represent our perspective, and therefore different perspectives on the same situation are not only possible, but equally “real” (or rather, equally lacking in substance). Reappraisal and perspective taking both involve dereification as a first step through questioning and setting aside an initial appraisal (or perspective) (Lutz et al., 2015). Indirect evidence that mindfulness training enhances dereification is provided by research showing that reappraisal mediates improvements in mindfulness with mindfulness-based interventions (Garland et al., 2011). Changes in self-distancing – the ability to separate oneself from one’s emotions, which is highly overlapping with the construct of dereification in the clinical literature – has also been shown to improve with mindfulness-based interventions (Lao et al., 2016).

A non-judgmental stance is a core feature of the instructions across meditation practices in styles considered within the family of mindfulness meditation, which can be understood as **nonreactivity** by refraining from elaborative thought, particularly in terms of value judgments (Kabat-Zinn, 1990; Lutz et al., 2015; Santorelli, 2014; Wielgosz et al., 2019). One of the goals and potential outcomes of a non-judgmental, or nonreactive, stance is to reduce the propensity to avoid negative or distressing experiences, or to cling to positive experiences – rather to simply observe such experiences as they arise, and inevitably pass (Wielgosz et al., 2019). Practicing non-reactivity in this way entails repeated exposure to affective experience without the subsequent elaborative, and potentially distressing, progression of thoughts and feeling states that may have usually followed, prior to mindfulness training. There is a striking similarity between the change in conditioned responding that is practiced in mindfulness meditation, and extinction learning (Hofmann, 2008), such as has been used in exposure therapy for anxiety

(Kummar, 2018; Tang et al., 2015) – except in the case of mindfulness, both the stimulus and response are internally-generated (e.g., negative affect and distressing elaboration). Changes in habitual emotional responding are an aim of practicing nonreactive (i.e., nonjudgmental) awareness in mindfulness meditation, and given the efficacy of exposure therapy and extinction learning to improve outcomes in clinical settings (Cusack et al., 2016; Parsons and Rizzo, 2008; Powers et al., 2010), provide a potential mechanism for mindfulness-related improvements in emotion regulation and affective processing. More research is needed to examine this hypothesis, and the potential for changes in habitual emotion responding with nonreactive awareness to further support the development of dereification and meta-awareness abilities (e.g., by releasing cognitive/ attentional resources).

The present-centered attentional focus of mindfulness meditation, paired with increased meta-awareness and dereification, may provide a foundation for an enhanced ability to notice and **label emotional states**. The combination of noticing and labeling emotions is another potential mechanism for mindfulness meditation-related affective change. Labeling emotions can reduce neural reactivity to negative emotional stimuli in the amygdala (Lieberman et al., 2016), and labeling emotions is a part of mindfulness meditation practices and mindfulness-based interventions (Lutz et al., 2008). According to the Conceptual Act Theory of Emotion, conceptualization of emotion determines affective experience (Barrett, 2006), and thus, labeling emotion could directly alter affective reactivity and its neural correlates. Conversely, labeling emotions could indirectly reduce reactivity through triggering emotion regulation processes, such as reappraisal. Preliminary evidence provides support for labeling emotions in the context of meta-awareness and dereification as a mechanism for affective changes: the availability of "meta-cognitive sets" was associated with improvements in depression following a mindfulness-based intervention (Teasdale et al., 2002). Meta-cognitive sets were scored based on participants' ability to label their emotional states, question their own perspective, and to discriminate their self and their thoughts (i.e., self-distancing) on the Measure of Awareness and Coping in Autobiographical Memory (Teasdale et al., 2002).

Target brain networks – meta-awareness, mind-wandering and rumination

Lateral prefrontal cortex (LPFC), and in particular dorsal sub-regions (DLPFC), corresponding to Brodmann's areas 9 and 46 and overlapping with middle frontal gyrus (Figure 1a, based on the Harvard-Oxford cortical atlas, at 50% threshold [Desikan et al., 2006]), are implicated in multiple aspects of working memory (for meta-analysis, see Wager et al., 2004). Specifically, transcranial stimulation and lesion studies demonstrate a role for DLPFC in manipulating information in working memory (Barbey et al., 2013; Postle et al., 2006), and neuroimaging evidence indicates associations with internal information monitoring (Petrides, 2005) consistent with a role in meta-awareness. Rostral LPFC, corresponding to Brodmann's area 10 (Figure 1a), has been implicated in attentional switching and is thought to support awareness of the *source* of cognitive representations, according to the Gateway Hypothesis (Burgess et al., 2007). This proposed function of rostral LPFC is consistent with the idea of "background meta-awareness" by which a meditator practices broadly monitors the contents and tone of conscious experience (e.g., for the presence of mind-wandering) while simultaneously maintaining focus on a task set (Lutz et al., 2015). However, further research is needed to directly probe the neural mechanisms underlying the core cognitive capacities targeted by mindfulness meditation training – dereification and meta-awareness – including task development.

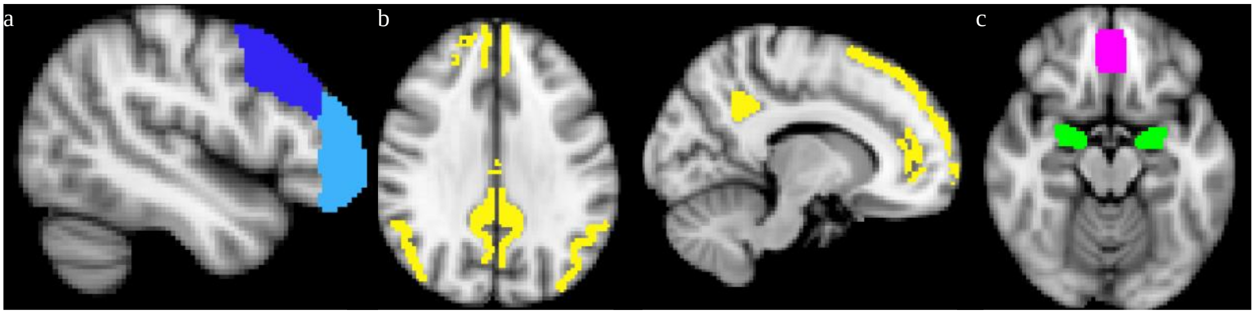

Figure 1. Potential neural targets of mindfulness meditation. Dorsolateral prefrontal cortex (PFC) and rostrolateral PFC are implicated in executive functions and attentional processes related to meta-awareness (in violet and blue, respectively) (a). The default mode network is associated with mind-wandering and self-related processing (in yellow) (b). Amygdala (in green) and ventromedial PFC (in fuchsia) support affective processing and regulation (c).

Mind-wandering, which often includes self-related thought that is predominantly future-oriented (Baird et al., 2011), is one major target of the meta-awareness that is practiced in mindfulness meditation (Wielgosz et al., 2019). In depressed individuals, mind-wandering involves predominantly negative and past-oriented self-related thought (Hoffmann et al., 2016), referred to as rumination when it is repetitive in nature. The default mode network (DMN) is a set of brain regions that takes its name due to its greater activation at rest than during tasks, and in particular the posterior cingulate cortex (PCC) as a node within the DMN (Figure 1b, based on the Gordon atlas [Gordon et al., 2016]), is implicated in mind-wandering, self-related thought and rumination (Andrews-Hanna et al., 2010; Hamilton et al., 2015; Mason et al., 2007; Spreng et al., 2008).

Target brain networks – Emotion labeling & reactivity

Research on amygdala-VMPFC connectivity and function offers insights into potential neural mechanisms for improving emotion regulation (Figure 1c, based on the Harvard-Oxford subcortical atlas, at 50% threshold [Desikan et al., 2006]). Research examining the neural circuitry underlying affective processing has focused on the amygdala due to its central role in emotion generation and regulation (Phelps and LeDoux, 2005). There is also evidence of a role for VMPFC in implicit emotion regulation through extinction of conditioned stimuli (Phelps et al., 2004), and explicit regulation during cognitive reappraisal (Urry et al., 2006). Moreover, amygdala-VMPFC functional coupling during emotion regulation has important functional consequences: stronger coupling is associated with lower self-reported negative affect (Banks et al., 2007), and patients suffering from depression have reduced amygdala-VMPFC connectivity during rest and passive viewing of emotional pictures compared to non-depressed, healthy adults (Anand et al., 2005). Moreover, patients with generalized anxiety disorder have reduced structural connectivity of a tract connecting amygdala and VMPFC (Tromp et al., 2012), and anxiety has also been linked to lower amygdala-VMPFC connectivity during resting state fMRI (Kim et al., 2011a).

Bidirectional connectivity between the amygdala and VMPFC thus provides a potential pathway by which VMPFC could be recruited by amygdala to update the salience or value of a stimulus (Price, 2005). This sort of value updating may be a mechanism underlying automatic down-regulation of amygdala activation. Non-human primate research of amygdala-prefrontal circuitry reveals a specific mechanism for these effects whereby direct, monosynaptic input to the basolateral amygdala from MPFC inhibits activity in the central nucleus of the amygdala (Kim et al., 2011b). Moreover, non-human primate research demonstrates a causal role for VMPFC in value updating (Rudebeck et al., 2013), and in a recent meta-analysis of the human neuroimaging literature the VMPFC was identified as the brain region representing value computations across stimulus modalities (Levy and Glimcher, 2012), providing additional support for the idea that VMPFC may automatically process information regarding the changing affective value of stimuli.

Current evidence for neural changes with mindfulness meditation training

The posterior cingulate cortex (PCC) is activated during rumination and de-activated during mindfulness meditation, providing a potential neural target for mindfulness meditation interventions (Brewer and Garrison, 2014). Moreover, mindfulness meditation practice is associated with increased resting state functional connectivity between PCC and dorsolateral prefrontal cortex (DLFPC) (Brewer et al., 2011; Creswell et al., 2016a; Kral et al., 2019), which is a node of the frontoparietal control network (FPN) associated with attentional control (MacDonald et al., 2000; Smallwood et al., 2012). Mindfulness meditation-related increases in PCC-DLPFC resting state functional connectivity may reflect a neural mechanisms underlying reductions in mind-wandering and rumination through increased attentional control (Brewer et al., 2011), however further research is needed to support this interpretation.

There is a growing literature describing neurobiological changes underlying affective processing following mindfulness meditation training – including increased insula and decreased amygdala activation to affective stimuli (Allen et al., 2012; Desbordes et al., 2012). Changes in gray matter volume have also been found following MBSR practice in left caudate (Farb et al., 2013), left hippocampus, temporoparietal junction (TPJ), cingulate and cerebellum (Hölzel et al., 2011). However, prior research was limited by small sample sizes with a limited range of training, and lack of a rigorously matched active control group. The literature is still lacking a precise cognitive mechanism and neurobiological explanation of how training in MBSR causes changes in emotion and its regulation.

Current Research

This dissertation rigorously tested the impact of mindfulness meditation training on brain measures associated with the cognitive mechanisms hypothesized to support cognitive and affective benefits of mindfulness training, in addition to behavioral and self-report measures. We utilized a study design combining a randomized controlled trial of MBSR, which included a well-matched, validated active control group (the Health Enhancement Program [HEP]), and a cross-sectional analysis of individuals with long-term training in mindfulness meditation compared to meditation-naïve participants. This unique design allowed us to examine the effects of both short- and long-term practice in mindfulness meditation with the same set of multi-modal measures. In addition, we collected practice logs in order to determine whether the amount of practice time, either in early stages of training or cumulatively over the lifetime, was associated with the degree of change in each of our measures.

Chapter 1 identifies a neurobiological mechanism for MBSR-related improvements in emotion regulation and **nonreactivity**. This research sought to conceptually replicate and extend prior work demonstrating mindfulness meditation training-related reductions in emotional reactivity. Improved emotional reactivity in this context was defined as reduced amygdala activation and increased amygdala-VMPFC functional connectivity to affective pictures, and was assessed with an implicit emotion regulation paradigm that we have previously demonstrated to have convergent validity with self-report measures (Schuyler et al., 2014). First, I tested whether a similar pattern of reduced emotional reactivity following mindfulness meditation training occurred in long-term meditators and following MBSR compared to control participants, as has been seen in previous research (Desbordes et al., 2012b). Then, I determined whether long- and short-term mindfulness meditation were associated with increased amygdala-VMPFC functional connectivity during affective images, possibly reflecting inhibitory control of amygdala by VMPFC. Finally, I examined relationships between the aforementioned measures of amygdala reactivity and functional connectivity, and individual differences in both self-reported emotional reactivity and mindfulness meditation practice time. I predicted that there would be a dosedependent relationship, whereby larger changes in amygdala reactivity and connectivity were associated with more time practicing mindfulness meditation. This research will extend understanding of the neural dynamics of emotion regulation and mechanisms underlying mindfulness meditation-related improvement.

Chapter 2 examines mindfulness meditation-related differences in resting state functional connectivity of brain networks associated with **mind-wandering and meta-awareness** – namely, posterior cingulate and the DMN, and DLPFC and the FPN, respectively. The task-free context of the resting state scan is optimal for inducing mind-wandering and rumination in a naturalistic way, and examining changes in the associated brain networks. This study aims to replicate prior research showing mindfulness training-related strengthening of resting state functional connectivity of the DMN (based on a posterior cingulate seed) with the DLPFC (Brewer et al., 2011; Creswell et al., 2016b). In addition, I assessed relationships with meditation practice time, structural connectivity of a white matter tract connecting PCC and DLPFC, and behavioral measures of attention and mind-wandering. The latter results will provide an important test of the interpretation that increased RSFC between PCC and DLPFC reflects better attentional control of mind-wandering.

Chapter 3 combines data from the participant samples used in Chapters 1 and 2 to determine whether mindfulness meditation was associated with **structural neuroplasticity**, as assessed with gray matter volume from MRI anatomical scans. Since the process of neuroplasticity was discovered, numerous studies have demonstrated the efficacy of magnetic resonance imaging (MRI) for measuring experience-dependent alterations in brain structure, which are detectable within weeks of behavioral change (May and Gaser, 2006). The current study tested for neuroplasticity in gray matter volume of affective regions such as amygdala and insula, and sought to replicate prior effects of increased gray matter volume in caudate, hippocampus and TPJ associated with mindfulness meditation training (Farb et al., 2013; Hölzel et al., 2011). Additionally, we examined associations between individual differences in practice duration with degree of change in gray matter volume, and hypothesized that a larger degree of change would be associated with more practice. We also expected that larger reductions in amygdala volume would be associated with improved measures of negative affect and anxiety, in line with prior research (Hölzel et al., 2009).

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Chapter 1

Impact of short- and long-term mindfulness meditation training on amygdala reactivity to emotional stimuli.

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Abstract

Meditation training can improve mood and emotion regulation, yet the neural mechanisms of these affective changes have yet to be fully elucidated. We evaluated the impact of long- and short-term mindfulness meditation training on the amygdala response to emotional pictures in a healthy, non-clinical population of adults using blood-oxygen level dependent functional magnetic resonance imaging. Long-term meditators (N=30, 16 female) had 9,081 hours of lifetime practice on average, primarily in mindfulness meditation. Short-term training consisted of an 8-week Mindfulness- Based Stress Reduction course (N=32, 22 female), which was compared to an active control condition (N=35, 19 female) in a randomized controlled trial. Meditation training was associated with less amygdala reactivity to positive pictures relative to controls, but there were no group differences in response to negative pictures. Reductions in reactivity to negative stimuli may require more practice experience or concentrated practice, as hours of retreat practice in long-term meditators were associated with lower amygdala reactivity to negative pictures – yet we did not see this relationship for practice time with MBSR. Short-term training, compared to the control intervention, also led to increased functional connectivity between the amygdala and a region implicated in emotion regulation – ventromedial prefrontal cortex (VMPFC) – during affective pictures. Thus, meditation training may improve affective responding through reduced amygdala reactivity, and heightened amygdala–VMPFC connectivity during affective stimuli may reflect a potential mechanism by which MBSR exerts salutary effects on emotion regulation ability.

Introduction

Mindfulness meditation practices, which aim to cultivate an accepting awareness of the present moment (Bishop et al., 2004; Brown and Ryan, 2003; Kabat-Zinn, 1990) can improve emotion regulation, ameliorate symptoms underlying anxiety and depression and boost positive mood (Goyal et al., 2014; Hofmann et al., 2010; Jain et al., 2007). Mindfulness based stress reduction (MBSR) is a widely used form of meditation practice taught by a trained professional that involves didactics, individual and group practices including: breath awareness meditation, body scans, walking meditation and yoga. Each of the practices involves focusing attention on present- moment experience (Kabat-Zinn, 1990). MBSR has been shown to lead to reduced negative experience (Goldin and Gross, 2010; Kaviani et al., 2011) and quicker recovery from a negative challenge (Britton et al., 2012; Raes et al., 2009). Improvements in affective responses following mindfulness meditation training have also been shown in non-clinical populations, including reductions in emotional interference (Ortner et al., 2007) and decreased negative mood (Jha et al., 2010). Research on the neural mechanisms underlying these affective changes that utilizes active control conditions is sparse, and systematic examination of the impact of mindfulness meditation training on functional connectivity in emotion regulation networks has not been investigated.

Allen *et al*. provide initial evidence that mindfulness meditation alters neural processing to affective stimuli following a short-term intervention – participants who practiced longer had more insula activation during negative pictures in a whole-brain analysis (Allen et al., 2012). The current study investigated a greater range of practice by including long- and short-term practitioners and expands upon prior work by probing the neural response to positive pictures – in addition to negative pictures – in an important emotion regulation circuit using *a-priori* amygdala and ventromedial prefrontal cortex (VMPFC) regions of interest (ROIs). Research using a similar approach demonstrated decreased amygdala activation during positive affective pictures following short-term mindfulness meditation training, however it was limited to 12 participants per group (Desbordes et al., 2012). Precisely how mindfulness-based meditation impacts the neural circuitry of emotion regulation remains unclear.

The amygdala is central to emotion generation (Phelps and LeDoux, 2005) and regulation (Buhle et al., 2014), and VMPFC is implicated in automatic emotion regulation (Phelps et al., 2004; Urry et al., 2006), possibly through functional coupling with the amygdala (Banks et al., 2007; Lee et al., 2012). Automatic, or implicit emotion regulation consists of processes that alter the course of affective experience outside explicit, conscious attempts to do so. Affect labeling is a process which may engage automatic emotion regulation even absent an intention to volitionally regulate emotion, as affect labeling has been shown to dampen the amygdala response (Lieberman et al., 2007). Extinction learning is another example of an automatic emotion regulation process and involves both amygdala and VMPFC (Phelps et al., 2004). Non-human primate research reveals a specific mechanism for these effects whereby monosynaptic input to basolateral amygdala from MPFC inhibits activity from the central nucleus of the amygdala (Kim et al., 2011). Moreover, research in non-human primates indicates functional specificity of VMPFC for value updating (Rudebeck et al., 2013), providing evidence that VMPFC automatically processes information regarding the changing salience of stimuli. Recent studies in humans have also found converging support for the role of VMPFC in value updating (Levy and Glimcher, 2012).

We systematically examined the impact of mindfulness meditation training on affective processing by assessing amygdala activation and amygdala-VMPFC functional connectivity during an automatic emotion regulation task in a non-clinical, healthy population of adults who were in a normal state, not explicitly practicing any form of meditation. Brain activation was assessed using blood-oxygen level dependent functional magnetic resonance imaging (BOLD fMRI). We employed a rigorous design combining cross-sectional analysis of long-term meditators (LTM) compared to meditation-naïve participants (MNP), and a randomized controlled trial (RCT) in which a subset of MNP completed either an 8-week intervention with MBSR or a validated, active control condition (the health enhancement program; HEP) that was matched for intervention effects non-specific to mindfulness meditation (MacCoon et al., 2012). The LTM had a daily sitting meditation practice of at least 30 minutes for at least three years, and the primary type of meditation practice was most similar to that taught in MBSR — Vipassana (i.e. open monitoring; OM). In this form of meditation, practitioners cultivate sustained awareness to experience without attempting to control the focus of attention, but rather maintaining openness to any feelings that arise in awareness (Lutz et al., 2015).

We hypothesized that mindfulness meditation training would decrease reactivity to affective stimuli, as assessed by amygdala activation to positive and negative pictures (relative to neutral). We further hypothesized that mindfulness meditation training would enhance automatic emotion regulation, as reflected by greater amygdala-VMPFC functional connectivity during affective pictures (negative, positive) relative to neutral. First, we tested for differences in LTM compared to MNP, and then we tested for a similar pattern of effects following training with MBSR compared to HEP, while controlling for pre-treatment baseline (i.e. data collected prior to randomization). Using RCT data to follow up on cross-sectional analyses allowed us to rigorously control for influential factors that may have systematically differed between LTM and MNP. Finally, we assessed the length and type of meditation practice to test how variations in practice predicted differences in the brain and behavior.

Methods

Participants

We recruited 158 healthy human subjects from a non-clinical population, comprised of 127 meditation-naïve participants (MNP) and 31 long-term meditators (LTM). The MNP (average age 48.1±10.7 years, 81 female) comprised a much larger group as they participated in both the RCT and cross-sectional arms of the study, and were recruited within Madison, WI and the surrounding community using flyers, online advertisements, and advertisements in local media. Recruitment materials requested participation in a study of "health and well-being" or the "benefits of health wellness classes." Following baseline data collection, a sub-set of MNPs who participated in the cross-sectional arm of the study were randomly assigned to mindfulness meditation training or an active control intervention for the RCT: Mindfulness Based Stress Reduction (MBSR; N=43, average age 48.2 ±10.0 years, 27 female) or the Health Enhancement Program (HEP; N=43, average age 48.0 ±12.2 years, 27 female), which has been validated in a separate study (MacCoon et al., 2012). The intervention and randomization procedures were identical to that detailed by MacCoon et al. (2012) Four participants did not complete the fMRI task following the intervention, and an additional 15 participants left the study prior to post-intervention data collection due to logistical reasons, resulting in 32 participants who completed MBSR (average age 50.8 ±8.8 years, 22 female) and 35 participants who completed HEP (average age 48.1 ±12.6 years, 19 female).

The LTM (average age 50.7±10.1 years, 17 female) were recruited at meditation centers and through related mailing lists throughout the United States, in addition to flyers and advertisements in newspapers similar to the recruitment strategy for MNP. The LTM did not differ from the MNP in terms of age, gender, motion during the fMRI task, level of education, or socio-economic status measured with the Hollingshead index (Hollingshead, 1975), nor were there statistically significant effects of any of these demographic factors on any of the outcome variables, except in 2 cases as described in the Results section. Meditation recruitment criteria include at least three years of daily practice (at least 30 minutes per day of sitting meditation), experience with Vipassana, concentration and compassion/loving-kindness meditations, and at least 3 intensive retreats lasting 5 or more days. LTM had an average of 9,081 lifetime hours of meditation practice, ranging from 1,439 to 32,612 total hours, and which primarily consisted of mindfulness-based practices (focused attention and OM; 86 % of daily practice hours), in addition to some practice with compassion/loving kindness meditations (14% of daily practice hours). Lifetime hours of practice were calculated based on subjects reports of their average hours of formal meditation practice per week and their total years of practice. Participants in either group were excluded if they had used medication for anxiety, depression, or other psychological issues, or had a psychiatric diagnosis in the past year. Participants were also excluded if they had any history of bipolar or schizophrenic disorders, brain damage or seizures.

The automatic emotion regulation task was one of a number of tasks administered during a 24-hour lab visit as part of a larger, multi-session study. Meditation-naïve participants completed one lab visit prior to randomization, and then following the 8-week MBSR or HEP intervention participants returned for a post-training visit during which the same measures were collected. Experimenters were blind to the group assignment of meditation-naïve participants during data collection for the RCT. Subjects also completed a series of questionnaires, including the Five Facet Mindfulness Questionnaire (FFMQ; Baer et al., 2006), which includes a sub-scale that was used as a self-report measure of non-reactivity. Example items from the non- reactivity scale include: “When I have distressing thoughts or images, I just notice them and let them go”, and “I watch my feelings without getting lost in them”. A panel of other domain-specific questionnaires was included to address hypotheses from aspects of the larger study that were focused on relationships between meditation and sleep, health, response to social stress and pain processing, and which were irrelevant to the current analysis. UW-Madison’s Health Sciences Institutional Review Board approved the protocol, and all participants provided consent and were given monetary compensation for their participation. Two MNPs were excluded due to brain abnormalities, one dropped out of the study before the task was completed, and three were unable to complete the task due to technical difficulties. This left a total of 121 MNP’s (78 female, 119 right-handed) with average age of 48.3 ± 10.8 years, and an age range of 26 – 66 years. One LTM was excluded due to a dental implant which distorted the functional fMRI data, leaving a total of 30 LTM’s (16 female, 29 right-handed) with an average age of 50.5 ± 10.2 years and an age range of 28-62 years. We recruited a larger sample of MNP than LTM, due to the unique study design that incorporated an RCT for which we needed at least 30 MNP participants per group following randomization. The MNP in the RCT were a subset of the participants whose pre-randomization baseline data were included in the cross-sectional arm of this study.

Automatic Emotion Regulation Task

Seventy-two pictures selected from the International Affective Picture Set (Lang et al., 2008) were presented during fMRI scanning, and evenly split between negative, neutral, and positive pictures. This resulted in 24 pictures in each of the three valence categories, and each picture was presented for 4 seconds. The average normative valence (V) and arousal (A) ratings of the pictures in the three categories were negative (V = 2.87±0.87, A = 5.51±0.47), neutral (V

= 5.08±0.60, A = 3.86±0.63) and positive (V= 7.10±0.47, A = 5.36±0.37), where both valence and arousal are measured on 9-point scales (1= *most unpleasant or least arousing* and 9 = *most pleasant* or *most arousing*, respectively). Valence order was pseudo-randomized and picture order was completely randomized within the task. The positive pictures did not include erotic images from the IAPS set, and all the pictures in this task were evenly split between social and non- social categories. The task also included the presentation of neutral (male and female) faces from the Extended Multimodal Face Database ("The XM2VTS Database," n.d.)**,** which were presented for 500 ms after the offset of the picture in two-thirds of the trials, and appeared either 1 s (8x per valence) or 3 s (8x per valence) post-picture offset. There were also eight trials in which a face did not follow the image. The faces were included as a way to probe emotional spillover from the preceding IAPS stimuli, however there were no differences in BOLD activation to faces following negative and positive pictures compared to faces following neutral pictures. Thus, we focused solely on the picture presentation period.

Automatic emotion regulation is assumed to be a process that occurs in the absence of volitional attention and without explicit instructions. Thus, the task used in the current study entailed examining automatic emotion regulation processes in response to emotional pictures in the absence of an explicit request to voluntarily regulate emotion. Participants were instructed to press a button indicating the valence category of the picture (either negative, neutral, or positive) to ensure they were paying attention. Button order was counterbalanced across subjects. Participants were instructed to passively view the faces following the images. All stimuli were presented using E-Prime software (*E-Prime*, 2012) and participants viewed these images with a fiber-optic goggle system (Avotec, Inc., Stuart, FL) while inside the MRI scanner. Due to a technical error the button box response was not recorded for 85 participants (19 LTM), in which case they were sent a link to complete the picture categorizations online at home following the scan. Sixty-one participants (11 LTM) completed the online task at home to categorize the pictures. The task consisted of 4 runs of approximately 5 minutes each. In order to familiarize participants with the task and the scanning environment, they completed 6 practice trials (with a separate set of pictures not included in the scanner task) in a mock scanner prior to beginning the experiment.

Image Acquisition

Images were acquired on a GE X750-3.0 Tesla MRI scanner device with an 8- channel head coil. Anatomical scans consisted of a high-resolution 3D T1-weighted inversion recovery fast gradient echo image (inversion time = 450 msec, 256x256 in- plane resolution, 256 mm FOV, 124x1.0 mm axial slices). Four functional scan runs were acquired for the Automatic Emotion Regulation Paradigm using a gradient echo EPI sequence (64x64 in-plane resolution, 240 mm FOV, TR/TE/Flip = 2000 ms/25 ms/60°, 40x4 mm interleaved sagittal slices, and 159 3D volumes per run).

Image Processing

Functional images were processed using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library) including a high pass temporal filter of 100 s, motion correction with MCFLIRT (Jenkinson et al., 2002), BET (Smith, 2002) brain extraction, spatial smoothing at 5 mm FWHM, and FILM prewhitening (Woolrich et al., 2001). Transformation matrices for registration were computed at the first level (within scan run) and applied at the second level using FSL in a two stage process where the Boundary Based Registration (BBR) approach (Greve and Fischl, 2009) was used to register the subject's time series data to their anatomical template, and a 12 DOF affine transformation was used to register the subject's anatomical scans to Montreal Neurological Institute (MNI) space using FLIRT

(Jenkinson et al., 2002).

Experimental Design and Statistical Analysis

The functional data from individual subjects were analyzed using a General Linear Model (GLM) in 3 levels, where the first level (within-scan) modeled stimulus presentation with a double-gamma hemodynamic response function as defined in FSL. Each trial type was modeled with up to two regressors for each of two events; the 4 s presentation period of the IAPS image, and the 0.5 s presentation of the neutral face on the 2/3 of trials in which a face was presented (1/3 of trials did not have a face) for a total of 9 regressors (trials with faces were modeled separately but were collapsed together in the higher level analysis). Additional regressors of no interest were included to model the 24 total motion-related parameters (the standard plus extended parameters, which include the squares, derivatives, and squares of derivatives). To further address motion, high motion time points with a framewise displacement larger than 0.9 mm (Siegel et al., 2014) were modeled out of the data with an individual regressor. There were no scans with greater than 25% of the data censored for motion, and thus no participants or scans were excluded for excessive motion. The second level combined data within subject and across scans using a fixed effects modeling approach. Group analysis to check for whole-brain, voxelwise effects was done at the third level in which we modeled data across subjects using the Flame1 mixed effects model estimation. Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z>3.1$ and a (family-wise error corrected) cluster significance threshold of $p=0.05$ (Worsley, 2001) for the voxelwise, whole-brain analyses.

Functional amygdala ROIs were identified from the contrast of Negative>Neutral IAPS pictures during a 4 s picture presentation period (analogous to the task used in the current study except participants did not make a button press response) and using data from an independent sample of 90 participants enrolled in a separate study (mean age(SD)=45.1(10.0) years, 52 female) (Grupe et al., 2017). The right and left functional amygdala ROIs were then masked with anatomical amygdala ROIs from the Harvard- Oxford Atlas (Desikan et al., 2006) with a threshold at 50% probability to remove any non-amygdala voxels, resulting in a left and right amygdala ROIs of 104 and 164 voxels, respectively. Mean percent signal change across each amygdala ROI was extracted for all participants from the output of the second level model.

The functional ROI for the right amygdala was used to extract the timeseries, which was deconvolved (Gitelman et al., 2003) prior to computing the psychophysiological interaction (PPI) for the connectivity analysis. We then ran a second model to examine right amygdala-VMPFC connectivity, which included (in this order) all the regressors as the basic task model described above (9 task regressors and 24 motion regressors), a PPI regressor for each of the 3 task conditions (negative, neutral and positive IAPS), as well as the right amygdala seed timeseries, otherwise known as the gPPI approach (McLaren et al., 2012). The regressors were not orthogonalized. We computed contrasts for negative versus neutral and positive versus neutral PPIs, which were then used for a group level connectivity analysis from which we extracted values from a MPFC ROI based on the Harvard-Oxford Atlas containing 976 voxels (depicted in purple in Figure 2a). We used an anatomical mask for MPFC as we did not have an independent, functional localizer for this region. We also did not want to restrict the ROI based on a different task or analysis, which may not include regions of VMPFC that are differentially connected to amygdala due to training in mindfulness meditation. Statistical analysis for regions of interest was done using the lm function from the stats package in the statistical analysis software R (R Core Team, 2015; version 3.2.2), and *p*-value computation used the modelSummary function of the lmSupport package (Curtin, 2015).

Results

All results are reported after removing outliers based on Cook's D using a cutoff threshold of 4/(N-P) for data points disconnected from the distribution, where N and P correspond to the sample size and number of model parameters, respectively. When outliers were removed from the analysis they were also removed from the corresponding figure(s). Across all tests, 4 model outliers had extreme motion and 2 model outliers had extreme values in change in FFMQ non-reactivity, where points greater than 3 standard deviations (SD) from the mean are considered extreme. Thirty- seven percent of model outliers were outliers in multiple statistical tests. All findings remain consistent when outliers are included in the model, except in 3 cases as noted in the text. When comparing model outliers from all tests (N=38) we found that the outlier participants had significantly more motion (p=0.02) and lower non-reactivity (p=0.003) compared to participants who never appear as outliers (N=117). Descriptive statistics for all variables are available in Table 1.

Mindfulness meditation practice and amygdala reactivity: Analytic strategy.

We tested for group differences in amygdala reactivity to affective pictures, as assessed via BOLD fMRI signal for the contrast of negative (NEG) and positive (POS) picture trials minus neutral (NEU) picture trials (hereafter referred to as NEGPOS > NEU). As our hypotheses were specific to the amygdala (and its connectivity with VMPFC), we focused on the ROI analysis. However, a whole-brain, voxelwise analysis did not reveal any regions in which there were differences between groups (neither for LTM versus MNP, nor MBSR versus HEP post-intervention controlling for baseline). Significant clusters for LTM and MNP for each contrast of interest are presented in Table 3, and for MBSR and HEP participants at T2 (controlling for T1) in Table 4. Un-thresholded statistical maps for each group for these contrasts are also available at NeuroVault: https://neurovault.org/collections/PFXOLGSS/.

Long-term mindfulness meditation practice and amygdala reactivity.

Long-term meditators had significantly lower right amygdala activation than meditation-naïve participants for the contrast NEGPOS > NEU (*t*(145)=2.66, *b*=0.09, *p*=0.01, CI=[0.02,0.15], 1 LTM and 3 MNP outliers removed, partial *r* = 0.20). In order to decompose this effect, we conducted separate analyses on the negative and positive picture trials. Long-term meditators had significantly lower right amygdala activation for POS > NEU than meditation-naïve participants (*t*(147)=3.86, *b*=0.13, *p*=0.001, CI=[0.06,0.21], 2 MNP outliers removed, partial *r* = 0.26; Figure 1b), however there was only a marginal, non-significant difference for the contrast NEG > NEU (*t*(145)=1.65, *b*=0.06, *p*=0.10, CI=[-0.01,0.14], 1 LTM and 3 MNP outliers removed, partial *r* = 0.11; Figure 1a). When outliers were included in the model the group difference for NEG > NEU was significant (*p*=0.05). Results of all statistical tests, including non-significant results for the left amygdala, are reported in Table 2.

While participants' valence categorization of the pictures was intended to keep their attention on-task, rather than as a behavioral assay of emotion regulation or reactivity, we examined these behavioral responses in an exploratory fashion. Due to a technical error, button presses for 84 participants were not recorded during the scan: 23 participants (5 LTM) were completely missing the categorization data, and 61 participants (11 LTM) categorized the pictures online after the scan. We included a covariate to control for whether participants' behavioral data was collected concurrently in the scanner, or online afterward, and the results are consistent whether or not this covariate is included. Including all the available data, LTM categorized a significantly higher proportion of the pictures as neutral compared to MNP (*t*(122)=-2.52, *b*=-0.07, *p*=0.01, CI=[-0.12, -0.02]). Upon further inspection of this group difference broken down by the standardized IAPS valence categories, we found that the LTM categorized significantly more pictures as neutral than MNP for the negative IAPS pictures (*t*(119)=- 2.18, *b*=-0.04, *p*=0.03, CI=[-0.08, -0.004], 2 MNP and 1 LTM outliers removed), and positive IAPS pictures (*t*(122)=-3.77, *b*=-0.14, *p*<0.001, CI=[-0.22, -0.07]). There was no difference between groups in the percentage of neutral IAPS pictures categorized as neutral (*t*(120)=-0.45, *b*=-0.02, *p*=0.65, CI=[-0.11, 0.07], 2 LTM outliers removed). The percent of negative and positive pictures that LTM categorized as neutral did not relate to the intensity of amygdala activation for NEGPOS > NEU (*t*(20)=0.87, *b*=0.15, *p*=0.39, CI=[-0.21, 0.52]).

Short-term mindfulness meditation practice and amygdala reactivity.

In order to determine whether short-term meditation training would lead to similar reductions in amygdala reactivity to affective pictures as seen with long-term training, we compared post-training right amygdala activation in participants randomized to MBSR with the active control condition (HEP), while controlling for pre-treatment baseline activation (i.e. an analysis of covariance approach – ANCOVA). Since participants were randomized to either MBSR or HEP subsequent to pre-treatment (i.e. baseline) data collection we were able to leverage this more powerful, and more statistically appropriate, ANCOVA analytical approach as opposed to a repeated measures analysis of variance (ANOVA) (Van Breukelen, 2006). We computed the contrast NEGPOS > NEU to examine the impact of MBSR on emotional reactivity, and there was no post-intervention group difference in right amygdala activation between MBSR and HEP (*t*(64)=-1.45, *b*=-0.06, *p*=0.15, CI=[-0.01,0.47], partial *r* = 0.23; controlling for pre-treatment baseline). In light of the differences between long-term meditators and meditation-naïve participants specific to positive pictures, we also conducted analyses separately for each valence contrast. Participants who trained in MBSR had significantly lower right amygdala activation for the contrast POS > NEU compared to participants who trained in

HEP (*t*(58)=-2.65, *b*=-0.10, *p*=0.01, CI=[-0.18,- 0.02], 3 MBSR and 3 HEP outliers removed], partial *r* = 0.31; Figure 2b), while controlling for pre-treatment baseline. There were no baseline differences in right amygdala activation for POS > NEU in the same set of participants (*t*(59)=-0.38, *b*=- 0.01, *p*=0.71, CI=[-0.09,0.06]). Similar to the results for long-term practice, participants who trained in MBSR had only marginally, and non-significantly lower right amygdala activation to the contrast NEG > NEU compared to HEP training (*t*(57)=-1.84, *b*=-0.06, *p*=0.07, CI=[-0.13,0.01], 4 MBSR and 3 HEP outliers removed], partial *r* = 0.37), while controlling for pre-treatment baseline. When outliers were included in the model the group difference for NEG > NEU was non-significant (*p*=0.46). There were no significant differences in activation for the left amygdala (Table 2). We were unable to compare post-training differences in behavioral categorizations while controlling for baseline, due to a technical error by which many of the button presses were not recorded, and most participants are lacking data at both time-points.

All subsequent analyses focused on the right amygdala, since the group differences in amygdala reactivity were limited to the right side, and the contrasts were kept separate by valence due to differential effects by valence.

Practice duration and amygdala reactivity.

The long-term meditator group had considerable variability in self-reported total lifetime daily practice (mean=4,281(2,817) hours, range=954 – 13,605 hours) and in total lifetime retreat practice (mean=4,658 hours, range=258 – 29,710 hours), which allowed us to test whether the amount of self-reported practice accounted for significant variance in amygdala activation. The distribution of lifetime hours of practice was skewed, so all practice variables were log-transformed prior to analysis. We controlled for age in the following analyses, as it was correlated with log total practice time (*r*=0.30 with daily practice, and *r*=0.15 with retreat practice), and 2 outliers were removed from all of the analyses with practice hours. One of the two model outliers was also an extreme point (greater than 3 SD from the mean) with regard to amygdala activation for NEG > NEU.

Long-term meditators with greater total lifetime hours of retreat practice had the lowest amygdala activation for the contrast NEG > NEU (*t*(25)=-2.10, *b*=-0.03, *p*=0.05, CI=[-0.07,-0.001]). Due to the heterogeneous nature of meditation practices employed on retreats, we also conducted post-hoc analysis to test whether this relationship was true specifically for the type of meditation practice that is most similar to that taught in MBSR — Vipassana (i.e. OM) retreat practice. Greater total lifetime hours of OM retreat practice were negatively associated with amygdala activation for the contrast NEG > NEU (*t*(23)=-2.62, *b*=-0.04, *p*=0.02, partial *r*=-0.47, CI=[-0.06,-0.01]; Figure 1c)*. This relationship was not significant for total lifetime hours of daily practice (*t*(25)=-0.48, *b*=-0.02, *p*=0.64, CI=[-0.09,0.05]). There were no significant relationships between amygdala activation for the contrast POS > NEU and any of the measures of lifetime practice (Table 2). All the results remained the same when age was not included as a covariate.

The total minutes of MBSR training (during class and at home) was not associated with amygdala activation for NEG > NEU following training (*t*(26)=-1.19, *b*<0.01, *p*=0.24, CI=[0.00,0.00], 3 outliers removed), nor with amygdala activation for POS > NEU (*t*(28)=0.17, *b*<0.01, *p*=0.87, CI=[0.00,0.00], 1 outlier removed), while controlling for pre-treatment baseline.

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* Two participants did not report retreat hours specific to OM practice, and so the degrees of freedom are different for models that include this variable.

Relation between amygdala activation and self-reported reactivity

We tested whether amygdala activation was associated with a self-report measure of emotional reactivity – the non-reactivity sub-scale of FFMQ (Baer et al., 2006). Example items from the non-reactivity scale include: “When I have distressing thoughts or images, I just notice them and let them go”, and “I watch my feelings without getting lost in them”.

Participants (across LTM and MNP groups) with greater self-reported emotional non-reactivity had lower amygdala activation for the contrast POS > NEU ($t(148)=-3.20$, $b=-5.38$, $p<0.01$, CI=[-8.70,-2.06], partial $r = 0.24$), and this relationship remained significant when controlling for social desirability as measured with the Marlowe-Crowne Social Desirability questionnaire (MCSD; Figure 3a; $t(147)=-3.05$, $b=-4.79$, $p<0.01$, CI=[- 7.88,-1.69]). This relationship was specific to the non-reactivity sub-scale, as total FFMQ scores, which served as a measure of “mindfulness”, were not associated with amygdala activation for the contrast POS > NEU ($t(148)=-1.57$, $b=-9.70$, $p=0.12$, CI=[-21.9,2.54]). There was no relationship between amygdala activation for NEG > NEU with FFMQ non-reactivity ($t(148)=-1.33$, $b=-2.19$, $p=0.19$, CI=[-5.43,1.06]), nor with total FFMQ scores ($t(148)=-0.33$, $b=-1.92$, $p=0.74$, CI=[-13.44, 9.60]). One MNP model outlier was removed from each of these analyses, and this participant was also an extreme point with regard to self-reported non-reactivity. In line with the group differences in emotional reactivity as measured by amygdala activation, the long-term meditators reported greater non-reactivity on the FFMQ than the meditation naïve participants while controlling for social desirability from MCSD (Figure 3b; $t(151)=-7.25$, $b=-5.36$, $p<0.001$, CI=[-6.83,-3.90], 1 LTM outlier removed, partial $r = 0.53$).

The post-training amygdala activation for the contrast POS > NEU in MBSR and HEP participants did not predict post-training self-reported non-reactivity (FFMQ; $t(53)=1.67$, $b=2.81$, *p*=0.10, CI=[-0.57,6.20], 4 MBSR and 5 HEP outliers removed), nor for NEG > NEU (*t*(60)=1.51, *b*=3.10, *p*=0.14, CI=[-0.99,7.00], 1 MBSR and 1 HEP outlier removed), while controlling for baseline activation and baseline self-reported non- reactivity. Nor was there a group difference between MBSR and HEP in self-reported non-reactivity following training while controlling for baseline (*t*(57)=-0.28, *b*=-0.28, *p*=0.64, CI=[-1.48,0.91], 3 MBSR and 3 HEP outliers removed).

Amygdala–VMPFC functional connectivity during affective pictures: Analytic strategy.

We tested for group differences in amygdala functional connectivity with an anatomically defined VMPFC ROI in order to understand the potential impact of mindfulness meditation practice on amygdala reactivity in the context of functional networks associated with emotion regulation. We used the same functionally defined amygdala ROI as a seed for a psychophysiological interaction (PPI) analysis to compare the relationship of the seed activation during negative versus neutral images, and positive versus neutral images by extracting the mean *Z*-values from VMPFC ROI for each PPI. A positive PPI result implies the slope between the BOLD response in the amygdala and the target voxels in VMPFC was larger during negative than neutral pictures (or during positive versus neutral pictures).

Long-term practice and amygdala functional connectivity.

Long-term meditators had a significantly positive right amygdala-VMPFC PPI during NEG > NEU (*t*(27)=2.17, *b*=0.13, *p*=0.04, CI=[0.01,0.24], 2 outliers removed), while for MNPs the right amygdala-VMPFC PPI for this contrast was not significant (*t*(118)=1.17, *b*=0.04, *p*=0.25, CI=[-0.03,0.10], 2 outliers removed), though the difference between the groups was not significant (*t*(145)=-1.29, *b*=-0.09, *p*=0.20, CI=[- 0.23,0.05], 2 LTM and 2 MNP outliers removed). When outliers were included in the model the group difference for PPI effect of NEG

> NEU was significant (*p*=0.05). One LTM and one MNP model outlier were also extreme points with regard to the NEG > NEU PPI effect. There was also no group difference in amygdala-VMPFC PPI for the contrast of POS > NEU (*t*(149)=-0.71, *b*=-0.05, *p*=0.48, CI=[-0.17,0.08]), nor was there significant amygdala-VMPFC PPI for this contrast for either LTM (*t*(29)=0.04, *b*=0.002, *p*=0.97, CI=[-0.10,0.10]), nor MNP (*t*(120)=-1.47, *b*=-0.04, *p*=0.14, CI=[-0.10,0.02]). Since there was a significant difference in the POS > NEU PPI effect by gender we also tested for group differences controlling for gender, and there were no changes in the result. There was no relationship between long-term meditators' amygdala-VMPFC PPI and any of the measures of lifetime practice (Table 2). We examined the association between amygdala-VMPFC connectivity and the behavioral categorization of affective stimuli. There was no relationship between the percentage of positive IAPS categorized as neutral and the amygdala-VMPFC PPI effect for POS > NEU (*t*(20)=-1.05, *b*=-0.13, *p*=0.31, CI=[-0.37,0.13]), and there was only a trend-level effect for percentage of negative IAPS categorized as neutral and the PPI effect for NEG > NEU (*t*(19)=1.73, *b*=0.12, *p*=0.099, CI=[-0. 02,0.27]; 1 outlier removed).

Short-term practice and amygdala functional connectivity.

We also investigated amygdala-VMPFC functional connectivity to determine if MBSR training enhanced connectivity of this emotion regulation circuit. These analyses utilized the identical VMPFC ROI used in the analyses with the LTM. Participants randomly assigned to MBSR had greater post-treatment amygdala-VMPFC PPI than HEP participants during NEG > NEU (*t*(62)=3.99, *b*=0.29, *p*<0.001, CI=[0.15,0.44], 2 MBSR outliers removed, partial *r* = 0.42; Figure 2b), as well as for POS > NEU (*t*(58)=2.57, *b*=0.16, *p*=0.01, CI=[0.04,0.29], 4 MBSR and 2 HEP outliers removed, partial *r* = 0.30; Figure 2c), while controlling for pre-treatment baseline. Since there was a significant difference in the POS > NEU PPI effect by gender (at baseline), and a significant difference in change in the NEG > NEU PPI effect by age, we also tested for group differences controlling for these variables in each model (respectively), and there were no changes in the results. There was no relationship between amygdala-VMPFC PPI connectivity and total practice with MBSR (Table 2). There were no significant differences in baseline PPI measures between groups in the same sample of participants for NEG > NEU (*t*(63)=-.53, *b*=-0.05, *p*=0.60, CI=[-0.23,0.13]), or for POS > NEU (*t*(59)=-1.92, *b*=-0.14, *p*=0.06, CI=[-0.28,0.01]).

Subsequent to the publication of these findings, we conducted additional analyses to examine whether there was a relationship between changes in amygdala reactivity and functional connectivity during NEG>NEU in a post-hoc analysis. Decreased amygdala reactivity from T1 to T2 was associated with increased amygdala-VMPFC PPI (NEG>NEU), regardless of intervention group (t(59)=-2.15, p=0.04, b=-0.12; Figure 4).

Discussion

We rigorously tested the relationship between mindfulness meditation training and affective processing by combining a cross-sectional design comparing long-term meditators to non-meditators with an RCT comparing MBSR and HEP. We were able to examine mechanisms underlying changes in affective processing by examining both amygdala reactivity and connectivity with VMPFC during automatic emotion regulation, and in a much larger sample size than has been previously reported in the literature on the effect of mindfulness meditation on automatic emotion regulation (Desbordes et al., 2012). The automatic emotion regulation task used in this study was designed to specifically probe participants' automatic response to emotional pictures, whereby the degree to which emotion regulation processes were automatically engaged would be reflected by individual differences in the amygdala response to affective stimuli. We have argued in other contexts that this form of emotion regulation is ubiquitous and is likely the most common form of emotion regulation in everyday life (Lapate et al., 2014). The current study was limited to inferring that regulation occurred, as there were no measures of participants’ emotional experience or regulation strategies during the task. Inclusion of trial-by-trial questions about regulation strategy and/or affective experience may have altered subsequent responses or primed participants to use an emotion regulation strategy, which we wanted to avoid in order to measure participants’ natural response.

Emotional reactivity, as assessed via right amygdala activation to affective pictures (combined across positive and negative), was lower in participants who engaged in long-term mindfulness meditation training compared to controls. We tested separately by valence to decompose this effect and found that LTM had lower amygdala activation than MNP specifically to positive pictures, but not negative pictures (for which there was only a marginal difference between groups). The LTM also reported less emotional reactivity than MNP on a questionnaire measure (FFMQ), however we did not find a difference in self-reported emotional reactivity following MBSR compared to HEP. It is possible that it requires more practice or practice over a longer period of time to see movement on self-report measures of emotional reactivity in healthy, non-clinical populations, particularly in comparison to a well-matched active control intervention.

In an exploratory analysis we also found that the LTM categorized more affective pictures as neutral compared to MNP. This pattern of behavioral results mirrors the pattern of results in the fMRI data and lends further support to the hypothesis that long-term mindfulness meditation training improves emotion regulation through a reduction in emotional reactivity. However, we did not find a relationship between the percent of negative and positive pictures that LTM categorized as neutral and the amygdala activation, and there was only a trend-level relationship with amygdala-VMPFC connectivity during negative versus neutral pictures. The exploratory nature of our behavioral analysis, and the technical difficulties with recording button responses in the scanner, greatly limited inference from these results, as well as our power to detect an effect. Future research should follow up to more carefully examine whether (and how) behavioral changes in valence categorization are associated with mindfulness training- related changes in self-report and neurobiological measures of emotion regulation.

Participants naïve to meditation who were randomly assigned to short-term mindfulness training showed a similar pattern: a reduction in amygdala reactivity specifically in response to positive images but not negative images when compared to HEP and controlling for the pre-treatment baseline activation. These results are consistent with prior work that meditation training decreased amygdala reactivity to positive pictures (Desbordes et al., 2012). Mindfulness meditation provides training in paying attention to thoughts and emotions as they unfold naturally and allowing them to pass without trying to avoid discomfort or to grasp onto pleasant experiences. One mechanism that could lead to decreased emotional reactivity with practice in mindfulness meditation is through increased exposure to the arising and passing of emotions accompanied by this new habit of attending to the experience non-reactively. Recent evidence that behavioral measures of mindfulness were associated with decreased attentional capture to rewarding stimuli provide additional evidence for this interpretation (Levinson et al., 2014). Importantly, decreased non-reactivity of the amygdala to positive stimuli does not preclude increased pleasure or liking of positive stimuli, similar to that found in prior work showing increased positive mood with mindfulness training (Jain et al., 2007). Prior research has dissociated the neural basis of pleasure from that of the "wanting" associated with approach behavior (Smith et al., 2011). It is likely that the amygdala, a key node in the salience network, marks wanting more than liking whereas the work of Berridge and colleagues suggests that local circuitry within the ventral pallidum is uniquely associated with liking (Smith et al., 2011). Therefore, our findings of decreased amygdala activation to positive stimuli are most consistent with a decreased desire to approach (or grasp at) a stimulus rather than a change in pleasure or appreciation.

Over time the practice of observing thoughts non-reactively may lead to greater automatic emotion regulation similar to extinction processes that recruit VMPFC (Phelps et al., 2004; Hölzel et al., 2011). The process strengthened by mindfulness meditation is not a specific decrease in reactivity to negative stimuli but rather may impact emotional responding more generally and is consistent with the view from contemplative traditions that this practice decreases “stickiness” – the power of emotions to linger and alter subsequent experience – by reducing the propensity to avoid unpleasant and grasp at pleasant experiences. This is summarized nicely by Hölzel: “nonreactivity leads to unlearning of previous connections (extinction and reconsolidation) and thereby to liberation from being bound to habitual emotional reactions” (2011). The fact that MBSR significantly increased the amygdala-VMPFC PPI effect relative to HEP training for both negative versus neutral pictures and positive versus neutral pictures lends further support to this explanation, and implies a similar emotion regulatory mechanism for reducing reactivity to affective stimuli independent of valence.

While prior research has provided evidence that amygdala-VMPFC functional connectivity correlates with improvements in emotion regulation, the current study had no direct, objective measures of emotion regulation behavior. Future research would benefit from including such behavioral measures. The paradigm used in the current study could also be improved in future research by including stimuli that are more naturalistic and personally relevant to participants, as the small-to-medium effect sizes seen in the current study may in part reflect the generic nature of the stimuli and less likelihood to elicit strong, self-relevant affective experiences.

The fact that short-term MBSR training affected amygdala-VMPFC functional connectivity in ways that we did not detect in participants with long-term training is revealing and implies a curvilinear trajectory in the neural changes required for learning the skill of non-reactivity. On this view, with long-term training the reduction in reactivity to affective stimuli becomes more automatic such that VMPFC recruitment is less necessary. This explanation aligns with subjective reports from practitioners, and with the goals and expectations of mindfulness meditation practice: to practice being aware and accepting of (affective) experience so that over time this process becomes more automatic. The fact that we found a relationship between hours of retreat practice and reduced amygdala reactivity, but not with amygdala-VMPFC connectivity lends further support to the idea that increased connectivity may only occur at earlier stages of practice, and fits with the nonlinear dosage-response curve of mindfulness meditation training (Brefczynski-Lewis et al., 2007). Moreover, the lack of relationships between individual differences in amygdala reactivity and the amount of MBSR practice (e.g. variation in the degree to which participants engaged in at-home practice, or ‘homework’ during MBSR) may also indicate an important distinction between short- and long-term practice: it may be that there is a more “all-or-nothing” effect of exposure to MBSR in terms of short-term effects while long-term changes may be more dependent upon practice dosage. Longitudinal research that follows practitioners as they progress from initial, to intermediate, and more advanced practice will be critical to provide insight into the trajectory of affective change with mindfulness meditation.

Of course, the fact that short-term MBSR training produced alterations in amygdala-VMPFC connectivity not seen among the LTM can also be due to other factors. All participants in the MBSR group received the same intervention and were recruited from the same geographic community (in Madison, WI), whereas the LTM had larger variation in practice type, duration and geographic location. The LTM likely also had greater variability in their motivations for engaging in practice. We attempted to minimize such variation by recruiting LTM who had similar practice that included primarily mindfulness-based meditation.

Interestingly, long term meditators' total lifetime practice while on retreat predicted reduced amygdala activation to negative pictures, while total hours of non-retreat practice (i.e. totally daily practice hours over the lifetime) was not associated with amygdala reactivity. This effect was stronger when looking specifically at retreat practice in OM meditation. All the long-term meditators had a daily practice that included OM meditation, and so engaging in this style of practice while on retreat appears to be particularly beneficial. The inverse relationship between OM retreat practice and reactivity to negative IAPS, combined with non-significant group differences in amygdala reactivity to negative IAPS, implies that altering the response to negative affect may require more intense practice over time (specifically in OM meditation). Retreats tend to differ from daily practice in many ways; the duration of practice is longer, the context promotes a continual focus on the goals of the training with fewer distractions than is typical for daily life, there is usually greater social support or accountability, and there is a release from daily work or family expectations. Any one or a combination of these and other factors that allow for a more intense focus on OM practice may facilitate change. Additionally, individual differences in factors relating to participants' choice and ability to go on retreat may be important to consider in relation to their emotional reactivity, though all the LTMs in this study by design were required to have participated in at least a few retreats. Future research could better assess the impact of retreat practice by assessing the different components of retreat practice, and by measuring responses before and after meditation retreats, as well as longitudinally across the development of participants' meditation practice from novice to expert.

We had also planned to test the impact of mindfulness meditation training on amygdala recovery to affective stimuli, as assessed by the intensity of amygdala activation during neutral faces following positive and negative pictures compared to neutral pictures. However, none of the groups had amygdala activation for the contrast NEGPOS > NEU during the face period with which we could examine group differences. There are a couple potential reasons why we failed to find a main effect of the preceding IAPS valence during the face period (i.e. emotional spillover from the affective stimuli onto the neutral faces that immediately followed). A subsequent study in our lab has shown that conscious awareness of affective stimuli dampens affective spillover onto subsequent neutral stimuli (Lapate et al., 2014). Since participants in this study were instructed to make a button press to indicate the valence of the IAPS, this brought awareness to the source of affective information very explicitly. Thus, the affective experience induced from the IAPS was less likely to be misattributed to the neutral face stimuli. Furthermore, research has shown that affect labeling can serve as a form of implicit emotion regulation and result in dampening of the amygdala response, which was associated with increased ventrolateral PFC activation mediated by VMPFC (Lieberman et al., 2007). While the valence labeling aspect of the task likely contributed to the lack of a valence effect on the subsequent neutral face response, it also likely allowed us to more easily assess individual differences in the ability to engage with automatic (i.e. implicit) emotion regulation processes during the IAPS presentations.

Labeling experiences – including affective experiences – is also a component of mindfulness meditation training, and has been previously proposed as one possible mechanism by which such practices train emotion regulation (Lutz et al., 2008). Prior research has found associations between self-reported dispositional mindfulness and reduced amygdala activation during affect labeling tasks (Creswell et al., 2007). Therefore, participants with mindfulness meditation training may have more easily or robustly engaged this automatic form of emotion regulation due to the affect labeling component of the task, possibly leading to lower amygdala reactivity. The results of the current study extend this literature in novel ways by demonstrating similar effects with a much stronger, RCT design combined with cross-sectional data from LTM.

The present study provides evidence that long and short-term training in mindfulness meditation improves automatic emotion regulation and elucidates the neural correlates of this improvement. Long and short-term mindfulness meditation training were associated with lower amygdala activation while viewing affective pictures. Short-term mindfulness meditation training with MBSR was also associated with a stronger amygdala-VMPFC PPI effect during negative and positive pictures (versus neutral) compared to the active control condition (HEP), while controlling for pre- treatment baseline. These findings are consistent with other evidence in suggesting that engagement of prefrontal regulatory resources may be more pronounced in the earlier stages of mindfulness training and dissipate with longer-term practice. Taken together, these findings provide novel evidence that training in mindfulness meditation alters the neural circuitry of automatic emotion regulation, which may be instantiated early on by modulating connectivity between the VMPFC and amygdala.

Conflicts of Interest: Dr. Richard J. Davidson is the founder, president, and serves on the board of directors for the non-profit organization, Healthy Minds Innovations, Inc. In addition, Dr. Davidson serves on the board of directors for the Mind and Life Institute. No donors, either anonymous or identified, have participated in the design, conduct, or reporting of research results in this manuscript.

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Author Contributions: T.R.A.K. and B.S.S. collected and processed the data. T.R.A.K. analyzed the data and wrote the manuscript. B.S.S. contributed to planning the analysis. M.A.R. and A.L. contributed to the design of the study. J.A.M. contributed to data processing and statistical analysis. R.J.D. designed and supervised the study. All authors contributed to writing the manuscript.

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Figures

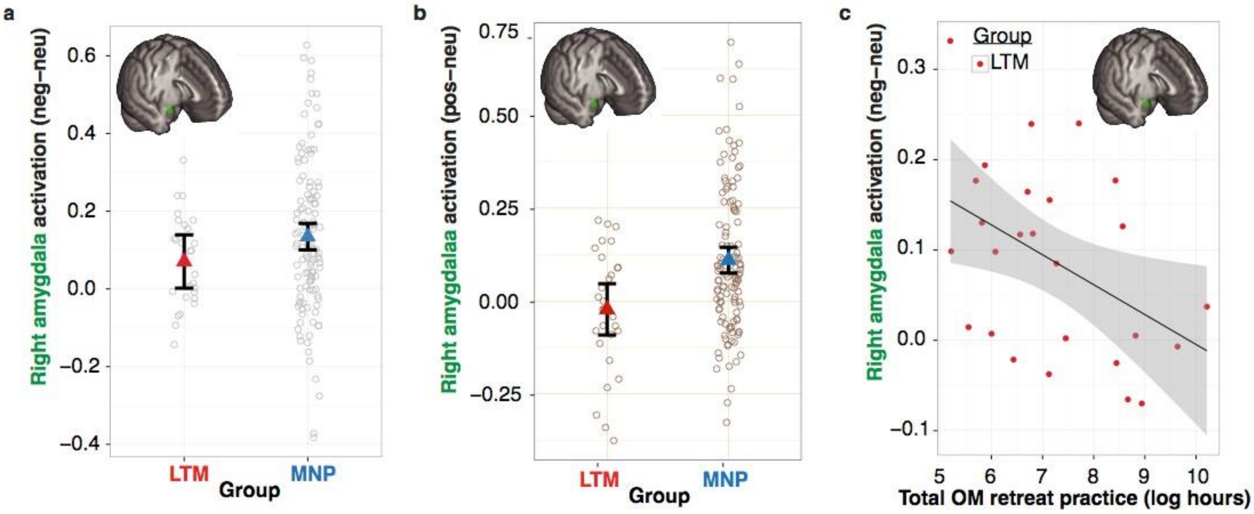

Figure 1. Long-term meditation training is associated with lower right amygdala reactivity to emotional pictures. Long-term meditators (LTM) had lower right amygdala activation for the negative versus neutral pictures at a trend level (a), and significantly lower activation for positive versus neutral pictures than mediation-naïve participants (MNP; b). Greater lifetime hours of open monitoring (OM) retreat practice predicted lower right amygdala activation for negative versus neutral pictures, controlling for age (c). The independently defined right amygdala region of interest from which mean percent signal change data were extracted is inset in each panel. Circles represent raw data points in a and b, and the raw data are adjusted for age in c. Error bars and envelopes are 95% confidence intervals around the point estimates.

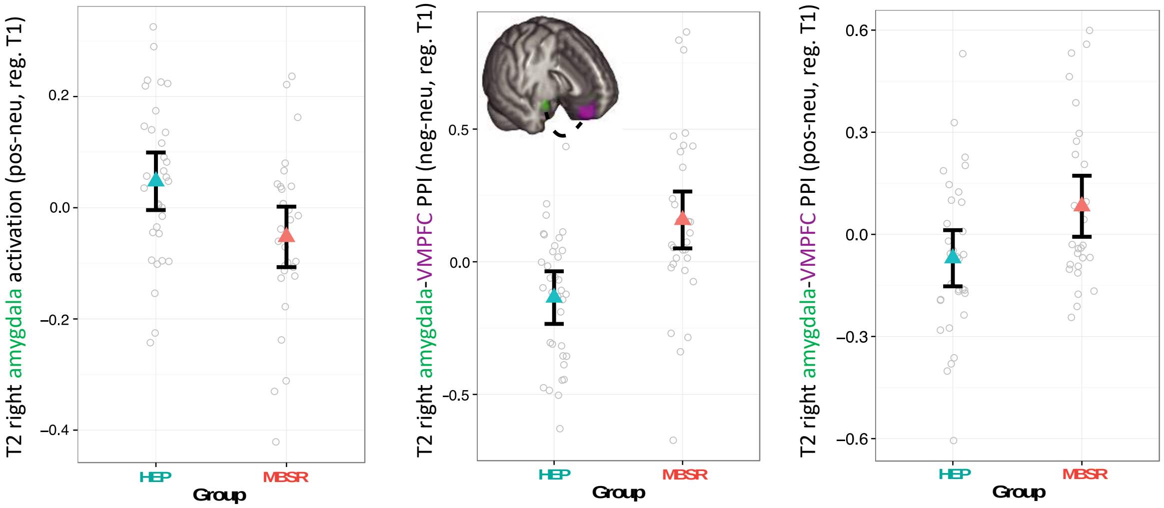

Figure 2. Short-term meditation training causes less right amygdala reactivity and greater connectivity with VMPFC in response to emotional pictures. The ventral medial prefrontal cortex (VMPFC) region of interest (purple) from which we extracted mean psychophysiological interaction (PPI) weights (represented by the dashed line) with the right amygdala ROI (green) is inset in panel b. Participants had lower right amygdala activation following Mindfulness-Based Stress Reduction (MBSR) compared to the Health Enhancement Program (HEP) for positive versus neutral pictures (a), and increased right amygdala-VMPFC PPI for negative versus neutral pictures (b), and for positive versus neutral pictures (c). Analyses and data points are adjusted for pre-treatment baseline activation, and error bars are 95% confidence intervals around the point estimates.

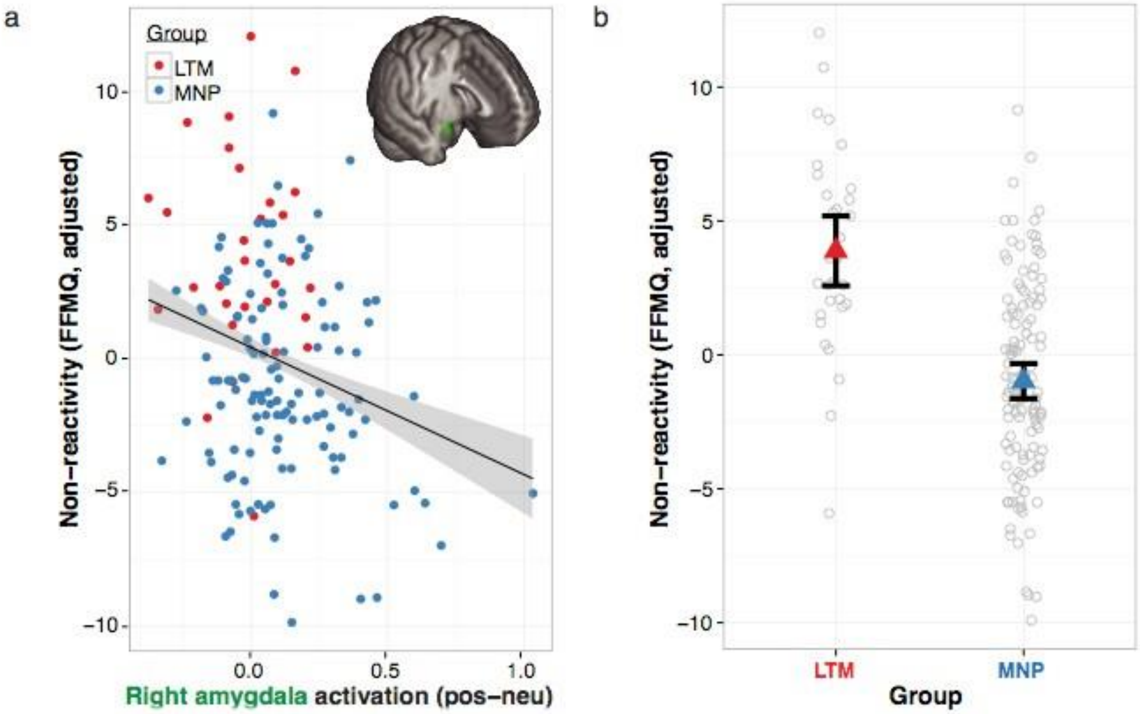

Figure 3. Reactivity in self-report and in right amygdala. Higher self-reported non-reactivity on the Five Facet Mindfulness Questionnaire (FFMQ) predicted lower right amygdala activation for positive versus neutral pictures (a). The independently defined right amygdala region of interest from which mean percent signal change data were extracted is inset in panel a. Long-term meditators (LTM) had overall higher self-reported non-reactivity than meditation-naive participants (MNP) (b). Example items from the non-reactivity scale include: “When I have distressing thoughts or images, I just notice them and let them go”, and “I watch my feelings without getting lost in them”. Error bars and envelopes are 95% confidence intervals around the point estimates.

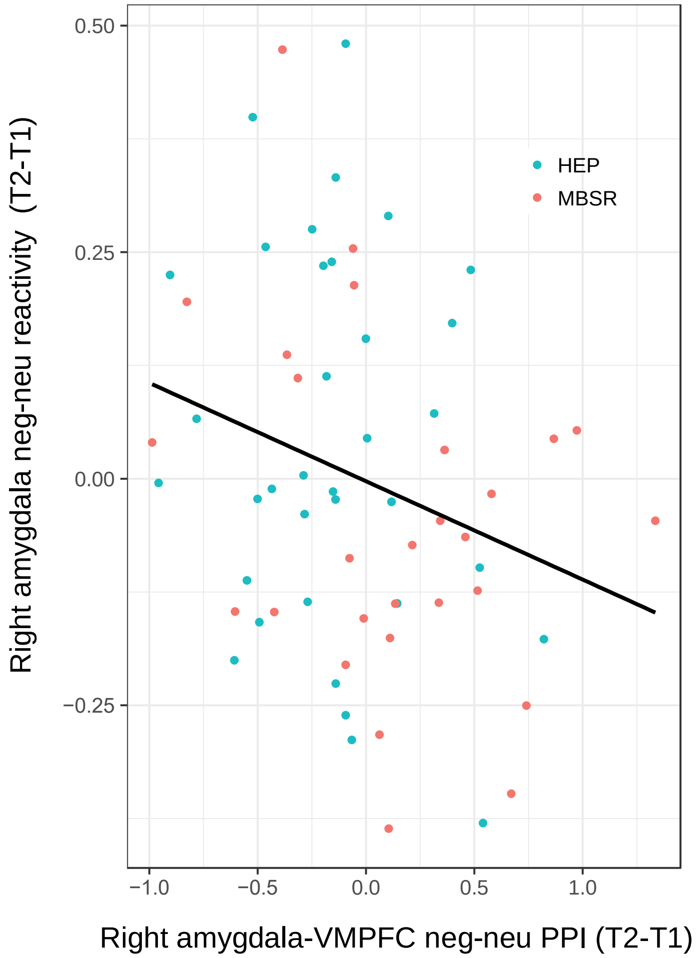

Figure 4. Post-hoc analysis of amygdala reactivity versus connectivity with VMPFC during NEG>NEU. Subsequent to the publication of these findings, we found decreased right amygdala activation from T1 to T2 was associated with increased amygdala-VMPFC functional connectivity (NEG>NEU), regardless of intervention group.

Tables

	Right amygdala activation											
	NEGPOS > NEU				NEG > NEU				POS > NEU			
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	0.02	0.13	-0.34	0.28	0.06	0.14	-0.37	0.33	-0.02	0.16	-0.38	0.22
MNP	0.12	0.22	-0.93	1.11	0.15	0.24	-0.51	1.13	0.11	0.25	-1.35	1.03
T1 MBSR	0.13	0.17	-0.32	0.60	0.16	0.21	-0.49	0.55	0.09	0.17	-0.16	0.60
T1 HEP	0.11	0.19	-0.23	0.80	0.11	0.25	-0.37	1.09	0.11	0.18	-0.16	0.53
T2 MBSR	0.05	0.20	-0.49	0.52	0.13	0.20	-0.20	0.71	-0.02	0.26	-0.80	0.75
T2 HEP	0.11	0.16	-0.35	0.41	0.15	0.19	-0.36	0.71	0.06	0.20	-0.45	0.59
	Left amygdala activation											
	NEGPOS > NEU				NEG > NEU				POS > NEU			
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	0.08	0.17	-0.45	0.42	0.10	0.22	-0.40	0.64	0.06	0.21	-0.61	0.43
MNP	0.11	0.25	-0.41	1.07	0.15	0.26	-0.58	1.01	0.08	0.28	-0.57	1.09
	Right amygdala–VMPFC PPI								Self-reported non-reactivity (FFMQ)			
	NEG > NEU				POS > NEU							
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	0.20	0.40	-0.42	1.34	0.00	0.27	-0.55	0.69	28.77	4.32	15	35
MNP	0.03	0.42	-2.18	1.77	-0.04	0.33	-1.12	1.64	23.42	3.91	14	33
T1 MBSR	0.02	0.61	-2.21	1.81	-0.10	0.46	-1.15	1.69	23.44	3.93	15	33
T1 HEP	0.08	0.38	-0.85	0.75	-0.01	0.24	-0.47	0.53	23.49	4.25	14	31
T2 MBSR	0.21	0.40	-0.88	0.93	0.08	0.34	-0.63	0.86	25.16	4.37	17	35
T2 HEP	-0.07	0.25	-0.58	0.52	-0.06	0.27	-0.64	0.72	24.63	4.14	9	32
	Percent pictures categorized as neutral											
	Negative				Positive				Neutral			
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	0.20	0.26	0.00	1.00	0.37	0.24	0.04	1.00	0.58	0.22	0.21	1.00
MNP	0.08	0.10	0.00	0.50	0.18	0.16	0.00	0.75	0.52	0.19	0.12	1.00

Table 1. Descriptive statistics by group including mean (M), standard deviation (SD), minimum (Min), and maximum (Max) for all variables reported in the Results section. Pre- and post-intervention statistics are presented for the right amygdala. LTM = long- term meditators; MNP = meditation-naïve participants; MBSR = randomized to Mindfulness-Based Stress Reduction; HEP = randomized to health enhancement program control intervention; NEG = negative IAPS; POS = positive IAPS; NEU = neutral IAPS.

Table 2. Summary of statistical results.

Right amygdala activation (NEGPOS > NEU)						Left amygdala activation (NEGPOS > NEU)					
	df	*t*	b	*p*	*CI*		df	*t*	b	*p*	*CI*
LTM vs MNP	145	2.66	0.09	0.01	[0.02, 0.15]	LTM vs MNP	146	-0.12	-0.01	0.90	[-0.09, 0.08]
MBSR vs HEP	64	-1.45	-0.06	0.15	[-0.01, 0.47]	MBSR vs HEP	64	-1.15	-0.07	0.25	[-0.19, 0.05]
Right amygdala activation (NEG > NEU)						Left amygdala activation (NEG > NEU)					
	df	*t*	b	*p*	*CI*		df	*t*	b	*p*	*CI*
LTM vs MNP	145	1.65	0.06	0.10	[-0.01, 0.14]	LTM vs MNP	145	0.53	0.03	0.60	[-0.07, 0.13]
MBSR vs HEP	57	-1.84	-0.06	0.07	[-0.13, 0.01]	MBSR vs HEP	63	-0.76	-0.05	0.45	[-0.18, 0.08]
Right amygdala activation (POS > NEU)						Left amygdala activation (POS > NEU)					
	df	*t*	b	*p*	*CI*		df	*t*	b	*p*	*CI*
LTM vs MNP	147	3.86	0.13	0.001	[0.06, 0.21]	LTM vs MNP	146	-0.33	-0.02	0.75	[-0.11, 0.08]
MBSR vs HEP	58	-2.65	-0.10	0.01	[-0.18, -0.02]	MBSR vs HEP	59	-1.54	-0.08	0.13	[-0.19, 0.02]
Right amygdala–VMPFC PPI (NEG > NEU)						Right amygdala–VMPFC PPI (POS > NEU)					
	df	*t*	b	*p*	*CI*		df	*t*	b	*p*	*CI*
LTM vs MNP	145	-1.29	-0.09	0.20	[-0.23, 0.05]	LTM vs MNP	149	-0.71	-0.05	0.48	[-0.17, 0.02]
LTM Retreat Practice	27	0.18	0.01	0.86	[-0.12, 0.14]	LTM Retreat Practice	28	0.08	0.003	0.93	[-0.08, 0.09]
LTM OM Retreat	25	0.37	0.02	0.72	[-0.10, 0.15]	LTM OM Retreat	26	0.17	0.01	0.87	[-0.07, 0.09]
LTM Daily Practice	26	0.35	0.05	0.73	[-0.23, 0.32]	LTM Daily Practice	28	-0.05	-0.003	0.96	[-0.16, 0.16]
MBSR vs HEP	62	3.99	0.29	<0.01	[0.15, 0.44]	MBSR vs HEP	58	2.57	0.16	0.01	[0.04, 0.29]
MBSR Practice	28	1.65	<0.01	0.11	[0.00, 0.00]	MBSR Practice	29	-1.20	<0.01	0.24	[0.00, 0.00]
Meditation time vs right amygdala activation (NEG > NEU)						Meditation time vs right amygdala activation (POS > NEU)					
	df	*t*	b	*p*	*CI*		df	*t*	b	*p*	*CI*
LTM Retreat	25	-2.10	-0.03	0.05	[-0.07, 0.00]	LTM Retreat	25	-0.72	-0.02	0.50	[-0.07, 0.03]
LTM OM Retreat	23	-2.62	-0.04	0.02	[-0.06, -0.01]	LTM OM Retreat	23	-0.95	-0.02	0.35	[-0.07, 0.03]
LTM Daily	25	-0.48	-0.02	0.64	[-0.09, 0.05]	LTM Daily	25	-0.51	-0.02	0.62	[-0.12, 0.07]
MBSR Minutes	26	-1.19	<0.01	0.24	[0.00, 0.00]	MBSR Minutes	28	0.17	<0.01	0.87	[0.00, 0.00]
Self-reported non-reactivity (FFMQ)											
	df	*t*	b	*p*	*CI*		df	*t*	b	*p*	*CI*
LTM vs MNP	151	-6.61	-4.92	<0.01	[-6.39, -0.36]	MBSR vs HEP	57	-0.48	-0.28	0.64	[-1.48, 0.91]
Non-reactivity vs right amygdala activation (NEG > NEU)						Non-reactivity vs right amygdala activation (POS > NEU)					
	df	*t*	b	*p*	*CI*		df	*t*	b	*p*	*CI*
LTM+MNP	148	-1.33	-2.19	0.19	[-5.43. 1.06]	LTM+MNP	148	-3.20	-5.38	<0.01	[-8.70, -2.06]
MBSR+HEP	60	1.51	3.10	0.14	[-0.99, 7.00]	MBSR+HEP	53	1.67	2.81	0.10	[-0.57, 6.20]
Percent pictures categorized as neutral											
LTM vs MNP	df	*t*	b	*p*	*CI*	LTM vs MNP	df	*t*	b	*p*	*CI*
NEG IAPS	119	-2.18	-0.04	0.03	[-0.08, -0.01]	All IAPS	122	-2.52	-0.07	0.01	[-0.12, -0.01]
POS IAPS	122	-3.77	-0.14	<0.01	[-0.22, -0.07]						
NEU IAPS	120	-0.45	-0.02	0.65	[-0.11, 0.07]						

Table 2. Summary of statistical results for all tests reported in the Results section, including degrees of freedom (df), *t-value*, *p-value*, parameter estimates (*b*), and confidence intervals (*CI*). LTM = long-term meditators; MNP = meditation-naïve participants; MBSR = randomized to Mindfulness-Based Stress Reduction; HEP = randomized to health enhancement program control intervention; NEG = negative IAPS; POS = positive IAPS; NEU = neutral IAPS

Table 3. Summary of significant clusters for LTM and MNP (T1).

		REGION (PEAK)	MAX (Z)	PEAK X	PEAK Y	PEAK Z	VOLUME (mm)
NEGPOS > NEU	LTM	Precuneus	5.8	-4	-60	22	1138
		Occipital Pole	4.5	4	-90	4	845
		Left Inferior Lateral Occipital Cortex	4.4	-50	-74	10	487
		Right Temporal Pole	4.2	48	22	-20	219
		Right Inferior Lateral Occipital Cortex	4.1	46	-62	8	177
	MNP	Right Amygdala	8.9	24	-10	-12	7561
		Right Superior Temporal Gyrus	9.1	48	-64	10	6628
		Precuneus	9.9	2	-60	28	3208
		Left Inferior Lateral Occipital Cortex	8.4	-48	-74	10	3123
		Superior Frontal Gyrus	6.5	6	56	24	2740
		Right Superior Parietal Lobule	4.2	22	-46	72	353
NEG > NEU	LTM	Right Superior Temporal Gyrus	4.8	58	-4	-14	455
		Precuneus	4.4	-6	-48	42	449
		Middle Temporal Gyrus	4.0	-56	-60	8	315
		Occipital Pole	3.9	4	-92	2	163
	MNP	Right Amygdala	9.3	24	-8	-10	3782
		Left Lateral Occipital Cortex	8.3	-54	-64	12	2767
		Middle Temporal Gyrus	7.8	46	-58	14	2330
		Precuneus	6.9	-4	-52	48	2050
		Left Amygdala	8.6	-28	-12	-14	1987
		Right Occipital Pole	6.3	24	-98	4	770
		Left Occipital Pole	5.9	-14	-100	0	546
		Superior Frontal Gyrus	5.6	6	50	32	511
		Right Anterior Supramarginal Gyrus	5.3	68	-24	34	429
		Left Anterior Supramarginal Gyrus	4.0	-58	-28	28	313
		Left Subcallosal Cortex	5.6	-2	0	-12	258
POS > NEU	LTM	Precuneus	5.7	-4	-60	24	1071
		Occipital Pole	5.0	2	-90	14	729
		Left Lateral Occipital Cortex	4.8	-40	-70	40	331
		Frontal Pole	4.8	-10	66	8	252
	MNP	Precuneus	11.2	0	-62	30	32,003
		Left Hippocampus	6.6	-24	-18	-12	933
		Right Fusiform Cortex	6.0	44	-54	-20	323
		Left Temporal Pole	4.5	-48	2	-24	271
		Left Middle Temporal Gyrus	5.1	-66	-10	-16	260

Table 3. Significant clusters for long-term meditators (LTM) and meditation-naïve participants (MNP) at baseline (T1) (cluster-forming threshold Z>3.1 and family-wise error corrected cluster significance threshold of *p*=0.05). NEG = negative IAPS; POS = positive IAPS; NEU = neutral IAPS.

Table 4. Summary of significant clusters for MBSR and HEP (T2 reg. T1).

		REGION (PEAK)	MA	PEAK X	PEAK Y	PEAK Z	VOLUME (mm)
NEGPOS > NEU	MBSR	Left Inferior Lateral Occipital Cortex	6.7	-58	-68	10	1864
		Right Middle Temporal Gyrus	5.6	50	-52	10	1741
		Right Superior Lateral Occipital Cortex	4.7	24	-82	42	260
		Left Superior Lateral Occipital Cortex	4.7	-18	-84	42	200
	HEP	Left Middle Temporal Gyrus	6.8	-48	-62	6	3439
		Right Inferior Lateral Occipital Cortex	5.7	50	-62	4	1859
		Right Superior Parietal Lobule	5.6	16	-56	60	1286
		Left Supramarginal Gyrus	4.6	-58	-30	36	573
		Right Supramarginal Gyrus	4.6	56	-30	38	448
		Right Superior Lateral Occipital Cortex	5.3	24	-82	40	439
		Left Occipital Pole	4.7	-20	-92	26	433
		Right Intracalcarine Sulcus	4.5	14	-82	12	266
		Left Amygdala	4.8	-26	-8	-12	258
		Right Amygdala	4.5	18	-2	-10	220
		Right Precentral Gyrus	4.4	50	6	28	175
NEG > NEU	MBSR	Left Inferior Lateral Occipital Cortex	6.3	-48	-64	12	5334
		Right Lingual Gyrus	6.6	16	-64	-6	4083
		Right Precentral Gyrus	4.8	48	8	28	898
		Midbrain	5.8	-2	-32	-6	285
		Right Putamen	4.3	30	-16	-8	167
		Precuneus	4.3	0	-54	60	154
	HEP	Left Intracalcarine Cortex	6.6	-8	-84	4	15,429
		Midbrain	6.2	-2	-30	-8	2627
		Precuneus	4.7	-8	-52	52	1343
		Right Precentral Gyrus	5.2	50	4	30	1254
		Left Supramarginal Gyrus	5.5	-60	-36	44	1066
		Left Precentral Gyrus	5.1	-46	0	36	1065
		Right Supramarginal Gyrus	4.3	64	-28	30	440
		Right Amygdala	5.8	18	-2	-10	414
		Occipital Pole	4.7	32	26	6	257
POS > NEU	MBSR	Right Inferior Lateral Occipital Cortex	5.5	52	-68	10	828
		Left Inferior Lateral Occipital Cortex	6.2	-58	-66	10	705
	HEP	Left Middle Temporal Gyrus	6.4	-46	-62	8	1109
		Right Inferior Lateral Occipital Cortex	6.2	48	-64	2	1108
		Right Superior Parietal Lobule	5.1	26	-44	46	1062
		Right Superior Lateral Occipital Cortex	4.7	24	-82	40	172

Table 4. Significant clusters for participants following training (T2) in Mindfulness-Based Stress Reduction (MBSR) or the health enhancement program (HEP) control intervention, controlling for baseline (T1) with a voxel-wise regressor (cluster-forming threshold Z>3.1 and family-wise error corrected cluster significance threshold of *p*=0.05). NEG = negative IAPS; POS = positive IAPS; NEU = neutral IAPS

Chapter 2

Impact of short- and long-term mindfulness meditation training on resting state brain connectivity.

Study 1 published as:

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Study 1: Mindfulness-Based Stress Reduction-related changes in posterior cingulate resting brain connectivity.

Abstract

Mindfulness meditation training has been shown to increase resting state functional connectivity between nodes of the frontoparietal executive control network (dorsolateral prefrontal cortex [DLPFC]) and the default mode network (posterior cingulate cortex [PCC]). We investigated whether these effects generalized to a Mindfulness-Based Stress Reduction (MBSR) course, and tested for structural and behaviorally relevant consequences of change in connectivity. Healthy, meditation-naïve adults were randomized to either MBSR (N=48), an active (N=47) or waitlist (N=45) control group. Participants completed behavioral testing, resting state fMRI scans, and diffusion tensor scans at pre-randomization (T1), post-intervention (T2) and approximately 5.5 months later (T3). We found increased T2-T1 PCC–DLPFC resting connectivity for MBSR relative to control groups. Although these effects did not persist through long-term follow-up (T3-T1), MBSR participants showed a significantly stronger relationship between days of practice (T1 to T3) and increased PCC–DLPFC resting connectivity than participants in the active control group. Increased PCC–DLPFC resting connectivity in MBSR participants was associated with increased microstructural connectivity of a white matter tract connecting these regions, and increased self-reported attention. These data show that MBSR increases PCC–DLPFC resting connectivity, which is related to increased practice time, attention, and structural connectivity.

Introduction

Mindfulness meditation practice aims to improve well-being through training attention to present-moment experience (Kabat-Zinn, 1990). There is growing evidence that mindfulness meditation training improves multiple aspects of attention and executive functioning, including improved working memory (Zeidan et al., 2010), decreased rumination and distraction (Jain et al., 2007), and decreased mind-wandering (Mrazek et al., 2013). Moreover, decreases in mind-wandering mediated improvements in cognitive performance following mindfulness meditation training (Mrazek et al., 2013). These behavioral changes following mindfulness practice suggest dynamic alterations in brain networks responsible for implementing attention and executive function.

The PCC is the central hub of the default mode network (DMN) (Spreng et al., 2013), which is a set of brain regions in which activation is correlated at rest and is associated with mind-wandering and self-referential processing in task-based fMRI (Andrews-Hanna et al., 2010; Mason et al., 2007). A cross-sectional study of long-term mindfulness meditation practitioners by Brewer *et al.* (2011) found reduced activation of this network at rest and during meditation practice, which was interpreted as reflecting reduced mind-wandering and increased focus on present moment experience. However, research to date has not provided direct, supporting evidence for this interpretation. Brewer *et al.* (2011) also found increased resting state functional connectivity (RSFC) between the PCC and a node of the frontoparietal executive control network (FPN), the DLPFC, in long-term mindfulness practitioners compared to non-meditators, suggesting a functional mechanism by which attentional improvements and reductions in mind-wandering may manifest. The FPN coordinates activation between the DMN and the dorsal attention network, for example for top-down modulation of mind-wandering

(MacDonald et al., 2000; Smallwood et al., 2012). Nodes of the FPN, including DLPFC, are also active during mind-wandering, and one interpretation infers this activation as constraining or regulating mind-wandering (Christoff et al., 2016, 2009). A recent study found that PCC-DLPFC RSFC also increased following short-term mindfulness meditation training relative to an active control intervention (Creswell et al., 2016).

Despite this progress, prior research is limited by small sample sizes, use of non-standard interventions, lack of meaningful behavioral correlates and failure to include diffusion-weighted measures of structural connectivity. In the present study we evaluate the impact of the most commonly used mindfulness intervention – Mindfulness-Based Stress Reduction (MBSR). MBSR is manualized, widely available and has been adapted for use in a variety of populations and contexts (Kabat-Zinn, 1990). Furthermore, arguments regarding the practical significance of mindfulness meditation-related changes in functional connectivity would be more compelling if these findings were mirrored by changes in the microstructure of pathways connecting these regions, and/or accompanied by alterations in behavioral processes served by these networks.

In the current study we sought to conceptually replicate and extend prior work showing that short-term mindfulness meditation training increases PCC–DLPFC RSFC in a randomized controlled trial of MBSR. We employed a validated, active control intervention well-matched to MBSR, the Health Enhancement Program (HEP) (MacCoon et al., 2012), and utilized a sample size that was approximately double that used in prior research (Creswell *et al*., 2016). We defined a PCC seed and PFC target region of interest (ROI) based on coordinates reported in Creswell *et al*. (2016); we refer to this region as “LPFC” as these coordinates are anterior and ventral to the canonical DLPFC that is part of the FPN. We also investigated PCC connectivity with a more canonical, anatomically-defined DLPFC target ROI, the middle frontal gyrus (MFG)

(Kim et al., 2012; Petrides and Pandya, 1999; Ptak, 2012) from the Harvard-Oxford Atlas (Craddock et al., 2012). We examined individual differences in practice duration to determine whether there was a dosage effect on changes in RSFC. Finally, we investigated associations between increased PCC–DLPFC RSFC and decreased mind-wandering, and with microstructural connectivity of a white matter tract connecting PCC and DLPFC, the superior longitudinal fasciculus (SLF). Thus, the current study enables us to examine with rigorous design and methodology, the efficacy of mindfulness meditation training in altering the structure and function of brain circuits associated with mind-wandering and attention, and their behavioral correlates.

Methods

This study is registered as a clinical trial with ClinicalTrials.gov (NCT02157766).

Participants

We recruited 140 healthy human participants (average age 44.3±12.8 years, 83 female) from Madison, WI and the surrounding community using flyers, online advertisements, and advertisements in local media. Recruitment materials described the study as researching "the impact of health wellness classes on the brain and body". Sixteen participants had RSFC data excluded from baseline analysis due to excessive motion (described below; n=11) or anatomical brain abnormalities as determined by a radiologist (n=5). Participants were randomly assigned to one of three groups following baseline data collection: MBSR, the HEP active control group, or a waitlist control group (WL). Participants were block randomized following stratification based on gender (male, female) and age (25-39 years; 40-50 years; 51-65 years). The intervention procedures were identical to those detailed by MacCoon *et al.* (2012). Twenty-nine additional participants were excluded from analysis of pre/post differences: 16 participants withdrew prior to T2, 6 participants were excluded for failure to attend more than 1 HEP or MBSR class, 1 participant had poor data quality (severe signal dropout in PFC), 1 participant was excluded for medical reasons, and 5 participants were excluded due to excessive motion during the T2 scan. Thus, there were 31 MBSR (average age 41.4±12.9 years, 18 female), 34 HEP (average age 43.6±13.1 years, 22 female), and 30 WL (average age 43.0±12.0 years, 19 female) participants in analyses of T2-T1 RSFC. An additional 10 participants withdrew prior to T3 and 3 were excluded for excessive motion at the third scan, resulting in 29 MBSR (average age 41.1±13.5 years, 17 female), 27 HEP (average age 43.7±13.4 years, 16 female), and 29 WL (average age 44.0±11.7 years, 18 female) for T3-T1 RSFC analyses. A subset of participants completed the Emotional Styles Questionnaire (ESQ), which was introduced subsequent to the onset of data collection due to availability of the measure: 25 MBSR (average age 40.2±12.5 years, 12 female), 24 HEP (average age 42.0±13.5 years, 13 female), and 21 WL (average age 41.6±10.6 years, 12 female).

Participants were excluded if any of the following applied, due to their potential impact on the current analyses or other aspects of the larger study in which they were enrolled: regular use of psychotropic or nervous system altering medication; psychiatric diagnosis in the past year or history of bipolar disorder, schizophrenia or schizoaffective disorder; color blindness; currently participating in another clinical trial; current asthma diagnosis; currently diagnosed with a sleep disorder or regularly taking prescribed sleeping medications; current night shift worker; significant training or practice in meditation or mind-body techniques such as yoga or Tai-Chi; expert in physical activity, music or nutrition (for HEP); any history of brain damage or seizures; medical problems that would affect the participant’s ability to participate in study procedures. Written, informed consent was obtained from all participants according to the

Declaration of Helsinki (“WMA - The World Medical Association-WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects,” n.d.) and the study was approved by the Health Sciences Institutional Review Board at the University of Wisconsin–Madison.

Data collection

Participants completed a baseline data collection visit prior to randomization, a second visit following the 8-week intervention period and a third visit approximately 5 – 6 months after the second visit. At each of these times, participants attended a 24-hour lab visit that included an MRI scan and the ESQ (Kesebir et al., n.d.) among other measures as part of a larger multi-session, multi-project study. The ESQ consists of a 1 – 7 Likert scale with 1 = strongly disagree and 7 = strongly agree. One of the ESQ sub-scales provided a measure of attention that was most relevant to the hypotheses of the current study, and items included: “I do not get distracted easily, even when I am in a situation in which a lot is going on” and “I sometimes feel like I have very little control over where my attention goes” (reverse-coded). Experimenters were blind to the group assignment during data collection. All participants were given monetary compensation for their participation.

2.3 Experience sampling

Experience Sampling was conducted during the week prior to and following the intervention period (14 days total). Participants provided their cellular phone numbers and available 8-hour periods for each of the 14 days. Participants had a choice of receiving text messages 6, 7, or 8 times a day, and received a text message every 90 minutes on average. The text message contained a question assessing mind-wandering: “Was your attention on the activity you were performing?” Participants were asked to respond with a number from 1 (attention is not on the task) to 9 (attention is completely on the task at hand). On average participants responded to 82 percent of text messages they received. The response window was set to the time between two successive messages such that participants were given until the next message arrived to respond to the current message. If participants sent two responses in-between messages, the second response was discarded. The ratings across all 7 days of the week for each time period (pre/post-intervention) were averaged to obtain a mean attention rating for each participant at T1 and T2.

Image acquisition

Images were acquired on a GE MR750 3.0 Tesla MRI scanner with a 32-channel head coil. Anatomical scans consisted of a high-resolution 3D T1-weighted inversion recovery fast gradient echo image (inversion time = 450 msec, 256x256 in-plane resolution, 256 mm FOV, 192x1.0 mm axial slices). A 12-minute functional resting state scan run was acquired using a gradient echo EPI sequence (360 volumes, TR/TE/Flip = 2000 ms/20 ms/75°, 224 mm FOV, 64x64 matrix, 3.5x3.5 mm in-plane resolution, 44 interleaved sagittal slices, 3-mm slice thickness with 0.5 mm gap). The in-plane resolution was decreased after the first 21 participants from 3.5x3.5 mm to 2.33*3.5 mm to better address sinus-related artifacts, resulting in a matrix of 96x64. Diffusion weighted images (DWIs) were acquired with single shot spin-echo EPI sequence (TR/TE/Flip = 8575 msec /76.6 msec /90$_o$, 75x2 mm interleaved sagittal slices, and 2.0 mm isotropic voxels). In total, 63 DWIs were acquired along non-collinear diffusion encoding directions across three b-values of 500/800/2000 s/mm$_2$ (9/18/36 directions, respectively) while 6 additional images with no diffusion encoding (i.e. b=0 s/mm$_2$) were acquired. Parallel acquisition with a geometric reduction factor of two was used to reduce image acquisition time and distortions from magnetic field inhomogeneities. The total time for the multiple b-value DTI acquisition was 10 minutes.

Image processing: RSFC

Functional images were processed using a combination of AFNI (Cox, 1996) (versions 17.3) and FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library) (Smith et al., 2004), including the following steps: removal of the first 4 volumes; motion correction with MCFLIRT (Jenkinson et al., 2002); BET (Smith, 2002) brain extraction; registration of the subject's functional data with their anatomical image using the Boundary Based Registration approach (Greve and Fischl, 2009). A 12 DOF affine transformation using FLIRT (Jenkinson et al., 2002) was followed by FNIRT nonlinear transformation to register each subject's functional data to Montreal Neuroligical Insitute 152 space. Images were segmented into white matter, grey matter and cerebrospinal fluid with FAST for use as masks that were eroded using a 3x3x3 voxel kernel and then used to generate ROI-averaged time series serving as nuisance regressors (along with their derivatives and the 6 motion regressors) with AFNI's 3dDeconvolve. Images were smoothed with a 5-mm full-width half-maximum Gaussian kernel.

We extracted the time-series from a spherical PCC seed with a 4-mm radius defined based on coordinates from Creswell *et al*. (2016). We regressed this time-series back onto each subject's data using AFNI's 3dDeconvolve, which also censored high-motion time-points (greater than 0.2 mm framewise displacement) (Power et al., 2014). Participants were excluded from analysis if they had less than 6 minutes of data due to more than 50% of data points censored for motion. Two sets of target ROIs were defined: a bilateral DLPFC ROI, based on MFG from the Harvard-Oxford atlas (Craddock et al., 2012) thresholded at 50% probability for small-volume-corrected voxelwise analysis, which was split into left and right for ROI analysis;

and left and right LPFC ROIs defined as 10-mm spheres around coordinates provided in Creswell *et al*. (2016). Resting state fMRI connectivity was assessed based on the Fisher-Z transformed (FZT) correlation between the PCC seed and every other voxel in the brain for the voxelwise analysis, and separately for each of the target ROIs.

Image processing: DTI

DWIs were corrected for between-volume head motion and eddy currents using FSL's eddy tool (Andersson and Sotiropoulos, 2016), while diffusion encoding gradient directions were additionally corrected for rotations (Leemans and Jones, n.d.). Brain extraction was performed using FSL's BET tool (Smith, 2002) and maps of diffusion tensors were calculated using the robust estimation of tensors by outlier rejection (RESTORE) (Chang et al., n.d.) algorithm as implemented by the diffusion imaging in python (DIPY) open source package (Garyfallidis et al., 2014). Quantitative maps of functional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD), were derived from the diffusion tensors (Basser and Pierpaoli, 1996).

A population-specific template was created from a representative subset of the subject data to minimize coordinate system induced bias. Affine and diffeomorphic registration as implemented in DTI-TK (Zhang et al., 2006) was used for estimating the spatial transformations. Data from the individual subjects were spatially normalized to the estimated population-specific template. The JHU ICBM-DTI-81 FA template (Mori et al., 2005) was co-registered to our population-specific FA template using diffeomorphic registration as implemented by the Advanced Normalization Tools (ANTs) (Avants and Gee, 2004) software package. Deep white matter labels included as part of the JHU template were warped into the space of our population-specific template using nearest neighbor interpolation. Right and left hemisphere SLF ROIs were then inverse warped to the native space of each participant using the inverse of the subject-specific spatial transformation obtained from DTI-TK. Median FA, MD, RD, and AD in these subject level SLF ROIs were then calculated. The median was used over the mean in order to be robust to outlier voxels within the ROIs.

Statistical analysis

The analysis plan for this study was pre-registered on the Open Science Framework at: https://osf.io/vrmz9/register/5771ca429ad5a1020de2872e. The analytic plan said linear models looking at T2 and T3 adjusting for T1 would be used, but we instead used T3-T1 and T2-T1 differences in our models, for consistency with analyses in other studies that were part of the larger project in which this study was embedded. We also changed the cutoff for the maximum number of TRs censored for motion from 25% to 50%, given that the resting state scans were about twice as long in duration than those in prior studies on which this cutoff was defined. Analyses of baseline data regressed the T1 measures on group or other variables of interest. For each analysis examining changes due to MBSR, we computed difference scores (T2 - T1 or T3 - T1), which were regressed on group to test the effect of MBSR compared to HEP or to WL. Within-group analyses tested the intercept of the linear model for the difference score, reflecting whether or not the change in the dependent variable was significantly different than zero. Comparisons between two different variables regressed the difference score for one variable on the difference scores for the second variable. All tests were conducted using linear models from the lm function in the stats package in R (R Core Team, 2015). All analyses included covariates for age and gender, and analyses of RSFC included an additional covariate for the change in the resting state scan acquisition (as described above). Significance of results are consistent regardless of inclusion of the covariates, except where noted in the text. All results are reported after removing outliers based on Cook's D, with a cutoff threshold of 4/(N-P) for data points disconnected from the distribution (where N=sample size and P=number of parameters in the model) as determined by the modelCaseAnalysis function of the lmSupport package (Curtin, 2015) in R (R Core Team, 2015). The significance of results is consistent with or without outliers included, except where indicated. We used a false discovery rate (FDR) correction to control for multiple comparisons for each family of tests with the p.adjust function in the stats package in R (R Core Team, 2015), and the corrected *p*-values are indicated by *p**.

RSFC analysis

In order to test for intervention effects, we computed difference maps for the T2 or T3 map subtracting the T1 map. We assessed the effects of mindfulness meditation on PCC–DLPFC RSFC by extracting the average RSFC FZT difference score across two sets of ROIs: 1) the anatomically-defined DLPFC ROI (split between left and right hemispheres of the Harvard-Oxford-defined MFG) and 2) two spheres of 10-mm radius in left and right LPFC centered on coordinates provided by Creswell *et al* (2016). The anatomical DLPFC ROIs provided a means to examine connectivity in a more "canonical" DLPFC region than the spherical ROIs defined from Creswell *et al.* (2016), and were also broader than the spherical ROIs. In addition, we examined group differences in a voxelwise fashion within the DLPFC ROI. We conducted a secondary voxelwise analysis across the whole brain to identify other regions that might differ in connectivity between groups, and to test for specificity of changes to PCC–DLPFC RSFC. Voxel-wise analyses were thresholded at $p<0.05$ controlling for family-wise error using threshold-free cluster enhancement with FSL's Randomize (Winkler et al., 2014).

Within the MBSR group, we tested relationships between PCC–DLPFC RSFC and two measures of mind-wandering as potential behaviorally-relevant outcomes of stronger connectivity between these regions: self-reported attention on the ESQ (Kesebir et al., n.d.) and experience sampling of mind-wandering via text messaging. We included difference scores of the attentional measure as a covariate of interest for each participant in a voxelwise analysis within the anatomical DLPFC. We also examined this relationship in an ROI analysis by extracting the average PCC RSFC from the left and right anatomical DLPFC ROIs, which were entered as the independent variables in linear models with the attention measure (T2-T1 for both variables).

DTI analysis

We tested whether MBSR-related changes in PCC–DLPFC RSFC were associated with changes in the SLF using DTI measures. The median values for FA, MD, RD, and AD of the SLF ROIs were entered into a principle component analysis (PCA) (McLaughlin et al., 2018; Zeestraten et al., 2016), using the prcomp function in R (R Core Team, 2015) to construct a right and left hemisphere composite of the underlying microstructure. The first principle component was used as a representative composite of white matter microstructure as this accounted for the greatest covariance between the four DTI parameters (77.1% for the right, 80.0% for the left). The factor loadings for fractional anisotropy and for radial, mean and axial diffusivity were 0.44, -0.54, -0.56 and -0.45 for the right side, respectively; and 0.45, -0.53, -0.56 and -0.46 for the left side, respectively. Thus, larger values of the microstructure composite are indicative of higher anisotropy and lower diffusivity, which is generally interpreted as a superior white matter microstructure (Basser and Jones, n.d.; Jones et al., 2013). DTI values at T2 were computed using the weights from this first principle component of the PCA analysis using the predict function in R (R Core Team, 2015).

Results: Confirmatory analyses

Group differences in PCC RSFC.

ROI analysis

Using anatomically generated DLPFC ROIs, participants who completed MBSR had increased PCC-right DLPFC RSFC from pre- to post-intervention compared to participants who completed HEP ($t(59)=2.16$, $p=0.04$, $p^*=0.05$, $b=0.05$, CI=[0.004, 0.10]; 1 HEP outlier removed) and compared to WL ($t(55)=2.53$, $p=0.01$, $p^*=0.04$, $b=0.06$, CI=[0.01, 0.10], 1 WL outlier removed; Figure 1A). Post-hoc analyses revealed that the change in PCC–DLPFC RSFC was significantly positive for MBSR ($t(27)=3.66$, $p=0.001$, $p^*=0.003$, $b=0.05$, CI=[0.02, 0.08]) and non-significant for HEP ($t(29)=0.05$, $p=0.96$, $p^*=0.96$, $b=0.001$, CI=[-0.04,0.04]; 1 outlier removed) and WL ($t(25)=-0.25$, $p=0.81$, $p^*=0.96$, $b<-0.01$, CI=[-0.04,0.03]; 1 outlier removed). The difference in PCC connectivity with left DLPFC was significant for MBSR compared to HEP ($t(60)=2.13$, $p=0.04$, $p^*=0.05$, $b=0.06$, CI=[0.003,0.11]), but non-significant compared to WL ($t(52)=1.34$, $p=0.19$, $p^*=0.19$, $b=0.03$, CI=[-0.02,0.08], 2 WL and 2 MBSR outliers removed). There were no group differences in PCC–DLPFC RSFC at baseline for MBSR relative to HEP or WL in either hemisphere ($ts<-1.77$, $ps>0.10$).

We tested whether changes in PCC–DLPFC RSFC persisted to T3. There were no significant group differences in T3-T1 PCC–DLPFC RSFC for MBSR compared to HEP (right DLPFC: $t(50)=0.99$, $p=0.33$, $p^*=0.44$, $b=0.03$, CI=[-0.03, 0.08], 1 HEP outlier removed; left DLPFC: $t(50)=1.06$, $p=0.30$, $p^*=0.44$, $b=0.03$, CI=[-0.03, 0.08], 1 HEP outlier removed). There were also no group differences in T3-T1 PCC–DLPFC RSFC for MBSR versus WL (right DLPFC: $t(52)=1.12$, $p=0.27$, $p^*=0.44$, $b=0.03$, CI=[-0.02, 0.08], 1 WL outlier removed; left DLPFC: $t(53)=0.54$, $p=0.60$, $p^*=0.60$, $b=0.02$, CI=[-0.04, 0.07]).

Using ROIs generated from coordinates in Creswell *et al.* (2016), there were no group differences in PCC–LPFC RSFC for T2-T1 for MBSR compared to HEP (right LPFC: *t*(55)=-0.19, *p*=0.85, *p**=0.94, *b*=-0.01, CI=[-0.07, 0.05], 1 MBSR and 4 HEP outliers removed; left LPFC: *t*(59)=-0.08, *p*=0.94, *p**=0.94, *b*=-0.002, CI=[-0.06, 0.05]; 1 HEP outlier removed). There were also no group differences in PCC–LPFC RSFC connectivity from T1 to T2 for MBSR compared to WL (right LPFC: *t*(56)=1.50, *p*=0.14, *p**=0.56, *b*=0.05, CI=[-0.02, 0.11]; left LPFC: *t*(54)=-0.63, *p*=0.53, *p**=0.94, *b*=-0.02, CI=[-0.07, 0.00]; 2 WL outliers removed). Given that the effects of MBSR training were limited to PCC RSFC with the anatomically defined DLPFC, subsequent analyses were limited to examining PCC RSFC with this target ROI.

Voxel-wise analysis

There were no regions in which PCC RSFC differed for MBSR participants compared to HEP or WL at baseline in the wholebrain analysis. MBSR participants showed significant increases in PCC RSFC with a network of regions that included DLPFC (primarily inferior frontal gyrus [IFG]) from T1 to T2 in the voxel-wise, whole-brain analysis (Figure 2A). MBSR participants had significantly increased PCC RSFC with a region in right inferior temporal gyrus (ITG) compared to HEP (Figure 2B), and no significant differences compared to WL. No statistically significant differences were observed in PCC–DLPFC RSFC when comparing MBSR to control groups. However, at a whole-brain threshold of $p < 0.10$, MBSR participants had increased PCC RSFC with right DLPFC (primarily IFG) relative to HEP, and with left frontal pole relative to WL (Figure 1C-D), among other regions (see Table 1 for detailed cluster information). There were no regions with significant (or marginal) changes in PCC RSFC within HEP or WL, and no regions where HEP or WL increased in PCC RSFC relative to MBSR (either significantly or at a trend level).

MBSR participants had increased PCC RSFC with right DLPFC in the small-volume-corrected, voxelwise analysis (Figure 3A). No significant group differences were observed in the voxelwise analysis restricted to the anatomical DLPFC ROI when comparing MBSR to control groups. However, at a threshold of $p < 0.10$, MBSR participants had increased PCC RSFC with right DLPFC relative to HEP (Figure 3B). We observed the same pattern for MBSR compared to WL, however at baseline WL participants also had higher PCC RSFC than MBSR participants in an overlapping cluster of right DLPFC (Figure 3C). There was no difference in PCC–DLPFC RSFC between MBSR and HEP at baseline.

There were no regions across the whole brain or within the small-volume-corrected anatomical DLPFC that showed significant group differences in PCC RSFC from T1 to T3.

Experience sampling

Within the MBSR group, there was no significant relationship between changes in PCC–DLPFC RSFC and mind-wandering based on experience sampling in the voxelwise analysis (p<0.05 corrected for FWE), nor in the ROI analyses (right DLPFC: t(21)=-1.12, p=0.28, p*=0.28, b=-2.24, CI=[-6.43, 1.95], 1 outlier removed; left DLPFC: (t(21)=-1.95, p=0.07, p*=0.14, b=-2.62, CI=[-5.42, 0.18], 1 outlier removed). There was no difference in the change in mind-wandering between MBSR and HEP (t(62)=0.85, p=0.40, p*=0.40, b=0.17, CI=[-0.24, 0.58], 1 HEP and 1 MBSR outlier removed) or WL (t(73)=1.91, p=0.06, p*=0.12, b=0.31, CI=[-0.01, 0.64], 1 MBSR outlier removed).

Results: Exploratory analyses

Structural connectivity

There were no group differences between the change in SLF DTI for MBSR compared to HEP (right SLF: t(70)=0.08, p=0.94, p*=0.94, b=0.01, CI=[-0.21, 0.23], 4 MBSR outliers removed; left SLF: *t*(73)=0.94, *p*=0.35, *p**=0.47, *b*=0.10, CI=[-0.11, 0.31], 1 MBSR outlier removed) or compared to WL (right SLF: *t*(74)=0.94, *p*=0.35, *p**=0.47, *b*=0.13, CI=[-0.14, 0.40]; left SLF: *t*(72)=-1.31, *p*=0.20, *p**=0.47, *b*=-0.14, CI=[-0.35, 0.07], 1 WL and 1 MBSR outlier removed). However, within the MBSR group, increased PCC–right DLPFC RSFC was associated with increased SLF DTI from T1 to T2 (*t*(26)=2.93, *p*=0.01, *p**=0.02, *b*=0.06, CI=[0.02, 0.11]; Figure 1B). There was no relationship in the MBSR group with change in PCC–DLPFC RSFC and SLF DTI on the left side (*t*(25)=1.00, *p*=0.33, *p**=0.33, *b*=0.05, CI=[-0.05, 0.15], 1 outlier removed).

Practice time

The change in PCC–left DLPFC RSFC from pre- to post-intervention was associated with practice time to a significantly higher degree in MBSR relative to HEP on the left side (*t*(57)=2.22, *p*=0.03, *p**=0.06, *b*=0.004, CI=[0.0004, 0.01], 1 MBSR outlier removed; Figure 4A); however this relationship was non-significant when the influential outlier was included (*t*(58)=1.59, *p*=0.12, *p**=0.24, *b*=0.003, CI=[-0.001, 0.01]). Within the MBSR group the relationship between practice and PCC–left DLPFC RSFC was significantly positive (*t*(25)=2.12, *p*=0.04, *p**=0.08, *b*=0.003, CI=[0.0001, 0.01], 1 MBSR outlier removed), whereas there was no relationship for HEP (*t*(29)=-0.67, *p*=0.51, *p**=0.51, *b*=-0.001, CI=[-0.003, 0.002]). The group difference in the relationship was non-significant on the right side (*t*(55)=0.69, *p*=0.49, *p**=0.49, *b*=0.001 CI=[-0.002, 0.004], 3 HEP outliers removed).

There was no significant group difference in the relationship between practice and PCC–left DLPFC RSFC at T3 (*t*(47)=0.17, *p*=0.17, *p**=0.17, *b*<0.01, CI=[-0.0003, 0.001], 1 MBSR and 1 HEP outlier removed). The change in PCC–right DLPFC RSFC from T1 to T3 was associated with practice over the same time period to a significantly higher degree in MBSR compared to HEP (*t*(47)=2.34, *p*=0.02, *p**=0.04, *b*=0.001, CI=[<0.001, 0.002], 2 HEP outliers removed; Figure 4B).

Attention questionnaire

Increased self-reported attention on the ESQ was associated with stronger PCC–left DLPFC RSFC from pre- to post-MBSR (T2-T1) in the voxelwise analysis, *p*<0.05 corrected for family-wise error (FWE; Figure 5). There was no significant association between change in ESQ attention and PCC RSFC with the right (*t*(20)=-0.70, *p*=0.49, *p**=0.49, *b*=-1.05, CI=[-4.18, 2.08], 1 outlier removed) or left DLPFC (*t*(19)=1.09, *p*=0.34, *p**=0.49, *b*=1.08, CI=[-1.22, 3.40], 2 outliers removed) in the ROI analysis. There was no group difference in the change in self-reported attention alone between MBSR and HEP (*t*(54)=1.21, *p*=0.23, *p**=0.23, *b*=0.18, CI=[-0.12, 0.49]) or WL (*t*(58)=1.41, *p*=0.16, *p**=0.23, *b*=0.23, CI=[-0.10, 0.55]).

Discussion

In this study we found evidence that MBSR – one of the most widely used mindfulness meditation interventions – increased RSFC between nodes of the DMN and the FPN compared to well-matched active and passive control groups. Previous research has found similar effects following a brief mindfulness intervention relative to relaxation training (Creswell et al., 2016), as well as in cross-sectional research of long-term practitioners (Brewer et al., 2011). A large and growing body of research has implicated the DMN in mind-wandering, task-unrelated thought and self-referential processing, whereas the FPN is associated with externally-oriented and goal-directed attention (Barrett and Satpute, 2013; Spreng et al., 2013; Spreng and Grady, 2009). Given that one of the primary skills trained during mindfulness meditation is directing attention to present moment experience without elaboration (unlike during typical self-referential processing), increased PCC–DLPFC RSFC could reflect a greater ability to attend to task-related stimuli and increased control over self-referential processing and mind-wandering.

The group differences in RSFC were localized to MFG, which is a more canonical region of the DLPFC (and of the FPN) (Kim et al., 2012; Petrides and Pandya, 1999; Ptak, 2012) that is posterior and dorsal to the regions reported by Creswell et al. (2016). In contrast, we failed to replicate prior findings using an ROI based on the exact coordinates provided by this prior study. There is a growing body of evidence suggesting the DLPFC region should be involved in attentional processes that shift with mindfulness training (Fox et al., 2016), whereas the LPFC ROI is based on coordinates from a single study and is likely less reliable. Although there were no significant group differences in PCC–DLPFC RSFC in the voxelwise analyses, we report the sub-threshold results given that the pattern is consistent with the significant group difference in the anatomical DLPFC ROI analysis, and to provide a complete picture of the data. However, sub-threshold results alone are not evidence of a group difference, given the possibility of false positives. The significant increase in PCC–DLPFC RSFC within MBSR participants in the voxelwise analysis adds further evidence to this pattern of results implicating MBSR practice with improved connectivity between the DMN and FPN.

There was an unanticipated, significant group difference in the voxelwise analysis, whereby MBSR participants had increased PCC–ITG RSFC relative to HEP. While there was no difference between MBSR and WL in PCC–ITG RSFC, this result appears to be driven by the significant increase for the MBSR group, as there was no change for HEP (or WL). The ITG has been considered part of the FPN (Kim et al., 2012), and reduced ITG RSFC has been associated with aging-related cognitive impairment (Agosta et al., 2012; Han et al., 2011). Increased PCC–

ITG RSFC may therefore reflect improved cognitive function. However, future research is needed to replicate this finding in a confirmatory analysis.

Importantly, we also found a group difference in the relationship between practice and change in PCC–DLPFC RSFC, whereby it was stronger for MBSR relative to HEP. The more time participants spent practicing MBSR, the stronger their PCC–left DLPFC RSFC became – representing a linear dosage effect at this early stage of training. There was no significant relationship for HEP practice and PCC–DLPFC RSFC. Since participants in the MBSR group did not all engage to the same degree with practicing mindfulness meditation, the intervention should not lead to an equivalent change across MBSR participants as a group. This interaction of an individual differences measure with group is strong evidence that MBSR practice played a causal role in the RSFC changes, particularly given the significant main effect of group on change in PCC–DLPFC RSFC.

While the group difference in changes in brain connectivity were not sustained at the later follow-up, continued practice with MBSR may be necessary to maintain this effect. Our finding that days of MBSR practice between T1 and T3 were positively associated with increasing strength of RSFC of this network over the same time period adds support to this hypothesis. Future research should seek to replicate this effect since it was not pre-registered in this study, and to examine whether individuals who continue to practice mindfulness meditation following MBSR and incorporate the practices into their daily lives experience more lasting change. For example, future studies may extend this line of research by following participants further out in time and examining whether participants engage in mindfulness practices when dealing with stressors versus solely as a sitting practice.

Increased PCC–DLPFC RSFC following MBSR training was associated with increased white matter microstructure of a tract linking these regions, the superior longitudinal fasiculus, in an exploratory analysis. Decreased diffusivity and increased fractional anisotropy in white matter may reflect a more streamlined white matter fiber organization (Alexander et al., 2007; Beaulieu, n.d.; Zatorre et al., 2012). However, changes in DTI-based measures are non-specific and may stem from various alterations to the underlying microstructure (Jones et al., 2013; Jones and Cercignani, n.d.). Thus, the cellular and molecular mechanisms underlying such changes still require future investigation. While the white matter microstructure of the entire group of MBSR participants' SLF did not change significantly following the intervention, our findings highlight the importance of examining individual differences, since it was only for those who showed more substantial changes in functional connectivity that we observed alterations in white matter structural connectivity. Future research should examine relationships between structural and functional connectivity in this network in a confirmatory manner to replicate these findings.

While we also found an association between increased PCC–DLPFC RSFC and self-reported attention, as theorized, the relationship was only present in an exploratory analysis with a different measure than hypothesized in our pre-registration. The questionnaire measure utilized in the current study (ESQ) is also new and the nomological network associated with individual differences on this measure remains to be determined. It will be important for future research to conceptually replicate this result with a validated ESQ or other validated self-report measure. Moreover, there was no relationship between changes in PCC–DLPFC RSFC and mind-wandering as measured by experience sampling following MBSR, contrary to our hypothesis. It is possible that the experience sampling measure in the current study assays a different aspect of attention than that which is supported by PCC–DLPFC connectivity, and/or that this measure is insufficient for measuring MBSR training-related changes. It is critical to identify behavioral measures associated with MBSR-related increases in PCC–DLPFC RSFC in order to more fully understand the relevance of such changes in brain connectivity. The ideal test would identify behavioral improvements following MBSR that are mediated by increased PCC–DLPFC RSFC.

Limitations

In the current study we found a mixture of positive and negative results, across confirmatory and exploratory analyses. Strong conclusions regarding the effect of MBSR training on PCC–DLPFC RSFC are limited, given that group differences in the voxelwise analysis were non-significant and the *p-values* of the ROI analyses change from 0.04 to 0.05 in two cases when correcting for multiple comparisons. While our pre-registered analysis of the *a-priori* anatomical ROIs provide support for the conclusion that MBSR strengthens PCC - DLPFC RSFC, further research is needed to replicate these effects. Replication of the results of multiple exploratory analyses is also needed, including relationships with practice, structural change of the SLF, and increased self-reported attention. Moreover, the questionnaire measure we utilized (ESQ) remains to be validated. Finally, results of the wholebrain analysis revealed an unanticipated difference in PCC - ITG RSFC between MBSR and HEP, that was non-significant for MBSR compared to WL. Since this group difference in PCC - ITG RSFC was the result of a secondary, exploratory analyses, we are hesitant to over-interpret this novel result, which needs to be replicated in future confirmatory research. We offered a tentative interpretation that such RSFC changes may reflect improved cognitive function as an idea for further exploration.

This study provides evidence of training-related changes following practice with mindfulness meditation in brain networks important for executive control and modulation of mind-wandering with relevant outcomes in self-reported attention. Individual differences in practice time and structural changes associated with PCC–DLPFC RSFC changes suggest the importance of continued practice beyond the formal instruction period. Future research to examine potential longer-term changes may need to consider factors that contribute to adopting mindfulness meditation practice as a lifestyle change, similar to the need for continued exercise and healthy eating for maintenance of healthy weight. Potential avenues to facilitate long-term use of mindfulness meditation practice may be through tailoring practices for incorporation into activities of daily living, availability of ongoing training exercises, greater accessibility of training support through mobile platforms and a meditation community. Given the wide use and efficacy of MBSR for treating numerous mental health conditions (Goldberg et al., 2018), as well as the growing evidence of behaviorally-relevant biological changes as described in this study, the future of mindfulness meditation research should aim to determine factors that predict lasting change.

Conflicts of Interest: Dr. Richard J. Davidson is the founder, president, and serves on the board of directors for the non-profit organization, Healthy Minds Innovations, Inc. No donors, either anonymous or identified, have participated in the design, conduct, or reporting of research results in this manuscript.

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Figures

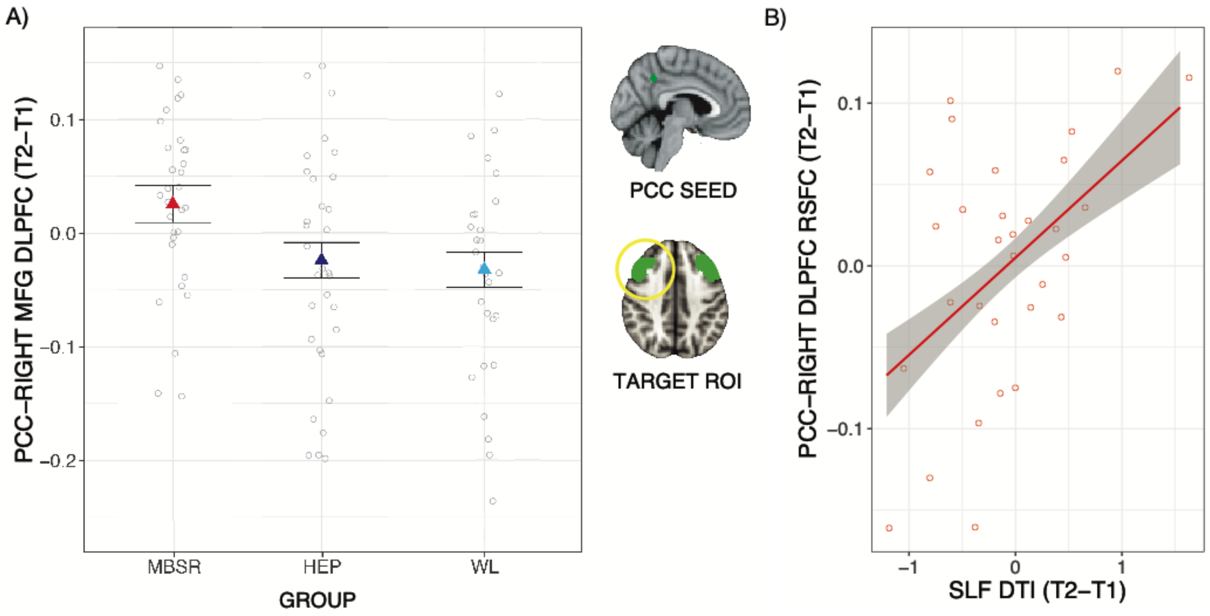

Figure 1. MBSR-related changes in PCC– DLPFC RSFC: ROI results. **(A)** Participants had increased PCC– DLPFC RSFC (time 2 - time 1) following MBSR compared to HEP and compared to WL. The PCC seed ROI and the anatomical DLPFC target ROIs are depicted in green with the right DLPFC circled in yellow. **(B)** A larger increase in MBSR participants' PCC–right DLPFC RSFC was associated with a larger increase in DTI integrity of a white matter tract connecting these regions (SLF) from pre- to post-intervention. Error bars/ envelopes represent 1 standard error above and below the point estimates of the means and raw data points are overlaid.

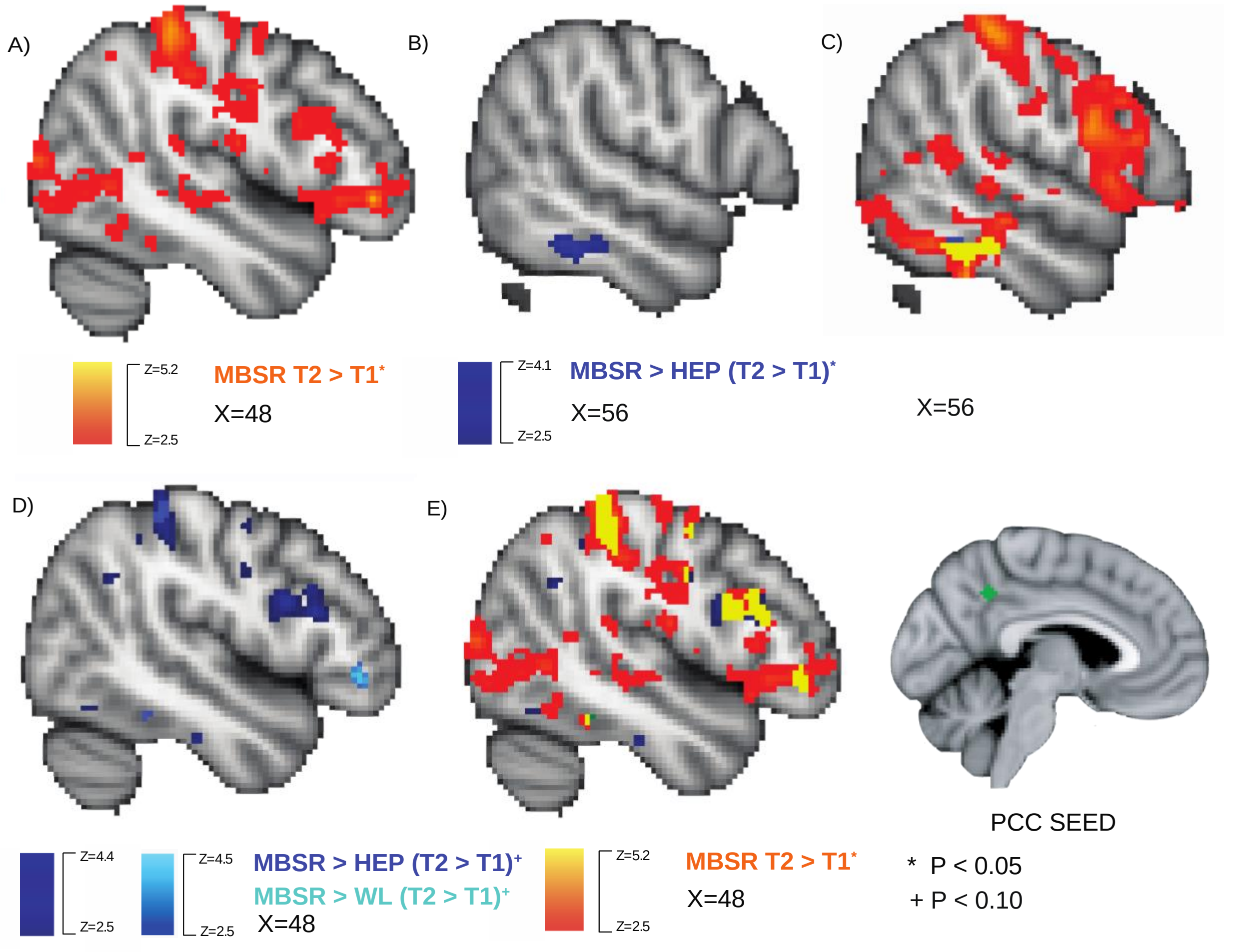

Figure 2. MBSR-related changes in PCC RSFC: Voxel-wise results. The PCC seed is inset in the lower right in green. **(A)** Brain regions where MBSR participants had increased PCC RSFC (time 2 - time 1) are depicted in yellow-orange (p<0.05 controlling for family-wise error using threshold-free cluster enhancement with FSL's Randomize). **(B)** The significant group difference between MBSR and HEP. **(C)** The overlap between A and B is depicted in yellow. **(D)** The marginal group differences for MBSR compared to HEP or WL are depicted in dark and light blue, respectively (p<0.10 corrected). **(E)** The overlap of from panels C and D is depicted in yellow.

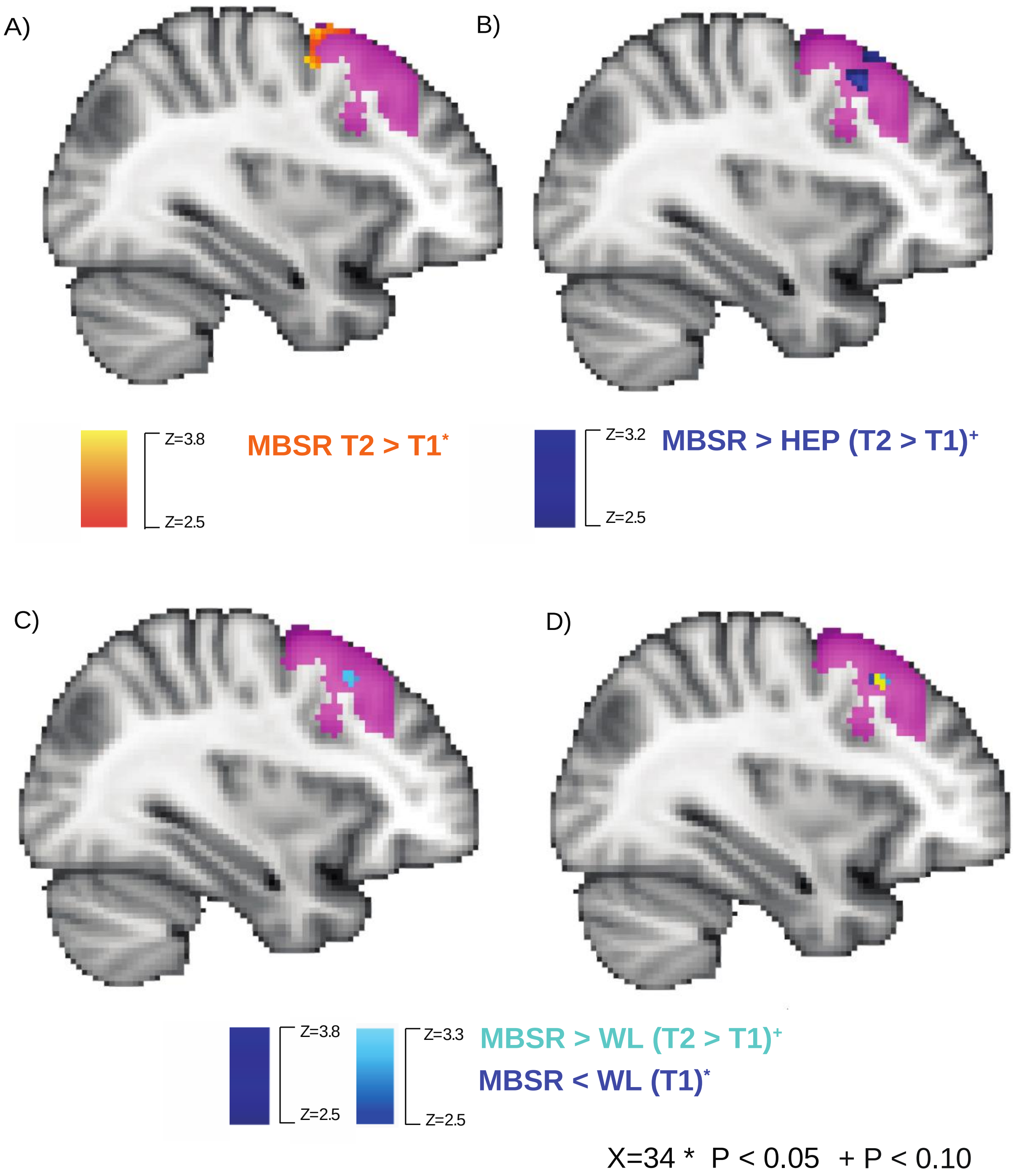

Figure 3. MBSR-related changes in PCC– DLPFC RSFC: Voxel-wise results. **(A)** MBSR participants had increased PCC RSFC (time 2 - time 1) (*p*<0.05, controlling for family-wise error using threshold-free cluster enhancement with FSL's Randomize). **(B)** The marginal group difference for MBSR compared to HEP (*p*<0.10, corrected). **(C)** The marginal group differences for MBSR compared to WL (*p*<0.10, corrected). **(D)** The overlap between C and time1 differences (dark blue, *p*<0.05, corrected) between MBSR and WL is depicted in yellow.

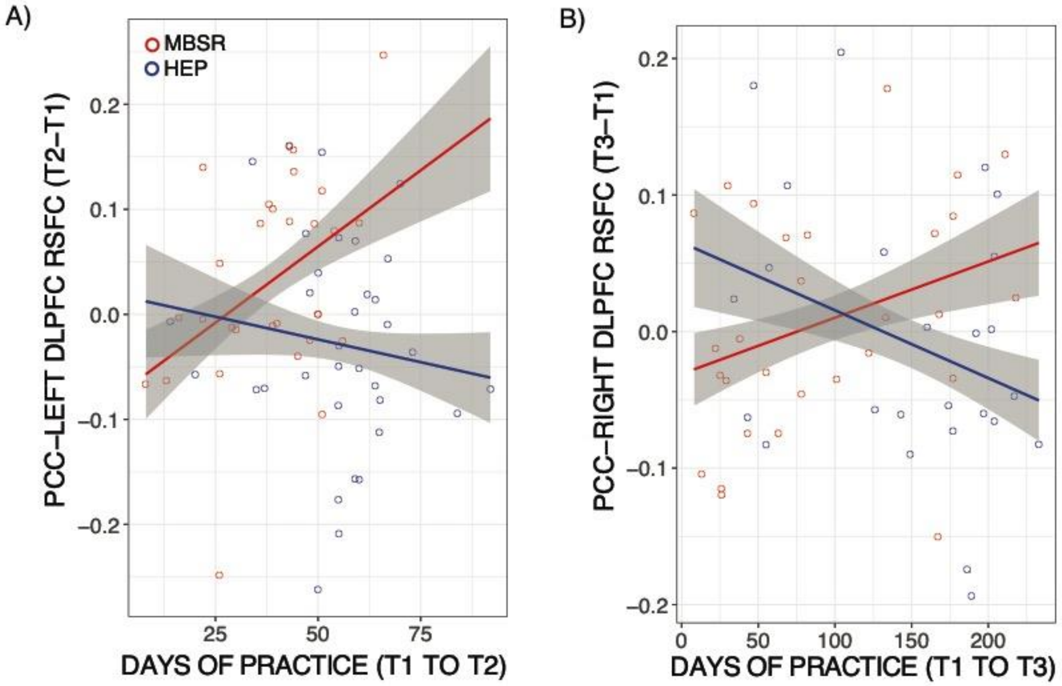

Figure 4. Effects of MBSR practice duration. **(A)** MBSR participants had stronger PCC–DLPFC RSFC with more total days of practice compared to HEP between T2 and T1 for the left DLPFC and **(B)** T3 and T1 for the right DLPFC. Envelopes represent 1 standard error above and below the point estimates of the means and raw data points are overlaid.

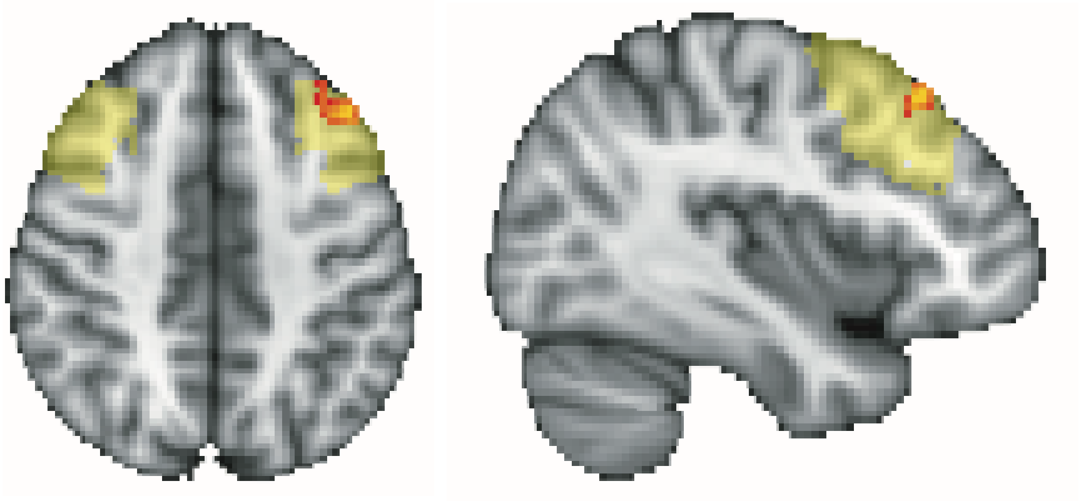

Figure 5. MBSR-related increased PCC–DLPFC RSFC and attention. The more participants' PCC–left DLPFC RSFC increased (time 2 - time 1) following MBSR, the more their self-reported attention also increased (red-yellow; $p<0.05$ controlling for family-wise error using threshold-free cluster enhancement with FSL's Randomize). The anatomical DLPFC ROI is underlaid in light yellow.

Tables

Table 1. MBSR-related increases in PCC RSFC: Cluster details (100 voxel minimum).

		PEAK COORDINATES			
	REGION	X	Y	Z	VOLUME (voxels)
MBSR vs HEP* (T2-T1)	Right inferior temporal gyrus ($p<0.05$)	56	-40	-18	218
	Right supramarginal gyrus	52	-34	58	3746
	Right inferior temporal gyrus	56	-40	-18	1849
	Left postcentral gyrus	-36	-32	50	941
	Right inferior frontal gyrus	44	12	20	826
	Right supplementary motor cortex	4	8	58	217
	Left precentral gyrus	-36	4	22	130
	Left inferior temporal gyrus	-46	-56	-12	123
MBSR vs WL* (T2-T1)	Right anterior cingulate gyrus	8	-8	34	968
	Left precentral gyrus	-36	4	22	299
	Left insula	-42	2	0	271
	Right postcentral gyrus	24	-32	42	231
	Left inferior frontal gyrus	-50	32	10	134
	Left insula	-36	4	-16	109
	Right middle temporal gyrus	60	-24	-14	102
MBSR (T2-T1)	Right postcentral gyrus	50	-32	56	25,200
	Left middle temporal gyrus	-42	-60	-10	769
	Left putamen	-30	2	8	197
	Left fusiform cortex	-18	-76	-14	160

Table 1. MBSR-related increases in PCC RSFC: Cluster details (100 voxel minimum). *Clusters for MBSR relative to control groups were marginal and non-significant with FWE cluster correction at $p<0.10$ except in 1 case as noted, and change within MBSR was significant corrected at $p<0.05$.

Study 2: Alterations in posterior cingulate cortex resting connectivity associated with long-term meditation training.

Abstract

Mindfulness meditation training has been associated with increased resting state functional connectivity (RSFC) between posterior cingulate cortex (PCC) and dorsolateral prefrontal cortex (DLPFC) with short-term training (Creswell et al., 2016; Kral et al., 2019), and among long-term meditators (Brewer et al., 2011). In the present study we sought to replicate these effects in 40 long-term meditation practitioners (LTM) with an average lifetime practice of 3759 hours, and to extend prior work by examining relationships between PCC RSFC, practice duration and attention measures, as well as examining network-level connectivity. DLPFC is involved in attentional control, while PCC is implicated in self-related processing. Compared to 124 meditation-naïve participants (MNP), LTM had stronger PCC RSFC with a left lateral PFC region of interest that was defined based on results from Creswell *et al.* (2016) (p=0.01), together with lower connector hub strength across the default mode network (p=0.02). LTM had better attention to task (i.e., less mind-wandering) than MNP based on both experience sampling and self-report (ps<0.05), although there was no relationship between these measures of attention and PCC RSFC with LPFC (ps>0.05). Given the role of PCC in self-related thought, and LPFC in attentional control, and that the practice of mindfulness meditation involves repeatedly returning attention to the present moment (e.g., away from self-related thought), stronger PCC–LPFC RSFC may reflect more effective attentional control of mind-wandering. Future research will need to isolate cognitive processes associated with PCC–LPFC RSFC to support this interpretation.

Introduction

A growing body of research provides evidence that mindfulness meditation practice is associated with alterations in activation and connectivity of the posterior cingulate cortex (PCC), which may reflect neural mechanisms and targets for change with mindfulness training (Brewer and Garrison, 2014). Mindfulness can be defined as the practice of focusing attention on present-moment experience (Kabat-Zinn, 1990), in contrast to mind-wandering or attending to thoughts about the past or future – activities that are associated with PCC activation (Fox et al., 2015; Spreng et al., 2008). A recent meta-analysis of functional neuroimaging studies of meditation practice found that open monitoring and focused attention meditations – both aspects of mindfulness practice – reduced activation of posterior cingulate cortex (PCC) while increasing activation of dorsolateral prefrontal cortex (DLPFC) (Fox et al., 2016). De-activation of PCC was associated with “undistracted awareness” and “concentration” based on a qualitative analysis of meditators’ subjective reports during an fMRI neurofeedback task (Garrison et al., 2013). Moreover, meditators were able to purposefully deactivate PCC through meditation during neurofeedback compared to meditation-naïve control participants (who were unable to do so) (Garrison et al., 2013). Research with individuals who have long-term mindfulness meditation training may thus provide unique insights into the effects of mindfulness meditation on brain function and connectivity, which may entail different mechanisms of change dependent on stage of training (Brefczynski-Lewis et al., 2007; Kral et al., 2018).

Short- and long-term mindfulness meditation training have both been related to increased resting functional connectivity between PCC and DLFPC (Brewer et al., 2011; Creswell et al., 2016; Kral et al., 2019), which is a node of the frontoparietal control network associated with attentional control (MacDonald et al., 2000; Smallwood et al., 2012). This increased coupling is thought to reflect better attentional control over mind-wandering, and the results of Study 1 provide initial evidence in support of this hypothesis, given the positive relationship between changes in PCC – DLPFC resting state functional connectivity (RSFC) and increased self-reported attentional control (Kral et al., 2019). Prior research with long-term mindfulness meditation practitioners lacked measures of attention to test this hypothesis, and was further limited by small sample sizes of less than fifteen participants per group (Brewer et al., 2011).

The current study builds on this literature by examining relationships between long-term mindfulness meditation practice and PCC RSFC in a much larger sample of 40 meditators (compared to 124 non-meditating controls who were later assigned to an intervention as part of a randomized controlled trial [RCT] arm of the same study, as detailed in Study 1, above). We expected to replicate prior results of increased PCC – DLPFC resting connectivity in this larger sample, as well as extending these findings in three important ways. First, in the current study, we tested relationships between PCC RSFC and total lifetime hours of meditation practice – both daily practice and meditation on retreat, separately. We hypothesized that meditators with more hours of lifetime meditation practice would have stronger PCC – DLPFC RSFC. Second, we examined relationships between PCC – DLPFC RSFC and measures of attention and structural connectivity of superior longitudinal fasiculus (SLF), a white matter tract connecting PCC and DLPFC, given that we found relationships among change these measures in the RCT arm of this study.

Finally, we added graph theoretical metrics indexing network topography to illuminate whether differences in RSFC associated with mindfulness meditation were specific to the PCC and DLPFC seeds, or reflective of differences in overall network dynamics. Two graph theoretical measures obtained from resting connectivity estimates provided assessments of within- and between-module hub properties: within-module degree and participation coefficient, respectively. In graph theory, modules correspond to resting state networks, and hubs or nodes correspond to brain regions within the network. Within-module degree indicates the localized degree of connectivity of a given node to other nodes within the same module, while the participation coefficient denotes the diversity of intermodular connections (see Figure 1 for an illustration). A provincial hub is a node with high within-module degree, and a low participation coefficient, and is thought to contribute to modular segregation. A connector hub has a high participation coefficient, and is hypothesized to contribute to global intermodular integration (Rubinov and Sporns, 2010). If higher PCC – DLPFC RSFC in meditators reflects a more general difference in network dynamics, such that these modules are more integrated, then we would expect to see higher participation coefficients for one or both of the corresponding networks (default mode and frontoparietal control networks, respectively). Given the interpretation of higher PCC – DLPFC RSFC as reflecting increased attentional control by the frontoparietal control network on default mode network, we hypothesized that LTM would have higher participation coefficients than MNP in the frontoparietal control network. We also assessed hub properties for the dorsal attention network, in which DLPFC also participates.

Methods

The methods exactly match those described in Study 1, above, with the exception of long-term meditators (LTM) and the addition of analysis with network measures based on a graph theory approach, which are described below.

Participants

We recruited 183 healthy human participants from a non-clinical population, comprised of 140 meditation-naïve participants (MNP) and 43 LTM. The MNP correspond to the same sample of participants described in Study 1, above. The baseline data for MNP served as a control in this cross-sectional study. Seventeen MNP participants had RSFC data excluded from analysis due to excessive motion (described below; n=11) or anatomical brain abnormalities as determined by a radiologist (n=6), resulting in 123 MNP (average age ± SD = 42.4 ± 12.4 years, 74 female, 49 male). LTM were recruited from meditation centers and through related mailing lists throughout the United States, in addition to flyers and advertisements in newspapers similar to the recruitment strategy for MNP. Meditation recruitment criteria included at least five years of daily practice (with an average practice of at least 200 minutes per week), experience with Vipassana, concentration and compassion/loving-kindness meditations, and at least 5 weeks of retreat practice. Meditation retreats involve spending a period of time away from home at a meditation or community center participating in meditation practice, and can vary in length from days to weeks or months (and in some cases, years). Retreats often include group practice, in addition to solitary practice, and may include periods of silence. Lifetime hours of practice were calculated based on subjects' reports of their average hours of formal meditation practice per week and their total years of practice (average = 3759 hours, range = 780 to 19,656 hours). Lifetime retreat practice hours were calculated by summing the practice hours that were reported for each retreat. Practice hours were log-transformed using the natural log, to correct for a highly right-skewed distribution. Three LTM had RSFC data excluded from analysis due to excessive motion (n=2) or anatomical brain abnormalities (n=1), resulting in 40 LTM in the final sample (average age ± SD = 44.1±11.8 years, 15 female, 25 male). A subset of MNP (n=93, average age ± SD = 44.1±11.8 years, 48 female, 45 male) completed the Emotional Styles Questionnaire (ESQ) (Kesebir et al., 2019), which was introduced subsequent to the onset of data collection due to availability of the measure. All LTM completed the ESQ.

Image processing & analysis

Functional images were processed using a combination of AFNI (Cox, 1996) version 17.3 and FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library) (Smith et al., 2004), using the following steps: removal of the first 4 volumes; motion correction with MCFLIRT (Jenkinson et al., 2002); brain extraction with BET (Smith, 2002) brain extraction; registration of the subject's functional data to their anatomical image using the Boundary-Based Registration approach (Greve and Fischl, 2009). A 12-degree of freedom affine transformation using FLIRT (Jenkinson et al., 2002) was followed by FNIRT nonlinear transformation to register each subject's functional data to Montreal Neurological Institute 152 space. Images were segmented into white matter, grey matter and cerebrospinal fluid with FAST for use as masks that were eroded using a 3x3x3 voxel kernel and then used to generate region of interest (ROI)-averaged time series serving as nuisance regressors (along with their derivatives and the 6 motion regressors) with AFNI's 3dDeconvolve. Images were smoothed with a 5-mm full-width half-maximum Gaussian kernel.

We extracted the time-series from a spherical PCC seed with a 4-mm radius defined based on coordinates from Creswell *et al*. (2016) (Figure 2a, in yellow). We regressed each time-series (separately) back onto each subject's data using AFNI's 3dDeconvolve, which also censored high-motion time-points (greater than 0.2 mm framewise displacement) (Power et al., 2014). Participants were excluded from analysis if they had less than 6 minutes of data due to more than 50% of data points censored for motion. Two sets of target ROIs were defined for assessing PCC RSFC: a bilateral DLPFC ROI, based on medial frontal gyrus (MFG) from the Harvard-Oxford atlas (Craddock et al., 2012) thresholded at 50% probability for small-volume-corrected voxelwise analysis (Figure 2a, in dark blue), which was split into left and right for ROI analysis; and left and right LPFC ROIs defined as 10-mm spheres around coordinates provided in Creswell *et al*. (2016) (Figure 2a, in light blue). RSFC was assessed based on the Fisher-Z transformed (FZT) correlation between the seed and every other voxel in the brain for the voxelwise analysis, and separately for each of the target ROIs. Voxel-wise analyses were thresholded at $p<0.05$ controlling for family-wise error using threshold-free cluster enhancement with FSL's Randomise (Winkler et al., 2014).

Graph theoretical network analysis

We calculated hub connectivity metrics for the default mode (Figure 2b), frontoparietal control, and dorsal attention networks based on the Gordon connectivity atlas (Gordon et al., 2016). First, the mean resting state time-series was extracted from each of the 333 nodes in the Gordon atlas, and then we constructed a correlation matrix for each subject by computing pairwise Pearson correlations for each set of nodes. We used the correlation matrix to calculate both provincial and connector hub properties of each node using within-module degree (WMD) and participation coefficient measures, respectively, (with nodes assigned to networks as defined in the Gordon atlas) following the procedures detailed by Hwang, Bertolero, Liu & D'Esposito (Hwang et al., 2017). We tested for group differences in hub connectivity across all nodes in the respective network (default mode, frontoparietal control, dorsal attention or salience network), separately, by estimating linear mixed-effects models with the lmer and anova functions in R statistics (Kuznetsova et al., 2017; R Core Team, 2013), which included by-subject random effects and covariates to control for age, gender and scan acquisition version.

DTI analysis

The DTI analysis was performed exactly as that in study 1 of this chapter (Kral et al., 2019), which stated the following: "The median values for FA, MD, RD, and AD of the SLF

ROIs were entered into a principle component analysis (PCA) (McLaughlin et al., 2018; Zeestraten et al., 2016), using the prcomp function in R (R Core Team, 2015) to construct a right and left hemisphere composite of the underlying microstructure. The first principle component was used as a representative composite of white matter microstructure as this accounted for the greatest covariance between the four DTI parameters (77.1% for the right, 80.0% for the left). The factor loadings for fractional anisotropy and for radial, mean and axial diffusivity were 0.44, -0.54, -0.56 and -0.45 for the right side, respectively; and 0.45, -0.53, -0.56 and -0.46 for the left side, respectively. Thus, larger values of the microstructure composite are indicative of higher anisotropy and lower diffusivity, which is generally interpreted as a superior white matter microstructure (Basser and Jones, n.d.; Jones et al., 2013). DTI values at T2 were computed using the weights from this first principle component of the PCA analysis using the predict function in R (R Core Team, 2015)."

Results

Group differences in PCC RSFC.

Voxel-wise analysis

There were no regions in which PCC RSFC differed for LTM participants compared to MNP in a wholebrain analysis. No statistically significant differences were observed in PCC RSFC between LTM and MNP in a small-volume-corrected, voxelwise analysis within the anatomically-defined DLPFC ROI. However, there was one 40-voxel cluster in the left DLPFC in which LTM had stronger RSFC with PCC compared to MNP at a sub-threshold level (p=0.10; peak voxel coordinates: -44, 18, 36; Figure 3).

ROI analysis

LTM had stronger RSFC than MNP between PCC and the left LPFC ROI that was defined based on coordinates from a study showing an effect of mindfulness meditation training on PCC RSFC (Creswell et al., 2016), and located rostral to the canonical DLPFC ($t(154)=-2.78$, $p=0.01$, $b=-0.06$, CI=[-0.10, -0.02]; 2 MNP and 2 LTM outliers removed; Figure 4A). However, there was no group difference in PCC RSFC between LTM and MNP using the right LPFC ROI ($t(158)=0.47$, $p=0.64$, $b=0.01$, CI=[-0.04, 0.07]). There were also no differences in PCC RSFC between LTM and MNP with the anatomically generated DLPFC ROIs based on the MFG from the Harvard-Oxford atlas (Craddock et al., 2012), (right DLPFC: $t(155)=-1.57$, $p=0.12$, $b=-0.04$, CI=[-0.08, 0.01], 1 MNP and 2 LTM outliers removed; left DLPFC: $t(157)=-0.79$, $p=0.43$, $b=-0.02$, CI=[-0.05, 0.02], 1 LTM outlier removed, Figure 5a).

Network analysis

LTM had significantly lower participation coefficients in default mode network compared to MNP ($t(164)=2.44$, $p=0.02$, $b=0.05$, CI=[0.01, 0.08]), while there were no differences between groups for participation coefficients in frontoparietal control or dorsal attention networks (ps>0.05). There were no group differences in WMD for any of the networks (ps>0.05).

Experience sampling

LTM reported significantly greater attention to task during one week of experience sampling than MNP ($t(161)=-3.45$, $p<0.01$, $b=-0.68$, CI=[-1.07, -0.29]; Figure 4B). There was no relationship between PCC–DLPFC RSFC and attention based on experience sampling in the voxelwise analysis ($p<0.05$ corrected for FWE). There was also no relationship between attention and PCC RSFC with the anatomically-defined DLPFC ROIs (right DLPFC: $t(142)=0.65$, $p=0.51$, $b=0.24$, CI=[-0.94, 1.86], 5 MNP outliers removed; left DLPFC:

(*t*(147)=0.15, *p*=0.88, *b*=0.13, CI=[-1.59, 1.84]), or the Creswell *et al.* LPFC ROIs (right LPFC: *t*(143)=0.06, *p*=0.95, *b*=0.04, CI=[-1.14, 1.21], 4 MNP and 1 LTM outlier removed; left LPFC: *t*(143)=-1.05, *p*=0.30, *b*=-0.80, CI=[-2.32, 0.71], 4 MNP and 1 LTM outlier removed).

Attention questionnaire

LTM reported higher attention on the ESQ than MNP (*t*(146)=-2.26, *p*=0.02, *b*=-0.40, CI=[-0.75, -0.05], Figure 4C). There was no significant association between ESQ attention and PCC RSFC in a wholebrain, voxelwise analysis (*p*<0.05 corrected for FWE). There was also no relationship with ESQ attention and the anatomically-defined ROIs (right DLPFC: *t*(129)=0.79, *p*=0.43, *b*=0.57, CI=[-0.86, 2.01], 1 MNP outlier removed; left DLPFC: *t*(128)=0.02, *p*=0.99, *b*=0.01, CI=[-1.67, 1.69], 2 MNP outliers removed), or the Creswell *et al.* LPFC ROIs (right LPFC: *t*(128)=1.56, *p*=0.12, *b*=0.96, CI=[-0.26, 2.18], 2 MNP outliers removed; left LPFC: *t*(129)=-0.05, *p*=0.96, *b*=-0.04, CI=[-1.57, 1.49], 1 MNP outlier removed).

Structural connectivity

There were no group differences in SLF DTI for LTM compared to MNP (right SLF: *t*(154)=0.35, *p*=0.73, *b*=0.07, CI=[-0.35, 0.50]; left SLF: *t*(153)=-0.68, *p*=0.50, *b*=-0.15, CI=[-0.59, 0.29], 1 MNP outlier removed). There was no relationship between SLF DTI and PCC–DLPFC RSFC based on the anatomical DLPFC ROIs (right SLF: *t*(152)=1.02, *p*=0.31, *b*=0.76, CI=[-0.71, 2.23] 2 MNP outliers removed; left SLF: *t*(152)=-0.14, *p*=0.89, *b*=-0.13, CI=[-1.95, 1.69], 2 MNP outliers removed), or the Creswell *et al.* LPFC ROIs (right SLF: *t*(152)=1.41, *p*=0.16, *b*=0.00, CI=[-0.34, 2.00] 2 MNP outliers removed; left SLF: *t*(150)=-0.63, *p*=0.53, *b*=-0.53, CI=[-2.19, 1.13], 4 MNP outliers removed).

Practice time

Retreat meditation practice

PCC–DLPFC RSFC was associated with log retreat practice time in LTM at a trend level (right DLPFC: (*t*(32)=1.95, *p*=0.06, *b*=0.03, CI=[-0.001, 0.06], 1 LTM outlier removed; left DLPFC: *t*(32)=2.00, *p*=0.05, *b*=0.03, CI=[-0.001, 0.06], 1 LTM outlier removed, Figure 5b). However, these relationships were non-significant when all data was used in the model (i.e, the influential outlier was included; right DLPFC: *p*=0.24; left DLPFC: *p*=0.28), or when the extreme point (with 135,000 hours of retreat practice) was removed (e.g., right DLPFC: *p*=0.17; left DLPFC: *p*=0.24). There were no relationships between log retreat practice and PCC–LPFC RSFC with the Creswell *et al.* ROIs (right LPFC: (*t*(31)=0.42, *p*=0.68, *b*=0.01, CI=[-0.03, 0.05], 2 LTM outliers removed; left LPFC: *t*(33)=0.45, *p*=0.66, *b*=0.01, CI=[-0.03, 0.04]).

Higher LTM retreat practice was associated with stronger DTI of the right SLF (*t*(30)=2.44, *p*=0.02, *b*=0.32, CI=[0.05, 0.58], 1 LTM outlier removed, Figure 5c). This relationship remained significant when excluding the extreme point (with 135,000 hours of retreat practice; *p*=0.03), however it was at a trend level when the influential outlier was included (*p*<0.10). There was no relationship between LTM retreat practice and DTI of the left SLF (*t*(31)=-0.82, *p*=0.42, *b*=-0.13, CI=[-0.47, 0.20]). There was no relationship between LTM retreat practice and attention based on experience sampling (*t*(34)=-1.59, *p*=0.12, *b*=-0.23, CI=[-0.52, 0.06], 2 LTM outliers removed), or for self-reported attention (*t*(36)=0.80, *p*=0.43, *b*=0.09, CI=[-0.14, 0.31]).

Home meditation practice

There was no relationship between daily, home meditation practice among LTM and PCC–DLPFC RSFC (right DLPFC: (*t*(32)=0.02, *p*=0.98, *b*=0.001, CI=[-0.07, 0.07], 1 outlier removed; left DLPFC: *t*(32)=-0.12, *p*=0.91, *b*=0.00, CI=[-0.06, 0.05], 1 outlier removed; right LPFC: *t*(33)=-0.82, *p*=0.42, *b*=-0.03, CI=[-0.12, 0.05]; left LPFC: *t*(31)=-0.52, *p*=0.61, *b*=-0.02, CI=[-0.09, 0.05], 2 outliers removed). There was also no relationship between daily, home meditation practice and SLF DTI (right SLF: *t*(30)=-1.17, *p*=0.25, *b*=-0.42, CI=[-1.16, 0.32], 1 outlier removed; left SLF: *t*(30)=-0.71, *p*=0.48, *b*=-0.32, CI=[-1.22, 0.59], 1 outlier removed), nor self-reported (*t*(35)=1.21, *p*=0.23, *b*=0.29, CI=[-0.19, 0.77s], 1 outlier removed) or experience sampling measures of attention (*t*(33)=0.31, *p*=0.76, *b*=0.09, CI=[-0.52, 0.71], 2 outliers removed).

Discussion

The current study partially replicated prior work (Brewer et al., 2011), showing stronger RSFC between PCC and LPFC in participants with a long-term practice in mindfulness meditation compared to meditation-naïve participants. The LPFC region in which we found a significant group difference was defined based on coordinates from a study showing stronger RSFC with PCC following a short-term mindfulness meditation intervention (Creswell et al., 2016). There was no difference between LTM and MNP in anatomically-defined ROIs, which include a more canonical “DLFPC” region, and in which we previously found increased RSFC with PCC following Mindfulness-Based Stress Reduction compared to controls (Study 1) (Kral et al., 2019). There is no overlap between the LPFC ROIs defined from the literature and the anatomically-defined DLFPC ROIs (Figure 2a, in light and dark blue, respectively). Total lifetime hours that LTM spent meditating while on retreat was weakly associated with PCC -– DLFPC RSFC in the anatomically-defined ROIs, although this relationship relationship was not robust to inclusion of outliers in the model. There was no relationship between LTM practice (either retreat or daily practice) and PCC RSFC with the other set of LPFC ROIs, nor with RSFC between PCC and any other brain region in a voxelwise analysis.

The LPFC region in which we found increased PCC RSFC in LTM relative to MNP is situated in rostral LPFC, or Brodmann's Area 10. While this region is activated across numerous cognitive tasks, taken together, the available evidence lends support to the hypothesis that the lateral aspect of rostral PFC may switch attention between internal and external stimlui (e.g., between self-related processing and task- or goal-oriented processing; Burgess et al., 2007). According to this hypothesis, rostral LPFC is part of a system that serves to determine the source of cognitive representations (e.g., internally or externally generated), exerting influence on attention allocation in open-ended situations when goals are self-generated or underspecified (e.g., the task-free setting of a resting state scan), or when sustained attention is required (Burgess et al., 2007). This hypothisized function for rostral LPFC is consistent with a role in meta-cognitive background awareness that is a core component of mindfulness meditation training. This form of background meta-awareness is specifically cultivated in "open monitoring" style practices that emphasize broad awareness to present-moment experience while simultaneously monitoring for the presence of mind-wandering, and returning attention to the "task" of focusing on present-moment experience (Lutz et al., 2015). Rostral LPFC activation and connectivity with PCC could thus serve as a neural mechanism underlying subcomponents of meta-awareness that support background awareness of the source of cognition, and the ability to re-allocate attentional resources to examine internally-generated cognitions as they arise, or conversely to return attentional focus to externally-generated phenomena. While there is a wealth of research and related paradigms examining phasic instances of meta-awareness in the context of error detection and noticing instances of mind-wandering (Hester et al., 2005; Schooler et al., 2011; Ullsperger et al., 2010), research and paradigms for assessing the neural basis for sustained meta-awareness is lacking and critical for our understanding of mechanisms of change with mindfulness-based meditation training.

We also examined resting connectivity at the network level, by examining provincial (i.e., within-module) and connector (i.e., between-module) hub properties. We found *lower* participation coefficients for the default mode network in LTM compared to MNP, reflecting *less* connector hubs, in contrast to the stronger PCC – LPFC RSFC we found for LTM in seed-based analysis. This may reflect reduced information flow from the default mode network to other networks, and highlights the potential specificity of increased RSFC between PCC and DLPFC in association with mindfulness meditaiton practice. There were no differences between groups for hub properties of attentional or salience networks, nor for provincial hub properties (i.e., within-module degree).

LTM had higher attention to task than MNP, as assessed with both a self-report questionnaire and experience sampling via text messaging. Higher attention to task, and conversely, less mind-wandering, is consistent with the goals of mindfulness meditation, namely, practicing keeping attention on present moment experience. These behavioral results are also consistent with the interpretation of higher PCC – LPFC resting connectivity among meditators as reflecting better attentional control of mind-wandering. However, there was no association between the RSFC and attentional measures in the current study, although we previously found that increased self-reported attention was associated with increased PCC – DLPFC RSFC following MBSR (Study 1) (Kral et al., 2019). Similarly, we previously found associations between MBSR-related increases in PCC – DLPFC RSFC and diffusion tensor imaging (DTI) measures of the superior longitudinal fasiculus (SLF), which is a white matter tract connecting PCC and DLPFC, among other regions (Study 1) (Kral et al., 2019). There was no relationship between DTI of SLF and PCC – DLPFC RSFC across participants, though the current study was based on a single scan rather than longitudinal data, as in the prior analysis. It is possible that changes in these measures of attention, functional and structural connectivity track together because they share underlying mechanisms of change, whereas the same measures examined cross-sectionally are uncorrelated due to different functionality. It is also possible that the within-subjects design in the prior RCT study may have been more sensitive to identifying correlations among changes without including variance in un-related between-subjects factors. The current study is limited given the cross-sectional nature of the data, and the associated lack of a baseline for LTM with which we could compare the current measures to examine change over time associated with long-term mindfulness meditation practice.

Interestingly, LTM total lifetime meditation practice during retreat was associated with higher right SLF DTI, and weakly associated with higher PCC – DLPFC RSFC (at a trend level), whereas there were no significant relationships for LTM daily meditation practice. Our previously reported results (from Chapter 1) were also specific to LTM retreat practice, with more lifetime hours of practice on retreat associated with reduced amygdala reactivity to negative pictures (Kral et al., 2018). Thus, the context and way in which meditation is practiced while on retreat may be particularly beneficial. However, future research is needed to disentangle the many, potentially interactive factors that differ between retreat and home practice. In addition to the longer duration and in-depth nature of meditation practice while on retreat, the context usually includes an aesthetically pleasing, natural environment in which practitioners are freed from work and stressors typical of daily life, often including “unplugging” from digital devices.

The results of the current study provide additional evidence consistent with prior work indicating a relationship between mindfulness meditation practice and increased resting connectivity between nodes of the default mode and frontoparietal control networks (Brewer et al., 2011; Creswell et al., 2016; Kral et al., 2019). While there was no evidence for a relationship between default mode network – frontoparietal network functional connectivity at rest and attentional measures in this cross-sectional dataset, we previously found positive relationships between changes in these measures (Study 1; Kral et al., 2019), and overall the LTM had higher goal-directed attention than MNP. Changes in the dynamic interactions of PCC and LPFC provide a potential neural mechanism underlying attentional improvements with mindfulness meditation training, although research is needed to identify the specific behavioral correlates of PCC–LPFC RSFC associated with long-term mindfulness meditation training. Further research is needed to determine the mechanisms and trajectory of change with mindfulness meditation practice, including contextual and individual differences factors that may influence its efficacy.

Conflicts of Interest: Dr. Richard J. Davidson is the founder, president, and serves on the board of directors for the non-profit organization, Healthy Minds Innovations, Inc. No donors, either anonymous or identified, have participated in the design, conduct, or reporting of research results in this manuscript.

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Figures

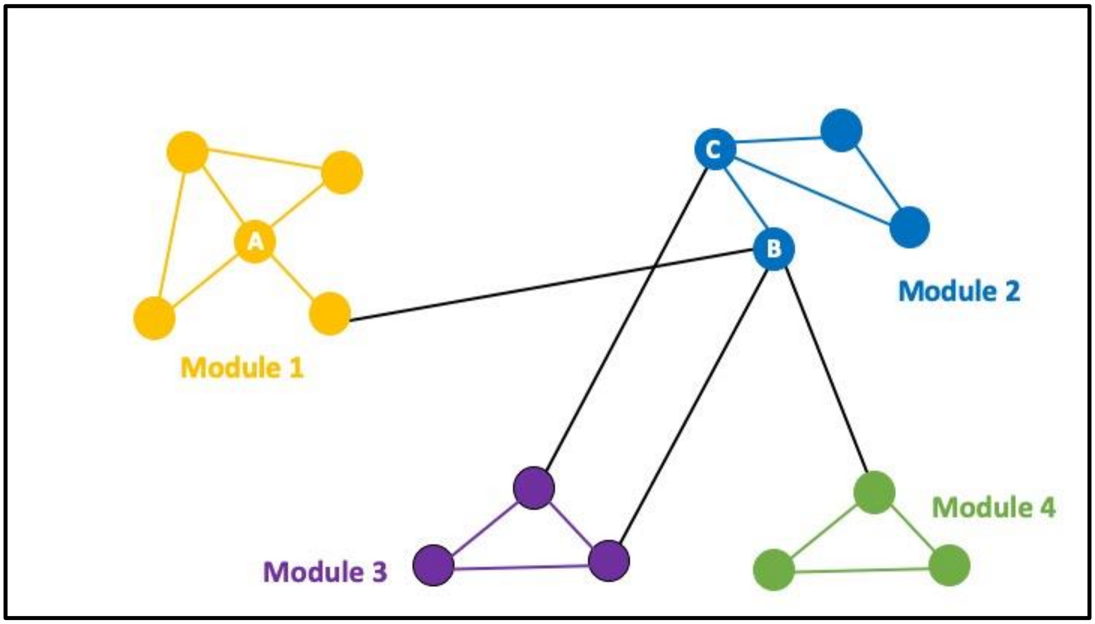

Figure 1. Illustration of graph theory concepts. Modules represent brain networks (e.g., default mode network). Nodes are represented by circles, and correspond to brain regions within a module. Lines represent connections between nodes, where black lines represent inter-modular connections and colored lines represent intra-modular connections (with all nodes within a module sharing the same color). Node “A” is an example of a provincial hub with many intra-modular connections and zero inter-modular connections, and would thus have a high within-module degree. Nodes “B” and “C” are examples of connector hubs with many inter-modular connections, and thus high participation coefficients. Node “B” has few intra-modular connections, and would have a low within-module degree, while node “C” has many intra-modular connections, and would have a high within-module degree.

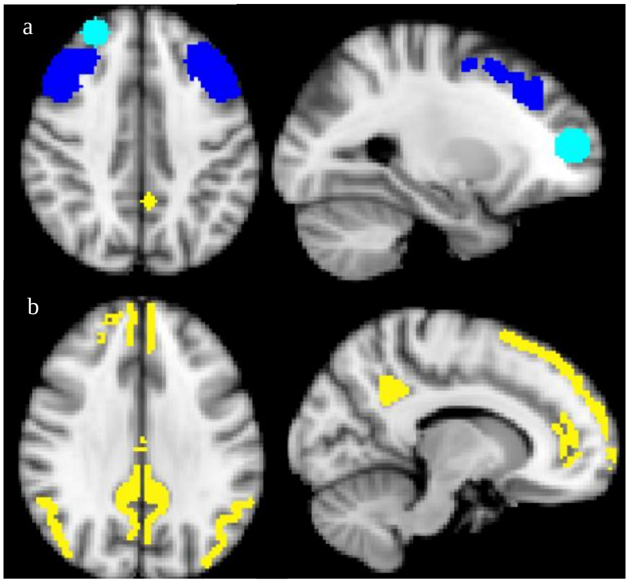

Figure 2. Regions of interest (ROIs). The dorsolateral prefrontal cortex (PFC) ROIs are in dark blue (based on the middle frontal gyrus from the Harvard-Oxford atlas [Craddock et al., 2012]), the lateral PFC ROIs are in light blue, and the posterior cingulate seed is in yellow (with the latter two based on coordinates from a prior study [Creswell et al., 2016]) (a). The default mode network is in yellow based on the Gordon atlas of resting state networks (Gordon et al., 2016) (b).

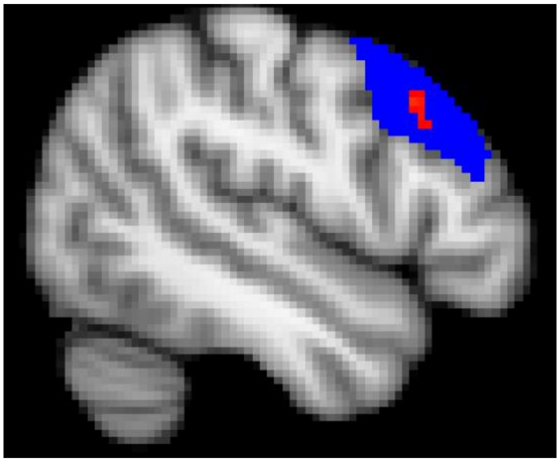

Figure 3. Results of masked, voxelwise analysis. A dorsolateral prefrontal cortex cluster (red; 40 voxels) in which long-term meditators had stronger resting state functional connectivity with posterior cingulate cortex (PCC) compared to non-meditators at a sub-threshold level (*p*=0.10), which was small-volume corrected for multiple comparisons within the middle frontal gyrus (blue), defined based on the Harvard-Oxford atlas (Craddock et al., 2012). Coordinates for the peak voxel: -44, 18, 36. The PCC seed (depicted in Figure 2a) was defined based on a prior study (Creswell et al., 2016).

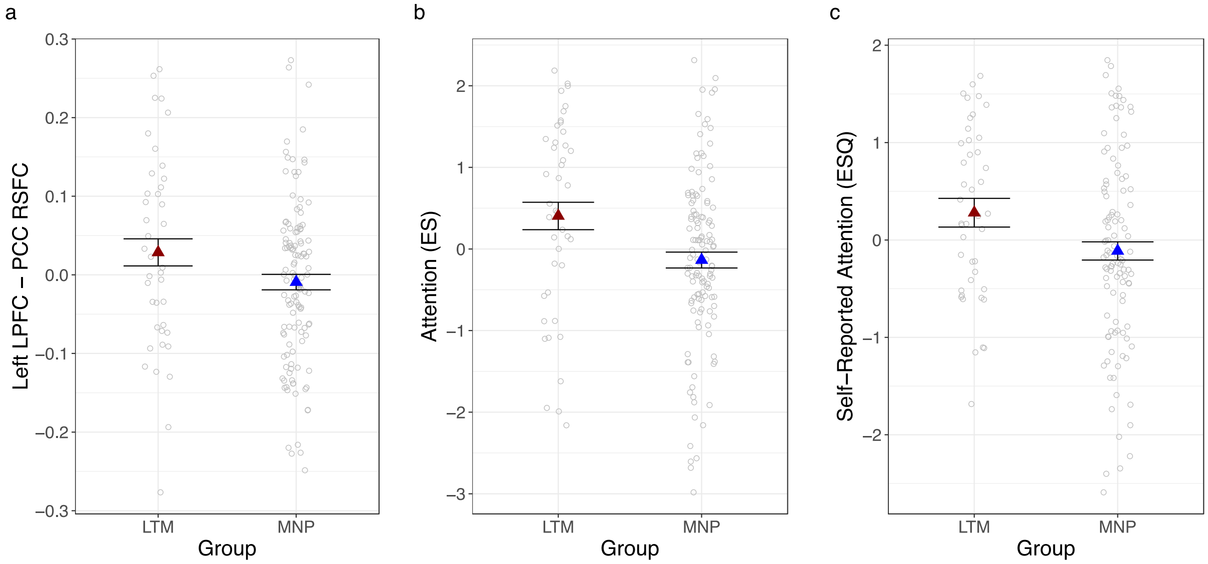

Figure 4. Long-term mindfulness meditation practice, attention and PCC RSFC. **(a)** LTM had higher PCC– LPFC RSFC compared to MNP. **(b)** LTM had higher attention to task compared to MNP based on experience sampling. (**c**) LTM had higher self-reported attention than MNP. Error bars represent 1 standard error above and below the point estimates of the means and raw data points are overlaid. Data are adjusted for covariates, including age and gender, and scan acquisition type for RSFC data. PCC = posterior cingulate cortex; RSFC = resting state functional connectivity; LTM = long-term meditator; LPFC = lateral prefrontal cortex; MNP = meditation naïve participant; ES = experience sampling; ESQ = Emotional Styles Questionnaire

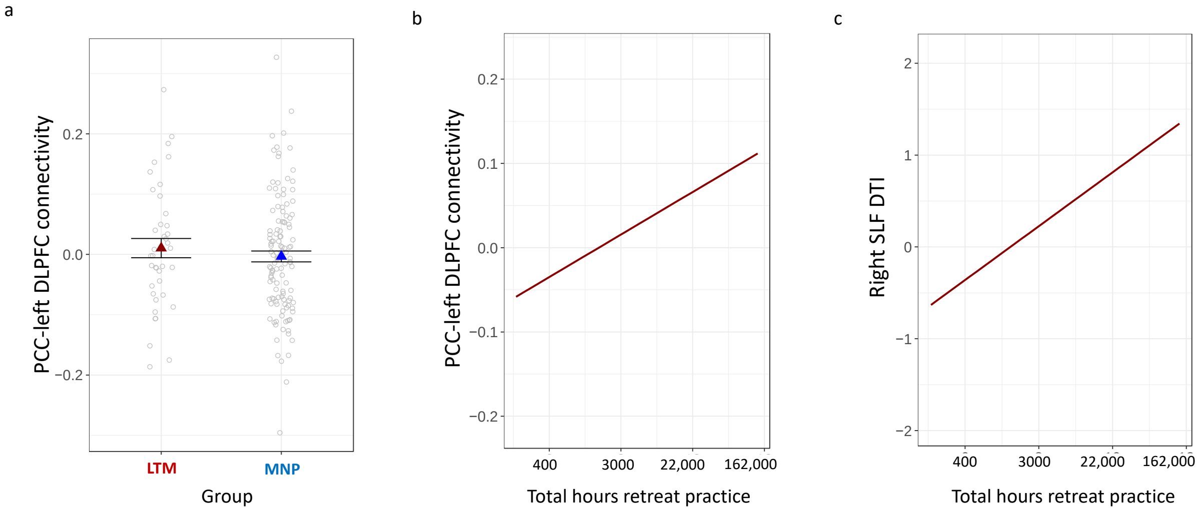

Figure 5. Long-term mindfulness meditation and PCC–DLPFC RSFC. **(a)** There was no difference in PCC– LPFC RSFC between LTM and MNP. **(b)** LTM total lifetime hours of retreat practice (ln-transformed) was associated with stronger PCC-left DLPFC RSFC at a trend level (*p*=0.05) and (**c**) significantly with higher right SLF DTI (*p*=0.02). Error bars and envelopes represent 1 standard error above and below the point estimates of the means and raw data points are overlaid. Data are adjusted for covariates, including age and gender, and scan acquisition type in (a). PCC = posterior cingulate cortex; RSFC = resting state functional connectivity; DLPFC = dorsolateral prefrontal cortex; LTM = long-term meditator; MNP = meditation naïve participant; SLF = superior longitudinal fasiculus; DTI = diffusion tensor imaging

Chapter 3

Impact of short- and long-term mindfulness meditation training on regional brain volume.

Abstract

As research on mindfulness meditation gains popularity, replication becomes increasingly important. Many papers and popular press articles have cited findings of changes in brain morphometry associated with mindfulness training, which were assessed in a meta-analysis (Fox et al., 2014). The current study aimed to replicate findings specific to changes in regional gray matter volume (GMV) following a mindfulness meditation intervention (Farb et al., 2013; Hölzel et al., 2011, 2009) using a more rigorously-designed randomized controlled trial (RCT) with an active control intervention. Meditation-naive participants (MNP, n=256) and long-term meditators (LTM, n=71) completed structural MRI scans. After baseline data collection (T1), MNP were randomly assigned to either wait-list control (WL, n=70) or one of two 8-week interventions: Mindfulness-Based Stress Reduction (MBSR, n=76) or the previously validated Health Enhancement Program (HEP, n=78) active control (MacCoon et al., 2012), and completed another data collection session (T2) following the intervention period. We examined group differences in GMV in atlas-based regions of interest (ROIs) for amygdala, hippocampus, caudate, and insula in each hemisphere. Additionally, we defined 2 cortical ROIs based on the statistical map of the MBSR effect (T2-T1) from Hölzel *et al*. (2011). There were no significant group differences between LTM and MNP at T1, or for change (T2-T1) between MBSR and either control group in GMV in any of the ROIs. Interestingly, MBSR practice time was related to reduced right amygdala GMV, while LTM practice time was related to increased amygdala and hippocampus GMV. Using a well-matched RCT of MBSR, and a cross-sectional study of long-term meditators, these results fail to replicate findings of group differences in regional GMV. Evidence provided herein suggests associations between mindfulness training and change in amygdala and hippocampus GMV may be dependent on engagement with meditation practice.

Introduction

Research on mindfulness-based interventions has increased in response to a growing interest in alternative treatments for reducing stress and improving well-being. Mindfulness-Based Stress Reduction (MBSR) is a popular, manualized mindfulness intervention that was originally developed for use in clinical settings to improve patients abilities to cope with pain (Kabat-Zinn, 1982; Kabat-Zinn et al., 1985). MBSR is efficacious for ameliorating symptoms of multiple psychopathologies (Wielgosz et al., 2019) and for reducing stress (Chiesa and Serretti, 2009). Studies have begun elucidating neural mechanisms underlying mindfulness training-related changes in affect (Desbordes et al., 2012; Hölzel et al., 2013; Kral et al., 2018), cognition (Chiesa et al., 2011; Gallant, 2016; Jha et al., 2010; Mrazek et al., 2013), and pain (Gard et al., 2012; Zeidan and Vago, 2016), among other processes.

In light of numerous studies demonstrating changes in brain structure following behavioral training (Colcombe et al., 2006; Draganski et al., 2006, 2004; Ilg et al., 2008), recent studies have examined whether meditation practice leads to changes in brain structure, as described in a meta-analysis by Fox et al. (Fox et al., 2014). In particular, three of the studies included in the meta-analysis examined changes following short-term mindfulness training with MBSR. Participants who completed MBSR had increased gray matter volume (GMV) in the hippocampus, posterior cingulate cortex (PCC), temporo-parietal junction (TPJ), and cerebellum (Hölzel et al., 2011), and left caudate (Farb et al., 2013). Furthermore, improvements in perceived stress were associated with significantly reduced amygdala GMV, and were mildly correlated with time practicing MBSR, though not significantly (Hölzel et al., 2009).

Changes in GMV in association with mindfulness meditation training would provide evidence of structural neuroplasticity with potentially long-lasting effects. Hippocampus and insula were selected *a-priori* as brain regions of interest (ROIs) in prior work, due to their role in emotion control and awareness, respectively, their activation during meditative states, and prior associations with long-term meditation training and increased GMV in these regions (Hölzel et al., 2011). Insula is also part of the salience network, along with amygdala and anterior cingulate, which is associated with emotional reactivity and subjective awareness processes that are expected to change with mindfulness training (Lutz et al., 2015; Seeley et al., 2007). PCC and TPJ are major nodes within the default mode network, which is implicated in self-related thought and mind-wandering (Fox et al., 2015; Spreng et al., 2008). Mindfulness meditation practice aims to reduce mind-wandering and repetitive, self-related thought, and initial evidence indicates its efficacy in these areas (Jain et al., 2007; Mrazek et al., 2013). Given the evidence for mindfulness-related changes in function and related psychological processes for these brain regions (Allen et al., 2012; Brewer et al., 2011; Creswell et al., 2016; Desbordes et al., 2012; Fox et al., 2016; Kral et al., 2018; Wielgosz et al., 2019), structural changes in GMV might also be expected. Indeed, prior research provides evidence for mindfulness-related changes in brain structure in the default mode and salience networks, among other regions (Farb et al., 2013; Fox et al., 2016; Hölzel et al., 2011).

However, prior research on MBSR-related changes in GMV lacked active control groups and randomization, and had small sample sizes of less than 20 participants per group. Recent literature also stresses the need for replication (Ionnadis, 2005; Moonesinghe, 2007; Button et al., 2013), especially for meditation research (Fox et al., 2014). The current study aimed to address these limitations by integrating a waitlist and a well-matched active control group with larger sample sizes (i.e., at least 70 participants per group), and attempted to replicate prior findings of increased gray matter volume following MBSR in hippocampus, PCC, TPJ, and caudate.

We tested for structural changes in the amygdala and insula as well, which were regions of interest in the prior studies in which no change was detected, but which are involved in affective processing that may change with mindfulness training (Allen et al., 2012; Desbordes et al., 2012). Furthermore, prior results from a sub-sample of participants in the current study showed a reduction in amygdala reactivity and increased amygdala functional connectivity with ventromedial prefrontal cortex (VMPFC) during affective images (presented in Chapter 1) (Kral et al., 2018). Therefore, we tested for relationships between amygdala gray matter volume and these task-based measures in a sub-sample of participants for whom we had both measures (structural MRI and functional, task-based MRI with the emotion processing task).

The current study also included a cross-sectional arm with long-term meditators, who were compared to the non-meditators at baseline to test for group differences in GMV that may be present with a longer duration of meditation experience. While prior research did not find associations between structural changes and amount of MBSR practice, we tested for such associations with practice time for MNP and LTM, especially given the broader range of MBSR practice in the current study relative to prior work. We hypothesized that mindfulness meditation practice would be associated with increased GMV in all ROIs except amygdala, where we expected an inverse relationship between volume and practice, given the inverse relationship between changes in amygdala GMV and stress in prior research (Hölzel et al., 2009).

Methods

This study is registered with ClinicalTrials.gov, and combines data across two clinical trials (NCT01057368 and NCT02157766), which started approximately 5 years apart. The experimental design was comparable across both data sets, corresponding to the data described in Chapters 1 and 2 of this dissertation. The automatic emotion regulation task was only collected in sample one (the results of which are summarized in Chapter 1), and thus only data from sample one can be used in comparisons with the task data.

Participants

Long-term meditators (LTM) from samples one (n=31, average age 50.7 ± 10.1 years, 17 female) and two (n=43, average age 44.6 ± 12.0 years, 16 female) were recruited at meditation centers and through related mailing lists throughout the United States, in addition to flyers and advertisements in newspapers. As described in Chapter 1, meditation recruitment criteria for the LTM in sample 1included: “at least three years of daily practice (at least 30 minutes per day of sitting meditation), experience with Vipassana, concentration and compassion/loving-kindness meditations, and at least 3 intensive retreats lasting 5 or more days.” As described in Chapter 2, study 2, meditation recruitment criteria for sample 2 included: “at least five years of daily practice (with an average practice of at least 200 minutes per week), experience with Vipassana, concentration and compassion/loving-kindness meditations, and at least 5 weeks of retreat practice. Meditation retreats involve spending a period of time away from home at a meditation or community center participating in meditation practice, and can vary in length from days to weeks or months (and in some cases, years). Retreats often include group practice, in addition to solitary practice, and may include periods of silence.” LTM had an average of 3995 lifetime hours of daily meditation practice, ranging from 780 to 19,656 total hours. Lifetime hours of practice were calculated based on subjects reports of their average hours of formal meditation practice per week and their total years of practice. LTM completed a questionnaire to report on retreats they attended, and estimated the time they spent practicing meditation while on each retreat. LTM had an average of 5390 hours of lifetime retreat practice, ranging from 160 to 135,000 hours. Practice hours were log-transformed (using the natural log) to correct for a highly right-skewed distribution.

Meditation-naïve participants (MNP) from samples one (n=125, average age 48.1 ± 10.7 years, 79 female) and two (n=139, average age 44.1 ± 12.7 years, 82 female) comprised a much larger group than the LTM, as their data were included in both repeated measures RCT-related analyses and cross-sectional analyses. Following baseline data collection, a sub-set of MNPs who participated in the cross-sectional arm of the study were randomly assigned to one of three groups for the RCT: Mindfulness Based Stress Reduction (MBSR; N=90, average age 46.6 ± 11.8 years, 53 female), waitlist control (WL; N=84, average age 46.0 ± 11.7 years, 53 female) or the Health Enhancement Program active control intervention (HEP; N=90, average age 45.4 ± 12.5 years, 55 female), which has been validated in a separate study (MacCoon et al., 2012). The MBSR course consisted of practices and teachings aimed at increasing mindfulness, including yoga, meditation and body awareness. The HEP course served as an active control, which was matched to MBSR and consisted of practices aimed at exercise, music therapy and nutrition. The intervention and randomization procedures were identical to those detailed by MacCoon et al. (2012). MBSR and HEP participants recorded logs of the minutes they spent each day on the respective practices at home, which were summed to calculate a variable for total minutes of home practice for each participant (except those in the WL group). MBSR participants practiced an average of 32 hours (standard deviation=20 hours, range=2–85 hours), and HEP participants practiced an average of 56 hours (standard deviation=33 hours, range=7–255 hours).

Participants in either group were excluded from enrollment if they had used medication for anxiety, depression, or other psychological issues, or had a psychiatric diagnosis in the past year. Participants were also excluded if they had any history of bipolar or schizophrenic disorders, brain damage or seizures.

Data collection

MNP completed a baseline data collection visit prior to randomization, and a second visit 8 – 12 weeks later, following the intervention period. The second sample of MNP also completed a third, long-term follow-up session that was not included in the current analysis. LTM completed 3 visits: a baseline session and 2 sessions preceded immediately by a day of meditation practice. The current study focused on the baseline visit in comparisons of LTM and MNP. At each visit, participants attended a 24-hour lab session that included an MRI scan, the Five Facet Mindfulness Questionnaire (FFMQ; Baer et al., 2008), the Symptom Checklist-90 (SCL-90; DELOGATIS, 1977) and the Positive and Negative Affect Scales (PANAS; (Watson et al., 1988) among other measures as part of a larger multi-session, multi-project study. We examined the FFMQ to gauge the efficacy of MBSR for improving mindfulness.

Prior research found relationships with changes in amygdala gray matter volume and perceived stress (Hölzel et al., 2009), however we did not collect a measure of perceived stress in the current study. In order to attempt to conceptually replicate this prior finding, we used self-reported negative affect (from PANAS) and anxiety (from SCL-90) to approximate perceived stress. Self-reported negative affect and anxiety have been shown to have moderate to strong correlations with perceived stress (Lee, 2012; Watson et al., 1988). Experimenters were blind to the group assignment of MNP during data collection for the RCT. A series of other domain-specific measures was included to address hypotheses from aspects of the larger study that were focused on relationships between meditation and sleep, health, response to social stress, emotion regulation, and pain processing, which were irrelevant to the current analysis. UW–Madison’s Health Sciences Institutional Review Board approved the protocol, and all participants provided consent and were given monetary compensation for their participation.

Six MNP and two LTM were excluded due to brain abnormalities, two MNP and one LTM were missing structural data due to technical difficulties, and one LTM was excluded due to extreme distortion of the data from a dental implant, resulting in 70 LTM (average age 46.5 ± 11.4 years, 31 female) and 256 MNP (average age 45.7 ± 12.0 years, 156 female) with baseline (T1) structural MRI data. Eighteen MNP withdrew prior to T2 data collection (8 MBSR, 1 HEP, 9 WL), thirteen MNP were excluded because they failed to attend the intervention (9 HEP, 4 MBSR), and seven MNP were missing T2 structural MRI data due to technical difficulties, resulting in 75 MBSR (average age 47.3 ± 11.9 years, 43 female), 73 HEP (average age 46.0 ± 12.7 years, 43 female), and 70 WL (average age 46.8 ± 11.6 years, 46 female) participants with T2 structural MRI data.

Image Acquisition

Anatomical images for sample one were acquired on a GE X750-3.0 Tesla MRI scanner device with an 8- channel head coil, and consisted of a high-resolution 3D T1-weighted inversion recovery fast gradient echo image (inversion time = 450 msec, 256x256 in- plane resolution, 256 mm FOV, 124x1.0 mm axial slices). Anatomical images for sample two were acquired on the same scanner using a 32-channel head coil with the same scan sequence, except with 192x1.0 mm axial slices.

Anatomical Image Processing

Image processing was conducted in FreeSurfer and used the automated longitudinal pipeline (stable release version 6.0), which included skull-stripping, registration, intensity normalization, Talairach transformation, tissue segmentation, and surface tessellation (Fischl and Dale, 2000; Reuter et al., 2012). FreeSurfer’s automated brain segmentation tool (Aseg; (Fischl et al., 2002) was used to extract measures of gray matter volume from sub-cortical regions, and the Desikan-Killany atlas was used to extract gray matter volume for the insula. A mask of clusters with significant change in MBSR participants was provided by Hölzel (from Hölzel et al., 2011), and used to extract gray matter volume from the corresponding TPJ and PCC cortical ROIs using the FreeSurfer program mris_anatomical_stats.

Automatic emotion regulation task & functional MRI scan

Participants completed an emotion processing task during an fMRI scan, which was described in Chapter 1 and copied below:

> "Seventy-two pictures selected from the International Affective Picture Set (Lang et al., 2008) were presented during fMRI scanning, and evenly split between negative, neutral, and positive pictures. This resulted in 24 pictures in each of the three valence categories, and each picture was presented for 4 seconds. The average normative valence (V) and arousal (A) ratings of the pictures in the three categories were negative (V = 2.87±0.87, A = 5.51±0.47), neutral (V = 5.08±0.60, A = 3.86±0.63) and positive (V= 7.10±0.47, A = 5.36±0.37), where both valence and arousal are measured on 9-point scales (1= *most unpleasant or least arousing* and 9 = *most pleasant* or *most arousing*, respectively). Valence order was pseudo-randomized and picture order was completely randomized within the task. The positive pictures did not include erotic images from the IAPS set, and all the pictures in this task were evenly split between social and non- social categories. The task also included the presentation of neutral (male and female) faces from the Extended Multimodal Face Database ("The XM2VTS Database," n.d.)**,** which were presented for 500 ms after the offset of the picture in two-thirds of the trials, and appeared either 1 s (8x per valence) or 3 s (8x per valence) post-picture offset. There

> were also eight trials in which a face did not follow the image. The faces were included as a way to probe emotional spillover from the preceding IAPS stimuli, however there were no differences in BOLD activation to faces following negative and positive pictures compared to faces following neutral pictures. Thus, we focused solely on the picture presentation period… Participants were instructed to press a button indicating the valence category of the picture (either negative, neutral, or positive) to ensure they were paying attention. Button order was counterbalanced across subjects. Participants were instructed to passively view the faces following the images. All stimuli were presented using E-Prime software (*E-Prime*, 2012) and participants viewed these images with a fiber-optic goggle system (Avotec, Inc., Stuart, FL) while inside the MRI scanner."

Four functional scan runs were acquired using a gradient echo EPI sequence (64x64 in-plane resolution, 240 mm FOV, TR/TE/Flip = 2000 ms/25 ms/60°, 40x4 mm interleaved sagittal slices, and 159 3D volumes per run). Functional images were processed using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library) including a high pass temporal filter of 100 s, motion correction with MCFLIRT (Jenkinson et al., 2002), BET (Smith, 2002) brain extraction, spatial smoothing at 5 mm FWHM, and FILM prewhitening (Woolrich et al., 2001). Transformation matrices for registration were computed at the first level (within scan run) and applied at the second level using FSL in a two stage process where the Boundary Based Registration (BBR) approach (Greve and Fischl, 2009) was used to register the subject's time series data to their anatomical template, and a 12 DOF affine transformation was used to register the subject's anatomical scans to Montreal Neurological Institute (MNI) space using FLIRT (Jenkinson et al., 2002).

Statistical Analysis

Analyses were conducted using difference scores for each variable (post- minus pre-intervention) to examine change related to MBSR training, and using pre-intervention values for analyses addressing differences in brain structure between LTM and MNP. All analyses were performed using the lm function in R statistics software (R Core Team, 2013), and *p*-value computation used the modelSummary function of the lmSupport package (Curtin, 2015). Age, gender, and sample (i.e., one versus two) were included as covariates of no-interest in all models. In analyses examining gray matter volume, total brain volume was also included as a covariate of no-interest. Outliers were identified based on Cook's D using a cutoff threshold of 4/(N-P), where N and P correspond to the sample size and number of model parameters, respectively, and removed from analyses. We used a false discovery rate (FDR) correction to control for multiple comparisons for each family of tests (i.e., across all 10 ROIs), using the p.adjust function. Corrected *p*-values are indicated by *p** in the text. A summary of descriptive statistics for all variables is presented in Table 1.

We conducted cross-project analysis to examine relationships between change in amygdala GMV and previously reported changes in amygdala reactivity and connectivity with ventromedial prefrontal cortex (VMPFC) during affective images (Chapter 1; Kral et al., 2018). Statistical analysis of functional data was described in Chapter 1 and included contrast analysis for amygdala activation during Negative>Neutral and Positive>Neutral IAPS. The functional ROIs were described in Chapter 1 thusly: "Functional amygdala ROIs were identified from the contrast of Negative>Neutral IAPS pictures during a 4 s picture presentation period (analogous to the task used in the current study except participants did not make a button press response) and using data from an independent sample of 90 participants enrolled in a separate study (mean age(SD)=45.1(10.0) years, 52 female) (Grupe et al., 2017). The right and left functional amygdala ROIs were then masked with anatomical amygdala ROIs from the Harvard- Oxford Atlas (Desikan et al., 2006) with a threshold at 50% probability to remove any non-amygdala voxels, resulting in a left and right amygdala ROIs of 104 and 164 voxels, respectively. Mean percent signal change across each amygdala ROI was extracted for all participants from the output of the second level model." Psychophysiological interaction (PPI) effects were also calculated to examine alterations in amygdala-ventromedial prefrontal cortex (VMPFC) functional coupling during affective images. The details of PPI analysis are described in Chapter 1, including the following: "The functional ROI for the right amygdala was used to extract the timeseries, which was deconvolved (Gitelman et al., 2003) prior to computing the psychophysiological interaction (PPI) for the connectivity analysis. We then ran a second model to examine right amygdala-VMPFC connectivity… We computed contrasts for negative versus neutral and positive versus neutral PPIs, which were then used for a group level connectivity analysis from which we extracted values from a MPFC ROI based on the Harvard-Oxford Atlas containing 976 voxels. We used an anatomical mask for MPFC as we did not have an independent, functional localizer for this region."

Results

Wholebrain analysis

There were no significant group differences for LTM compared to MNP, nor for change in brain structure for MBSR compared to HEP or WL in the wholebrain analysis. Significant within-group volume increases (T2-T1) in the left hemisphere were present for MBSR in lingual gyrus, for HEP in rostral middle frontal gyrus, and for WL in precuneus, pars opercularis, and rostral middle frontal gyrus. Coordinates for significant clusters in the within-group tests are provided in Table 2. There were no associations between LTM practice and gray matter volume at T1. There were no significant associations between MBSR practice and change in gray matter volume (T2-T1), nor were there any significant interactions between MBSR and HEP practice in the wholebrain analysis.

ROI analysis

There were no significant group differences for LTM compared to MNP, nor for change in brain structure for MBSR compared to HEP or WL for any of the ROIs (ps > 0.1).

MBSR practice

Significant effects of MBSR practice were limited to the amygdala, and relationships with the other 8 ROIs were non-significant ($ps>0.05$). MBSR practice was associated with reduction in right amygdala volume significantly more than HEP practice ($t(128)=-3.30$, $p=0.001$, $p^{*}=0.01$, 8 MBSR and 3 HEP outliers removed; Figure 1a). Within MBSR, there was a significant relationship between practice time and right amygdala volume reduction ($t(62)=-2.55$, $p=0.01$, $p^{*}=0.13$, 6 outliers removed; Figure 1b). There was no relationship with HEP practice and change in right amygdala volume ($t(62)=0.80$, $p=0.43$, 5 outliers removed).

MBSR practice was also associated with reduction in left amygdala volume significantly more than HEP practice ($t(129)=-2.06$, $p=0.04$, $p^{*}=0.21$, 4 MBSR and 6 HEP outliers removed). However, there was no significant relationship between left amygdala volume change and MBSR practice ($t(64)=-1.22$, $p=0.23$, 4 outliers removed), or HEP practice ($t(63)=1.32$, $p=0.19$, 4 outliers removed).

MBSR practice was associated with reduced negative affect ($t(66)=-2.05$, $p=0.04$, 3 outliers removed, Figure 1c). There was no significant difference between MBSR and HEP practice in relation to reduced negative affect ($t(132)=-1.33$, $p=0.19$, 8 outliers removed) or anxiety ($t(128)=0.04$, $p=0.97$, 11 outliers removed). There was no relationship between MBSR practice and change in anxiety (*t*(63)=-0.89, *p*=0.38, 5 outliers removed).

LTM practice

Significant effects of LTM practice were limited to the left amygdala and left hippocampus, and relationships with the other 8 ROIs were non-significant (*ps*>0.05). Lifetime hours of LTM home meditation practice (log-transformed) were associated with larger left amygdala volume (*t*(58)=2.27, *p*=0.03, *p**=0.19, 3 outliers removed, Figure 2a). Similarly, lifetime hours of LTM retreat meditation practice (log-transformed) were associated with larger left hippocampal volume (*t*(57)=2.39, *p*=0.02, *p**=0.20, 4 outliers removed, Figure 2b). This relationship remained significant when the extreme point (with 135,000 hours of retreat practice) was excluded from the model (*p*=0.03).

There was no relationship between LTM practice and anxiety (retreat: *t*(62)=0.55, *p*=0.58, 2 outliers removed; daily practice: *t*(62)=0.40, *p*=0.69, 2 outliers removed) or negative affect (retreat: *t*(58)=1.33, *p*=0.19, 3 outliers removed; daily practice: *t*(58)=0.54, *p*=0.59, 3 outliers removed).

Automatic emotion regulation task

We examined relationships between change in amygdala gray matter volume and task-based measures for the right amygdala in sample one, in which we previously found decreased right amygdala reactivity (for the contrast positive plus negative minus neutral) for LTM and MBSR, and increased right amygdala functional connectivity with VMPFC for MBSR (for the contrast negative minus neutral).

Decreased right amygdala GMV was associated with increased right amygdala – VMPFC functional connectivity (T2-T1) across MNP (*t*(91)=-3.82, *p*<0.001, 9 outliers removed) (Figure 3a). There was no relationship between either baseline or change in right amygdala GMV and reactivity to affective images (*ps*>0.10).

Negative affect

There was no difference in negative affect for LTM compared to MNP (*t*(315)=1.65, *p*=0.10, 15 outliers removed). There were no group differences for change in negative affect for MBSR compared to HEP (*t*(200)=0.29, *p*=0.77) or WL (*t*(200)=0.11, *p*=0.91), with 6 MBSR, 3 HEP, and 5 WL outliers removed. There were no relationships between change in negative affect and amygdala volume (right: *t*(201)=0.30, *p*=0.77 with 4 MBSR, 2 HEP and 5 WL outliers removed; left: *t*(198)=-0.07, *p*=0.95 with 4 MBSR, 4 HEP, and 6 WL outliers removed), nor were there relationships between change in negative affect and amygdala volume within MBSR participants (right: *t*(66)=0.67, *p*=0.51, 3 outliers removed; left: *t*(67)=-0.45, *p*=0.66, 2 outliers removed).

Anxiety

There was no difference in anxiety for LTM compared to MNP (*t*(308)=-1.60, *p*=0.11, 15 outliers removed). There were also no group differences for change in anxiety for MBSR compared to HEP (*t*(199)=0.92, *p*=0.36) or WL (*t*(199)=1.16, *p*=0.25), with 6 MBSR, 3 HEP, and 5 WL outliers removed. There were no relationships between change in anxiety and amygdala volume (right: *t*(200)=0.27, *p*=0.79; left: *t*(200)=-1.53, *p*=0.13), each with 5 MBSR, 4 HEP, and 2 WL outliers removed. Nor were there relationships between change in anxiety and amygdala volume within MBSR participants (right: *t*(63)=-0.53, *p*=0.60, 5 outliers removed; left: *t*(64)=-1.85, *p*=0.07, 4 outliers removed).

Mindfulness

We examined self-reported mindfulness based on the FFMQ to gauge the effectiveness of the MBSR intervention. A prior study reported on the results for sample one, whereby MBSR was associated with increased mindfulness ($p<0.05$, within-group) that marginally differed from WL ($p=0.09$), but not from HEP ($p=0.33$) (Goldberg et al., 2015). In sample two, MBSR was associated with increased mindfulness ($t(37)=2.69$, $p=0.01$), and this change in MBSR was significantly larger than for HEP ($t(102)=-2.33$, $p=0.02$) or WL ($t(102)=-2.29$, $p=0.02$), with 1 MBSR, 2 HEP and 1 WL outlier removed. When collapsing across both studies, results were consistent with the prior report, whereby MBSR differed significantly from WL ($t(214)=-2.48$, $p=0.01$), but not from HEP ($t(214)=-0.66$, $p=0.51$), with 1 MBSR outlier removed. Across both samples, mindfulness increased following MBSR ($t(70)=3.89$, $p<0.001$, 2 outliers removed) and HEP ($t(65)=3.28$, $p=0.002$, 4 outliers removed).

Discussion

The current study sought to replicate and extend prior work demonstrating increased gray matter volume following mindfulness meditation training in hippocampus, caudate, posterior cingulate and temporoparietal junction (Farb et al., 2013; Hölzel et al., 2011). We combined data from two datasets to yield sample sizes of 70 or more participants per group. Both datasets were collected with the same rigorous methods and design, including two study arms: a randomized controlled trial of MBSR with a well-matched, active control (in addition to a waitlist control), and a cross-sectional analysis of long-term mindfulness training compared to controls. We expected to replicate prior results of increased volume following short-term training in MBSR in hippocampus, caudate, TPJ, and PCC (and reduced volume for amygdala), and we also hypothesized that these effects would be larger for participants who spent more time practicing mindfulness meditation (either MBSR practice or in long-term meditators). We failed to find any group differences in gray matter volume in support of our hypothesis, either between MBSR or LTM and the respective control groups. It is unlikely that the failure to replicate prior work was due to ineffective training. The MBSR intervention was effective with regard to expected changes in neural, psychological, and cognitive outcomes: MBSR reduced amygdala reactivity and increased amygdala-VMPFC functional connectivity to emotional stimuli in sample one (Kral et al., 2018), increased self-reported mindfulness (reported in Goldberg et al., 2015 for sample one, in addition to the results presented here), and increased PCC resting functional connectivity with dorsolateral prefrontal cortex (Kral et al., 2019) and attentional alertness in sample two (Patsenko et al., under review).

The more time participants spent practicing MBSR outside of class, the larger their reduction in right amygdala volume following the intervention, which was significantly different than HEP, the well-matched active control intervention. There was no relationship between time practicing HEP and change in right amygdala volume. Prior results from a subset of participants in the current study found MBSR-related reductions in right amygdala reactivity, and increased amygdala – VMPFC connectivity, during emotional stimuli (Kral et al., 2018). We tested whether the prior results for task-based changes in right amygdala reactivity and connectivity with VMPFC were associated with reductions in gray matter volume, and found an inverse association with right amygdala – VMPFC functional connectivity during negative versus neutral images. Given the evidence for inhibitory control of amygdala by VMPFC (Kim et al., 2011; Phelps et al., 2004), we interpreted the increased connectivity as reflecting greater emotion regulation via inhibition of amygdala, which may in turn lead to reduced amygdala volume via less amygdala neuronal firing, and a consequent loss of synapses and associated glial cells (Anderson, 2011). The current study thus provides evidence that MBSR-related reductions in amygdala volume may depend on the degree of engagement with practice, and are associated with increased amygdala-VMPFC functional coupling during emotion processing.

Decreased amygdala volume has also previously been associated with reductions in perceived stress following MBSR (Hölzel et al., 2009). We failed to find associations between changes in amygdala volume with negative affect or anxiety in the current study (as a proxy for perceived stress), though this may be partially due to floor effects in the current dataset, and reflective of the relative health of participants. The mean anxiety score across groups was 0.14, the mode was 0.00. The range of the measure is 0 to 4, with 0 indicating that anxiety symptoms are experienced “not at all” and a 1 indicating “a little bit”. Thus, participants in the current study reported very little to no anxiety, both at baseline and after the intervention (with a mean anxiety score of 0.16 and a mode of 0.00 across MNP at T2).

Conversely, among LTM, more hours of daily meditation practice across the lifetime were associated with *larger* left amygdala gray matter volume, although this result should be interpreted with caution since it did not survive corrections for multiple comparisons. If replicated, this somewhat counterintuitive finding is consistent with the non-linear dose-response trajectory of mindfulness meditation training proposed by Brefczynski-Lewis et al. (2007). Differential findings for associations between MBSR and LTM practice with amygdala volume are also consistent with our prior results showing increased amygdala–prefrontal cortex functional connectivity during negative picture viewing for MBSR participants, for which there was no difference between LTM and non-meditators (Kral et al., 2018). Increased amygdala volume has also been associated with “extraordinary altruists” – living individuals who donate a kidney to a stranger, who also show increased amygdala response to fear faces (Marsh et al., 2014). However, the amygdala is composed of multiple nuclei and sub-regions that are known to have differential functions and connectivity (Adhikari et al., 2015; Phelps and LeDoux, 2005; Tye et al., 2011; Tyszka and Pauli, n.d.). Thus, it is unknown whether the meditation practice-related amygdala volume differences correspond to the same sub-nuclei for short- and long-term practitioners, nor how they compare to other populations in which amygdala volume differences have previously been reported (e.g., in extraordinary altruists). Moreover, given the cross-sectional nature of the LTM data, it is possible that individuals with a larger amygdala are more drawn to meditation, or more likely to practice meditation. The interpretation of mindfulness meditation practice-related differences in amygdala volume is complex, and requires further research to fully elucidate.

The results of the current study failed to support the hypothesis that mindfulness meditation practice would be associated with significant group differences in regional brain volume compared to a well-matched, active control intervention or a waitlist control group in a rigorous RCT design, or in a cross-sectional analysis of long-term practitioners compared to controls. Despite previous research suggesting that mindfulness meditation training impacts the structure of the brain, results of the present study failed to replicate these group differences. While this highlights the importance of replication studies, it also raises new questions. There were important differences between the current study and prior work, including the populations from which participants were drawn, and differences in the study design and methods. Prior work recruited participants who elected to participate in an MBSR course (Farb et al., 2013; Hölzel et al., 2011), and were thus not randomly assigned, while the current study utilized a rigorous randomized controlled trial design. The participants in prior studies may have had more “room for improvement”, since they sought out a course for stress reduction, with some samples recruited specifically based on the presence of high stress in participants the month prior to study participation (Hölzel et al., 2009). While the RCT design employed here provides the strongest scientific methodology, this increased rigor likely comes at the expense of ecological validity –

the simple act of choosing to enroll in MBSR may be associated with increased benefit, and the same may be true for LTM. There may have also been important factors relating to individual differences of participants, teachers, or the context of the intervention that may have contributed to the difference in findings across studies.

It is notable that the current study also had sample sizes of over 3 times that of prior work (e.g., n=75 MBSR participants in our final sample compared to n=20 or less participants per group in prior work) (Farb et al., 2013; Hölzel et al., 2011). Given the low samples sizes of prior work, and the larger samples and lack of replication in the current study, there is a possibility that prior results suffered from inflated effect sizes and low positive predictive value (Button et al., 2013). As more research is conducted on this topic, the importance of reporting results of replication attempts should be emphasized in light of known publication bias for positive findings.

The results of the current study also highlight the importance of examining the effects of mindfulness meditation along a wide range of practice experience. While the prior work failed to find relationships between MBSR practice time and amygdala volume change, the present study included a much larger range of MBSR practice time (from 2 to 81 hours, compared to a range of 7 to 40 hours in prior work), and this increased range and associated variation of the sample may have allowed for a fuller characterization of practice effects on amygdala gray matter volume. The examination of long-term practice hours also uncovered a somewhat surprising relationship with increased hippocampus and amygdala volume – in the opposite direction of results for MBSR – suggesting a nonlinear relationship in the effects of mindfulness meditation from initial exposure to long-term practice, similar to that reported in our prior work (Kral et al., 2018). Future research should aim to examine the impact of mindfulness meditation practice longitudinally, in order to capture the shape of this relationship as individuals transition from short- to long-term practice, and possibly from strictly formal meditation to practice that is more integrated into daily life.

Conflicts of Interest: Dr. Richard J. Davidson is the founder, president, and serves on the board of directors for the non-profit organization, Healthy Minds Innovations, Inc. No donors, either anonymous or identified, have participated in the design, conduct, or reporting of research results in this manuscript.

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Figures

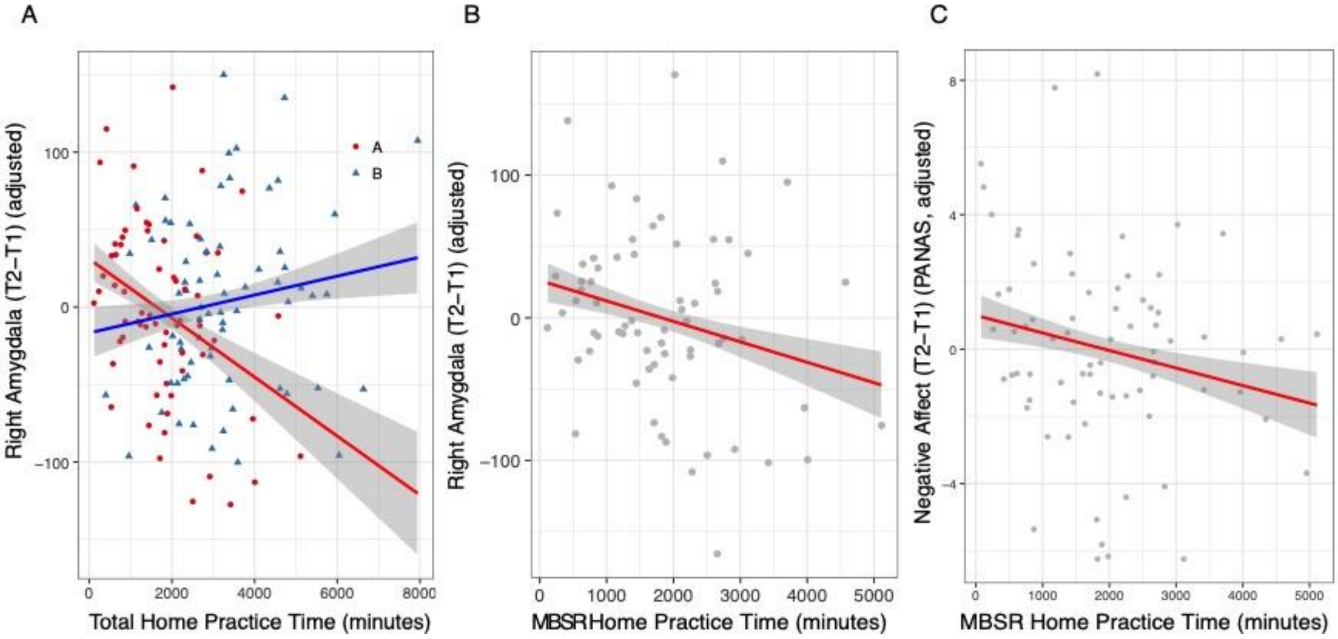

Figure 1. Effects of MBSR practice time. **(A)** MBSR practice was related to reduced right amygdala gray matter volume significantly more than HEP practice. **(B)** Within MBSR participants, more practice time was related to significantly smaller right amygdala gray matter volume following the intervention. **(C)** MBSR practice time was significantly associated with reduced negative affect following the intervention. Error envelopes represent 1 standard error above and below the point estimates of the means, the dependent variables are adjusted for covariates (e.g., age, gender, sample and total brain volume – in analysis with brain volume), and raw data points are overlaid.

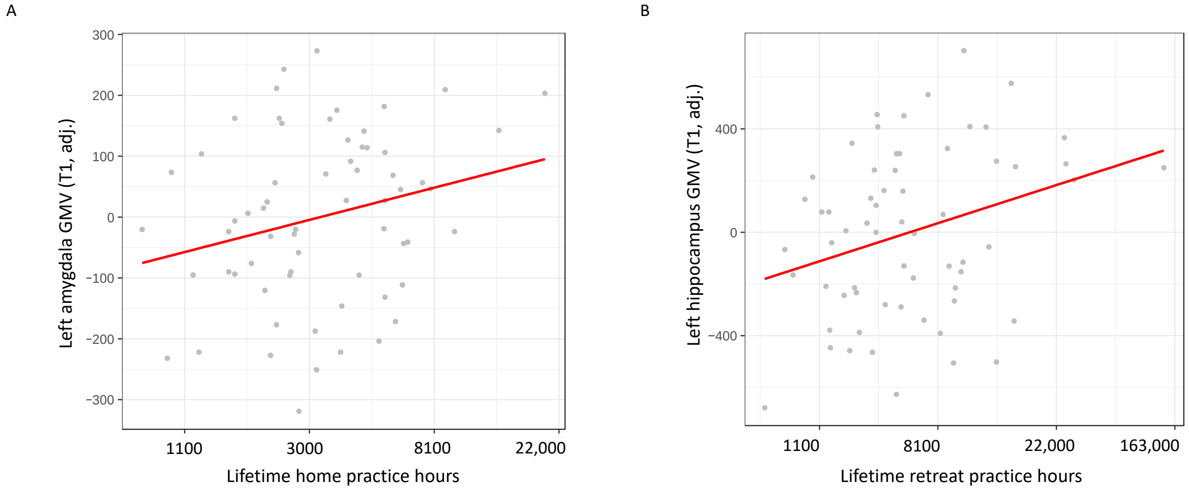

Figure 2. Effects of LTM total practice time. **(A)** Total lifetime hours of daily, home meditation practice (log-transformed) were associated with larger left amygdala gray matter volume among LTM. **(B)** Total hours of lifetime retreat meditation practice (log-transformed) were associated with larger left hippocampus gray matter volume. Error envelopes represent 1 standard error above and below the point estimates of the means, the dependent variables are adjusted for covariates (e.g., age, gender, sample and total brain volume), and raw data points are overlaid.

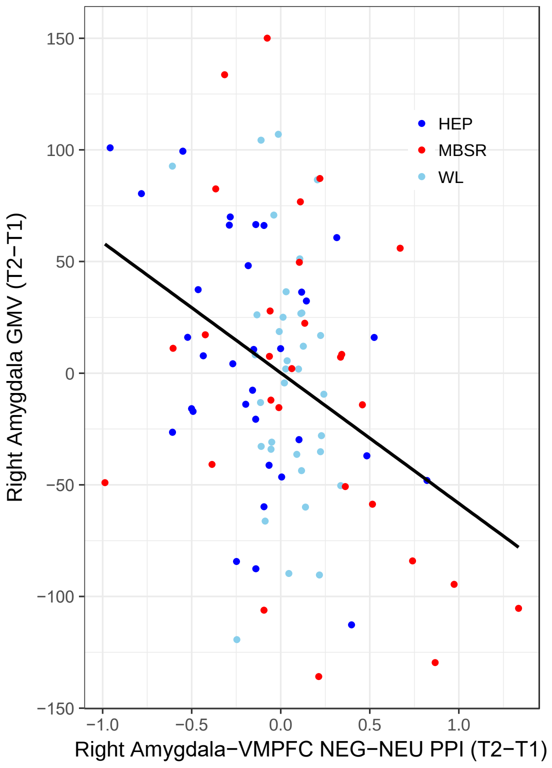

Figure 3. Increased right amygdala – VMPFC functional connectivity is associated with decreased right amygdala gray matter volume. Functional connectivity between right amygdala and ventromedial prefrontal cortex (VMPFC) was assessed via psychophysiological interaction (PPI) during negative versus neutral affective images. Error envelopes represent 1 standard error above and below the point estimates of the means, the dependent variables are adjusted for covariates (e.g., age, gender), and raw data points are overlaid. GMV = gray matter volume

Tables

Table 1. Descriptive statistics.

Measure:	Left amygdala GMV				Right amygdala GMV				Left TPJ GMV			
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	1587	190	1135	2105	1868	210	1138	2357	653	118	368	1012
MNP	1573	210	1107	2356	1857	230	1237	2632	639	139	371	1197
T1 MBSR	1541	191	1113	1882	1846	234	1272	2631	639	133	404	985
T1 HEP	1594	215	1134	2277	1873	226	1427	2511	624	143	389	1116
T1 WL	1565	216	1107	2101	1839	240	1237	2578	653	142	371	1197
T2 MBSR	1549	202	1103	1987	1843	239	1299	2577	640	127	410	929
T2 HEP	1591	222	1179	2430	1876	227	1437	2492	615	143	384	1106
T2 WL	1565	217	1059	2087	1847	221	1402	2439	641	130	405	1179

Measure:	Left hippocampus GMV				Right hippocampus GMV				Posterior cingulate GMV			
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	4309	436	3130	5262	4359	450	2969	5273	906	178	614	1449
MNP	4257	452	3131	5431	4316	431	3261	5720	933	201	491	1681
T1 MBSR	4212	465	3131	5162	4294	418	3283	5225	893	178	524	1305
T1 HEP	4284	433	3574	5367	4340	447	3546	5720	965	224	553	1681
T1 WL	4233	436	3320	5431	4282	420	3261	5407	925	201	491	1437
T2 MBSR	4190	484	3006	5190	4270	427	3288	5172	885	182	532	1396
T2 HEP	4273	439	3600	5440	4338	457	3470	5717	951	220	575	1682
T2 WL	4218	429	3234	5614	4263	418	3255	5491	929	193	518	1434

Measure:	Left caudate GMV				Right caudate GMV				Negative affect			
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	3698	418	2951	4928	3812	426	3084	5373	13.8	4.2	10	30
MNP	3596	475	2516	5275	3756	496	2563	5373	14.3	3.8	10	26
T1 MBSR	3561	483	3472	5275	3728	518	2563	5373	14.7	4.0	10	26
T1 HEP	3572	479	2516	4794	3731	502	2905	5043	13.8	3.5	10	26
T1 WL	3596	434	2731	4561	3741	428	2758	4897	14.3	3.7	10	24
T2 MBSR	3560	490	2516	5242	3734	521	2518	5416	15.0	5.2	10	31
T2 HEP	3554	490	2512	4864	3730	505	2912	5155	14.0	4.0	10	30
T2 WL	3594	435	2718	4601	3758	433	2734	4886	14.6	4.5	10	26

Measure:	Left insula GMV				Right insula GMV				Anxiety			
Group	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*	*M*	*SD*	*Min*	*Max*
LTM	7216	744	5813	8760	7234	814	5696	9468	0.18	0.25	0	1.4
MNP	7097	794	5248	10052	6997	811	5142	10023	0.13	0.2	0	1.2
T1 MBSR	7074	783	5335	8542	6997	750	5191	8512	0.14	0.2	0	0.8
T1 HEP	7189	816	5959	9221	7012	842	5656	10023	0.13	0.2	0	1
T1 WL	7017	744	5509	8834	6962	854	5390	9269	0.13	0.19	0	0.9
T2 MBSR	7103	823	5290	8856	6998	749	5140	8598	0.19	0.23	0	1.1
T2 HEP	7199	834	5801	9348	7066	880	5795	10565	0.14	0.2	0	0.9
T2 WL	7024	756	5515	8943	6933	847	5349	9170	0.14	0.17	0	0.7

Mean (M), standard deviation (SD), minimum (Min), and maximum (Max) for all measures. GMV = gray matter volume; TPJ = temporoparietal junction; LTM = longterm meditators; MNP = meditation-naïve participants; MBSR = randomized to Mindfulness-Based Stress Reduction; HEP = randomized to health enhancement program control intervention; WL = waitlist control.

Table 2. Within-group changes in gray matter volume: Cluster details.

	REGION	PEAK COORDINATES X	Y	Z	VOLUME (mm2)
MBSR	Left lingual gyrus	-16.8	-78.6	-11.2	309
HEP	Left rostral middle frontal gyrus	-22.1	46.5	25.6	196
WL	Left precuneus	-16.1	-46.0	50.8	163
	Left pars opercularis	-42.7	24.6	17.6	149
	Left rostral middle frontal gyrus	-26.9	42.4	17.0	170

General Discussion

The set of studies comprising this dissertation aimed to rigorously examine the effects of short- and long-term mindfulness meditation training on brain function during emotion processing, resting state functional connectivity, and structural neuroplasticity. Mindfulness meditation provides promise as an approachable, widely available practice for stress relief and coping that is critically needed. While research on mindfulness meditation has grown exponentially in the past two decades (Barrett et al., 2016), the vast majority of studies suffer from major limitations in design and insufficient sample sizes (Chiesa and Serretti, 2009; Davidson and Kaszniak, 2015; Fox et al., 2014; Khoury et al., 2015). We addressed many of these prior limitations through larger sample sizes with a minimum of 30 participants per group, a hybrid design combining an active-controlled randomized controlled trial (RCT) with a cross-sectional analysis of long-term training, and by utilizing complementary brain and behavioral measures.

The emotion processing task and task-free condition of the resting state fMRI scan were selected to isolate expected reductions in emotional reactivity and mind-wandering, respectively. In addition to wholebrain analyses, brain regions and networks of interest were selected based on evidence that their function supports the relevant cognitive processes (e.g. emotion regulation/ reactivity, and meta-awareness and self-related thought, respectively), as well as from prior research on mindfulness meditation-related changes in an effort at replication. We included analysis of the impact of practice duration on each of our measures of interest to more fully examine individual differences of the impact of mindfulness meditation training.

The results reported in Chapter 1 provided evidence that short- and long-term mindfulness meditation practice reduce amygdala reactivity in response to emotional stimuli.

The amygdala was selected as an *a-priori* region of interest (ROI), as there is extensive literature across human and non-human animal studies indicating its central role in emotion processing and regulation (Adhikari et al., 2015; Phelps and LeDoux, 2005). Amygdala connectivity with ventromedial prefrontal cortex (VMPFC) has also been implicated in implicit and explicit emotion regulation, including extinction learning and regulation deficits in anxiety disorders (Kim et al., 2011b, 2011a; Phelps et al., 2004). We found that short-term training in Mindfulness-Based Stress Reduction (MBSR) increased amygdala-VMPFC functional connectivity during negative pictures (relative to neutral) compared to a well-matched, active control intervention, while there was no difference in functional connectivity between long-term meditators (LTM) and non-meditators. The MBSR-related increase in amygdala-VMPFC functional connectivity was present for both negative and positive pictures (relative to neutral), which suggests that mindfulness meditation training may involve a similar emotion regulatory mechanism for reducing neural reactivity to affective stimuli independent of valence.

One interpretation of this pattern of findings is that it reflects increased emotion regulation via inhibitory control of amygdala by VMPFC, resulting in decreased emotional reactivity, as revealed by decreased amygdala activation following MBSR. Increased emotion regulation with MBSR, and decreased reactivity absent changes in regulation with long-term training, is consistent with the goals and expectations of mindfulness practice, whereby nonreactivity becomes more automatic over time. In this view, decreased emotional reactivity with practice in mindfulness meditation follows from increased exposure to the arising and passing of emotions while attending to experience non-reactively, which may initially involve explicitly identifying emotions through meta-awareness and distancing with dereification.

The post-hoc results whereby increased amygdala-VMPFC functional connectivity was associated with decreased amygdala reactivity for negative versus neutral pictures (regardless of intervention status), lend further support to the interpretation of amygdala-VMPFC connectivity reflecting emotion regulatory inhibition of amygdala. In addition, lower amygdala activation across participants at baseline was associated with higher self-reported *non*-reactivity, which provides further evidence for the interpretation of amygdala reactivity as a measure of psychological reactivity. However, these interpretations ultimately rest on reverse inference, despite the pattern of evidence supporting them, and the current study lacked direct measures to probe participants' engagement with emotion regulation processes. While LTM had less self-reported emotional reactivity than non-meditators, there were no differences for MBSR. It is possible that it requires more practice over a longer period of time to see changes on questionnaire measures, particularly in healthy, non-clinical populations with well-matched control groups. These results also highlight the importance of collecting outcome measures in multiple modalities (e.g., brain and behavior).

Chapter 2 examined the impact of mindfulness meditation training on resting state functional connectivity (RSFC) of posterior cingulate cortex (PCC) with lateral prefrontal cortex (LPFC), which are brain regions implicated in mind-wandering and self-related processing, and in executive functions and meta-awareness, respectively. Results indicated that MBSR increased RSFC between PCC and dorsal LPFC (defined from an anatomically-based middle frontal gyrus ROI), whereas long-term mindfulness meditation practitioners had stronger RSFC between PCC and rostral LPFC compared to non-meditators. One interpretation of these results is that increased RSFC between PCC and LPFC reflects higher attentional control and less mind-wandering, which is supported by results in Chapter 2 showing that increased RSFC between

PCC and DLFPC was associated with increased self-reported attention. This interpretation is consistent with one of the primary skills that mindfulness meditation aims to train – directing attention to present moment experience without elaboration. In addition, both questionnaire and experience sampling measures indicated that LTM had higher attention (or less mind-wandering) than non-meditators, although we did not see differences in these measures with MBSR training. Similar to the results for non-reactivity, it is possible that movement on self-report and behavioral measures (in healthy, non-clinical populations) requires longer-term training, and changes in the underlying brain networks may precede behavioral change.

The specific localization of differences in PCC RSFC associated with short- and long-term mindfulness meditation training may, in part, reflect interactions between differences in the stage of mindfulness training and the putative functions of dorsal and rostral LPFC. Early in mindfulness meditation training, meta-awareness is cultivated, in part, by noticing moments of mind-wandering in an acute way, which involves an inward turn of attention (Lutz et al., 2015). Dorsal LPFC has been implicated in awareness of mind-wandering and error detection (Hester et al., 2005; Schooler et al., 2011), consistent with this type of intermittent, or “phasic”, meta-awareness. With increased experience in mindfulness meditation, as in long-term training on the order of thousands of hours, meta-awareness is expected to become more sustained as a “background” process, whereby the practitioner is aware of the broader phenomenological experience (e.g., including mind-wandering) without turning attention away from its focal object or task (Lutz et al., 2015). While there is a dearth of research on this presumed form of “background” meta-awareness, evidence from cognitive neuroscience supports a hypothesized role for rostral LPFC in differentiating the source of cognitive representations and exerting influence on attention allocation in open-ended situations, such as the task-free context of a resting state scan (Burgess et al., 2007), which is consistent with a role in meta-cognitive background awareness. However, interpretations of the behavioral consequences of mindfulness meditation-related effects on PCC RSFC that were found in the current study represent only one potential explanation of the findings, and additional research is needed to test these hypotheses.

Analysis of network hub properties with measures derived from graph theory provided further characterization of mindfulness meditation-related effects on resting state connectivity. Within-module degree (WMD) assessed the degree of within-module connectivity of each node, while the participation coefficient assessed the degree of inter-modular connectivity, where modules correspond to different resting state brain networks and nodes correspond to regions within a module. The PCC is a central node of the default mode network (DMN), while dorsal LPFC participates in the frontoparietal control network (FPN) and dorsal attention network (DAN). Given the interpretation of increased RSFC between PCC and DLPFC following MBSR as reflecting better attentional control (by DLPFC) of mind-wandering (by PCC), we expected to see larger participation coefficients for DAN and FPN associated with mindfulness meditation training, reflecting higher intermodular integration (Rubinov and Sporns, 2010). However, there were no differences in WMD or participation coefficients for MBSR compared to controls for any of the networks. Interestingly, LTM had lower participation coefficients (i.e., less inter-modular connector hubs) than non-meditators within the DMN, and there were no differences in any of the other networks or measures. Therefore, mindfulness meditation training-related increases in PCC RSFC may be specific to LPFC, rather than reflecting more widespread integration of DMN and FPN. Moreover, fewer connector hubs in the DMN associated with long-term mindfulness meditation practice may reflect reduced information flow to other networks.

Chapter 3 attempted to replicate and extend prior research showing increased regional gray matter volume (GMV) associated with mindfulness meditation training. Prior research has shown increased GMV following MBSR in hippocampus, temporoparietal junction, brainstem, cerebellum, posterior cingulate, and caudate (Farb et al., 2013; Hölzel et al., 2011). There were no group differences in GMV in any region, including the former brain regions in which changes were previously reported, with either short-term MBSR training or long-term meditation practice compared to the respective, well-matched control groups. The combined datasets used in the current study resulted in sample sizes over 3 times the size of those used in prior work (e.g., n=75 MBSR participants in our final sample compared to n=20 or less participants per group in prior work) (Farb et al., 2013; Hölzel et al., 2011). Thus, there is a possibility that prior studies over-estimated the effect sizes due to low positive predictive value (Button et al., 2013). However, prior work also used a non-randomized, stressed sample of adults, while our samples were exceptionally healthy and had a truncated range on symptom questionnaires, which likely resulted in an underestimation of effect sizes due to ceiling effects in the current study (e.g., on anxiety symptom questionnaires). The simple act of choosing to enroll in MBSR, and the underlying motivations for doing so, may be associated with increased benefit and may explain the different results of the current study and prior work. Additional, rigorous research in community samples is therefore of utmost importance, as is reporting results of replication attempts and non-significant findings, given the known publication bias for positive findings.

Despite the lack of group differences in regional GMV, time spent practicing mindfulness meditation was associated with differences in amygdala and hippocampus GMV. Reductions in right amygdala GMV were dependent on practice time with MBSR – a relationship that differed significantly from HEP practice, with the latter having no association with changes in amygdala

GMV. Prior research indicated that stress reduction following MBSR was associated with decreased amygdala GMV (Hölzel et al., 2009). The current study also found that reduced amygdala GMV was associated with increased amygdala-VMPFC functional connectivity during negative (versus neutral) pictures, irrespective of intervention group. Therefore, one mechanism by which mindfulness meditation practice may reduce amygdala GMV is through VMPFC inhibition of amygdala activation, given the evidence for an inhibitory control of amygdala by VMPFC (Kim et al., 2011b; Phelps et al., 2004). Less amygdala neuronal firing could lead to reduced amygdala GMV through a consequent loss of synapses and associated glial cells (Anderson, 2011). This dissertation provides evidence that MBSR may reduce amygdala GMV dependent on engagement with meditation practice, and such changes may be associated with increased connectivity of brain networks underlying emotion regulation.

Conversely, long-term practice was associated with *larger* amygdala (and hippocampus) gray matter volume, despite a lack of differences between meditators and non-meditators. It is important to note that the amygdala is a heterogeneous structure, comprised of multiple sub-nuclei with differential functionality and connectivity (Adhikari et al., 2015; Kim et al., 2011a; Phelps and LeDoux, 2005). Thus, it is unknown whether the results reported in the current study are due to changes in the same portion of the amygdala, or the amygdala complex as a whole, compared to prior research (or even between results for MBSR and LTM within this study). Moreover, changes in GMV cannot be linear – while there may be a linear relationship within a certain range, there must be a limit in both growth and shrinkage (Wenger et al., 2017). Structural changes are caused by multiple factors, and different mechanisms may be underlying change in different groups (Anderson, 2011).

The increased amygdala volume associated with long-term meditation practice hours may, in part, relate to other forms of meditation practice with which the LTM engaged – namely, compassion and loving-kindness meditation. While the LTM practice history in the current study was predominantly comprised of meditation styles within the mindfulness family, participants all had substantial experience with compassion or loving-kindness meditation as well. The latter forms of meditation have been associated with *increased* amygdala reactivity to emotional sounds in meditating experts, as well as increased insula activation to negative sounds, in particular (Lutz et al., 2008). Short-term training in compassion meditation has also been associated with increased empathic accuracy, a behavioral measure of empathy, and increased medial PFC brain activation that was associated with the behavioral change (Mascaro et al., 2013). Therefore, it is possible that the associations between larger amygdala GMV and long-term hours of meditation practice are the result of the mixed training of LTM in both mindfulness and compassion-loving-kindness practices. Moreover, extraordinary altruism – living organ donation – has been associated with larger amygdala volume (Marsh et al., 2014). One interpretation is thus that larger amygdala volume can reflect greater attunement to the suffering of others, however, research is needed to directly test this hypothesis, which rests entirely on reverse inference.

In addition to the limitations of interpretations based on reverse inference, the current set of studies has a number of limitations, particularly related to the cross-sectional arms examining the impact of long-term meditation practice. While LTM did not differ from non-meditators in terms of age, gender, level of education or socio-economic status, there may have been other differences in the samples, and they were recruited in different ways (since LTM were specifically recruited from meditation communities). We also lacked baseline data for LTM, so it is unknown whether, e.g., their pre-training amygdala GMV was similar to or different than non-meditators. It’s possible that the lack of group differences in amygdala GMV, and presence of associations with lifetime practice hours, was due to LTM starting off with smaller amygdala GMV. A lack of differences between LTM and MNP could thus reflect the fact that LTM devoted thousands of hours practicing meditation to relieve suffering, which has brought them up to the “baseline” of non-meditators. However, one limitation of this dissertation is that we lack the ability to test this hypothesis. There was also likely higher variability in the meditation training received by LTM than the manualized training delivered in MBSR, and thus the long-term mindfulness meditation training of LTM in this dissertation does not reflect a simple extension of the short-term training in MBSR. Future research should aim to utilize a longitudinal design, following practitioners as they progress from initial, to intermediate and more advanced practice, which will provide insight into the trajectory and mechanisms of change with mindfulness meditation across stages of training.

One finding that was consistent across each chapter of this dissertation was the presence of a relationship between meditation practice time and neural changes. Individual differences in MBSR practice time were associated with increased RSFC between PCC and DLPFC, and reduced amygdala GMV. LTM lifetime hours of practice while on meditation retreat were associated with reduced amygdala reactivity to negative pictures, and increased hippocampus GMV, while LTM lifetime hours of home practice were associated with increased amygdala GMV. These results highlight the importance of continued practice, as well as the potential differential impact of practice in different contexts, at different stages of training. While not necessarily surprising – effective health interventions often entail sustained change or practice, such as the need for continued exercise and a healthy diet to maintain physical health – research and media coverage on the effects of mindfulness meditation should consider and emphasize the role of engagement with practice. Future research on mindfulness meditation should examine factors that contribute to adopting mindfulness meditation practice as a lifestyle change, and ways to facilitate long-term mindfulness meditation practice, such as: tailoring practices for incorporation into activities of daily living, availability of ongoing training exercises, and greater accessibility of training support through mobile platforms and a meditation community.

This dissertation rigorously tested the impact of mindfulness meditation training on the neural correlates of emotional nonreactivity, resting brain connectivity in networks associated with meta-awareness and mind-wandering, and structural neuroplasticity. Chapters 1 and 2 reported evidence in support of the hypothesized changes whereby mindfulness meditation training strengthens neural networks associated with emotion regulation in response to affective provocation while reducing reactivity, and increased connectivity between brain regions associated with self-related thought and executive control. Results of Chapter 3 failed to support the hypothesis of structural neuroplasticity associated with mindfulness meditation training using well-matched control groups and large sample sizes. However, individual differences in practice time were associated with changes in amygdala and hippocampus gray matter volume that suggest structural neuroplasticity is possible with sufficient time practicing (beyond the formal instruction period). MBSR, in particular, is widely used and efficacious for treating numerous mental health conditions (Goldberg et al., 2018), and there is growing evidence of its impact on behaviorally-relevant neural changes, as described in this dissertation and elsewhere (Creswell et al., 2016; Fox et al., 2016; Kral et al., 2019, 2018; Wielgosz et al., 2016). The future of mindfulness meditation research should aim to determine factors that predict individual differences in its efficacy, adoption and sustained practice.

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