Resting State Neural Correlates of Mindfulness: An fMRI Study

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Abstract

Since the development of magnetic resonance imaging (MRI), there have been many novel advances in our understanding of brain structure and function. More recently, functional MRI has revealed networks of spatially isolated brain regions with temporally correlated activity, forming resting state networks. Research has long shown that mindfulness can produce psychological improvements. A new wave of research is demonstrating how mindfulness is associated with alterations in these brain networks. The current thesis examined changes in patterns of functional connectivity associated with scores from a commonly used mindfulness questionnaire in three resting state networks: the default mode network, the central executive network, and the salience network. Independent component analysis data from 32 healthy participants revealed that mindfulness is associated with altered patterns of functional connectivity in all three networks. For example, decreased connectivity was observed in the precuneus in two of the networks, a region associated with mind wandering. This suggests that mindfulness has a physiological influence on the resting state functional connectivity of the brain that coincides with the underlying principles of mindfulness.
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Contribution of Authors on Manuscript

Drs. Jennifer Kornelsen and Stephen D. Smith acquired the data for this project and aided in the revision of the submitted document.

Elena Bilevicius entered, preprocessed, and analyzed the data, wrote the manuscript, created tables and figures, and prepared the manuscript for journal submission in the journal *Mindfulness*. 
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List of Abbreviations

fMRI: functional magnetic resonance imaging
MRI: Magnetic resonance imaging
MR: Magnetic resonance
RF: Radio frequency
TR: Repetition Time
TE: Echo Time
MPRAGE: Magnetization prepared rapid acquisition gradient echo
EPI: Echo planar image
BOLD: Blood oxygen level dependent
3D: Three-dimensional
ICA: Independent component analysis
ROI: Region of interest
IC: Independent component
Sog-ICA: Self-organizing grouped independent component analysis
BV: BrainVoyager
DMN: Default mode network
PCC: Posterior cingulate cortex
mPFC: Medial prefrontal cortex
ADHD: Attention deficit hyperactivity disorder
ACC: Anterior cingulate cortex
MFG: Medial frontal gyrus
CEN: Central executive network
dlPFC: Dorsolateral prefrontal cortex
SN: Salience network
MBSR: Mindfulness-based stress reduction
MAAS: Mindful Attention and Awareness Scale
GM: Gray matter
VBM: Voxel-based morphometry
FMI: Freiburg Mindfulness Inventory
FFMQ: Five Facet Mindfulness Inventory
ANCOVA: Analysis of covariance
OFG: Orbitofrontal gyrus
IFG: Inferior frontal gyrus
VOIs: Volumes of interest
BA: Brodmann area
rCEN: Right Central Executive Network
lCEN: Left Central Executive Network
STG: Superior temporal gyrus
Mindfulness is a state of being that has been positively associated with well-being. Substantial literature exists to support the psychological changes related to mindfulness; however, the anatomical and physiological alterations that accompany mindfulness have only recently been investigated. The purpose of this thesis is to contribute to the literature of the resting state neural correlates of mindfulness. To accomplish this goal, we used resting state functional magnetic resonance imaging (fMRI) scans to assess the changes in functional connectivity patterns associated with trait mindfulness, as measured by a commonly used questionnaire. In order to understand this contribution to the literature, a review of basic magnetic resonance imaging (MRI), fMRI, resting state fMRI, and mindfulness will precede a manuscript, which will then be followed by a brief discussion to conclude the thesis.

Chapter I: Literature Review

Magnetic Resonance Imaging

A number of resources exist to address the principles of magnetic resonance imaging (MRI; e.g., Huettel, 2004; Scilid, 1990). I focused on these two references to develop my understanding of the topic and presentation of this information.

MRI is a non-invasive imaging technique that uses information about atoms, such as hydrogen, and their magnetic properties to then graphically arrange them into images (Lauterbur, 1989). MRI typically relies on hydrogen atoms that contain a single proton and no neutrons; and because these protons have mass, a positive charge and nuclear spin, each one has angular momentum and a small electromagnetic field. When placed in an external magnetic field, the
protons precess, which can be thought of as a spinning top wobbling around its axis, and will align either parallel or anti-parallel to the main magnetic field. When protons precess parallel to the magnetic field, it is considered a low-energy state. As this parallel state requires less energy, it is intrinsically more stable and therefore, more protons precess in this direction. It is the greater proportion of parallel to anti-parallel states that creates a net longitudinal magnetization. The “magnetization” in MRI refers to the net magnetic moment of the longitudinal and transverse vectors. The static magnetic field in an MR scanner is referred to as $B_0$ (in line with the z-axis). The longitudinal vector is parallel to $B_0$, whereas the transverse vector is perpendicular to $B_0$. In normal conditions (i.e., not in a strong magnet), the transverse vector is cancelled out, as protons precess in different phase. In a strong magnetic field, there is a strong longitudinal vector and no transverse vector. This is important because both the longitudinal and transverse vectors and their relationship to each other are important for image acquisition.

As previously mentioned, protons precessing out of phase cancel each other out, resulting in no transverse vector. Therefore, the equilibrium state of the protons inhibits the ability to produce a detectable MR signal (Pykett, 1982). In order to produce a signal, the equilibrium of protons must be disrupted. Energy, in the form of radio frequency (RF) waves, is required to disrupt the equilibrium. Protons parallel to the magnetic field absorb the energy from the RF pulse and flip to the higher energy, anti-parallel state and are now in phase in the transverse plane. This results in the loss of the longitudinal vector and creation of the transverse vector. The energy needed to induce this transition is at the same frequency as the precessing protons, at the resonant frequency, creating resonance. This resonance frequency in proportional to $B_0$ and this is defined by the Larmor Equation:
\[ \omega_0 = \gamma B_0 \]

where \( \omega_0 \) is the resonant frequency and \( \gamma \) is a gyromagnetic constant. This converts the longitudinal magnetization into transverse magnetization, comprising the excitation portion of MRI. The RF pulse “whips” the protons into phase, resulting in protons precessing at the same frequency and in the same phase.

Once the RF pulse has been removed, excitation stops and image acquisition can begin. During this time, two things happen: the spins that were transformed into phase and in the antiparallel position become out of phase and return back to their stable, parallel position. There are two changes in magnetization that are occurring: (1) there is an increase in the longitudinal magnetization, or T1 relaxation, and (2) there is a decrease in the transverse magnetization due to the dephasing of spins, or T2 relaxation. Relaxation times vary depending on the specific tissue you are examining. However, regardless of the tissue type, relaxation ensures the system returns to its previous equilibrium state (Pykett, 1982).

In MRI research, the experimenter has the ability to manipulate the time between RF pulses, or repetition time (TR) and the time between the end of the RF pulse and the acquisition of the signal, or echo time (TE) in different pulse sequences. As a result, differently weighted images can be obtained (e.g., having a short TR will mean the spins do not have enough time to decay back to their original position before the next RF pulse). Differently weighted images serve different purposes. A T1-weighted image is dependent on how far the spins were tipped following the RF pulse and the length of TR. When the TR is short, a high resolution anatomical image can be acquired because the contrast is high as not all of the longitudinal magnetization has been restored. Commonly, a magnetization-prepared rapid acquisition gradient echo
(MPRAGE) sequence is used to obtain a T1-weighted image and serves as the high resolution anatomical image during data analysis. A T2-weighted image, in contrast, is dependent on TE. Once the RF is removed, spins become out of phase and as a result, the transverse magnetic moments cancel each other out. Therefore, there is more wait time in T2-weighted images compared to T1-weighted images to allow the spins time to dephase.

In order to localize where a specific signal originates in the three dimensions, spatial encoding is used. There are three gradients that can be applied intentionally at the discretion of the experimenter to isolate a particular signal. In order to excite a particular slab of tissue in the brain, a magnetic field gradient can be applied in a particular direction, and the RF pulse bandwidth can be tailored to match the precession frequency of the targeted area, thereby exciting only the nuclei within the slab of tissue. This is termed slice selection.

There are two types of within slice encoding that can be used to further spatially isolate spins. Frequency encoding applies an additional gradient during reception to cause spins in different locations to precess at different frequencies (Mansfield & Maudsley, 1977). This gradient is applied along one of the other axes (orthogonal to the slice-select gradient) that results in protons that are precessing at different frequencies within a slice. Phase encoding is similar in that it is performed by applying an additional gradient, but it occurs just prior to signal reception and is applied along a third axis that is orthogonal to both the slice-select and frequency-encoding gradients (Mansfield & Maudsley, 1977). This allows spatially dependent phases to be recorded.

*Functional Magnetic Resonance Imaging*
Functional magnetic resonance imaging (fMRI) is a newer imaging technique that can provide information about the neuronal function of the brain. It is a non-invasive, indirect measure that can provide both spatial and temporal information, something that is not possible in structural MRI discussed above. In order to obtain temporal data, a large number of MR images of an area are collected in rapid succession over a period of time. This allows the experimenter to differentiate between and isolate periods of time when a participant was completing a task, in different conditions of a task, or at rest. As previously mentioned, fMRI is an indirect measure of neural activity, based on changes in local blood flow and levels of blood oxygenation (Huettel, 2004).

There are different fMRI contrast mechanisms that are sensitive to particular aspects of cerebral blood volume, cerebral blood flow, and/or the cerebral metabolic rate of oxygen. However, for the purpose of this thesis, I will only be discussing blood oxygenation-level dependent (BOLD) fMRI contrast. According to the BOLD theory, the indirect measurement of fMRI is based on changes in blood flow and blood oxygenation (Ogawa, Lee, Kay, & Tank, 1990). When a neuron is active or fires, it requires more energy than it would if it were at rest in order to carry out its activity. In order to accommodate this neural activity, a hemodynamic change is elicited, resulting in an influx of blood to the local neural region or what is referred to as functional hyperemia (Huettel, 2004). This massive inflow of new blood results in a decreased ratio of deoxygenated hemoglobin to oxygenated hemoglobin (i.e., there is now more oxygenated hemoglobin compared to the deoxygenated hemoglobin at the location of the active neuron compared to its immediate surroundings). Although this area of the brain is active and more oxygen is available, not all of the oxygen is taken up by the cells, resulting in left-over oxygenated hemoglobin (Huettel, 2004). This is important because the MR properties of
oxygenated and deoxygenated hemoglobin are quite different; oxygenated hemoglobin is diamagnetic (having a negative and relatively low magnetic susceptibility), whereas deoxygenated hemoglobin is paramagnetic (having positive magnetic susceptibility). The paramagnetic properties associated with deoxygenated hemoglobin distort an MR image and causes the signal itself to affect the relaxation time and decay more rapidly (Pauling, 1936). It is this ratio of oxygenated to deoxygenated hemoglobin that ultimately affects the MR signal we detect in BOLD images.

**Functional Magnetic Resonance Imaging Preprocessing**

When data is acquired from an MR scanner, it is in a raw format. Before any analyses can be completed, the data must be preprocessed. There are a variety of functional preprocessing steps that can be used; however, the key is to be consistent within a dataset. Preprocessing steps exist to correct or adjust for certain factors that may have been present during the scan and/or differences between subjects. Slice scan time correction is used to adjust the order of the slices that are collected as the entire 3-dimensional (3D) brain is not collected at the same time. It is a form of temporal interpolation, as it uses nearby data to estimate a value that was not originally collected. Because the effects of the RF pulse can bleed into the next section, resulting in large noise for that slice, slices are often acquired in an interleaved manner (i.e., acquiring all the odd slices followed by all the even slices). Slice scan correction reorganizes the slices into the correct anatomical sequence and interpolates the values as though they were acquired simultaneously. A major step involved in fMRI study preprocessing is motion correction. Because the aim of an fMRI scan is to provide temporal and spatial information about the brain, it is essential that there is no major movement in any of the possible six rotations or translations (i.e., rotation in the x, y,
or z axis and translation in the x, y, or z axis). Every analysis software has its own motion correction technique, but motion is a major consideration involved in study design and analyses. Motion correction adjusts each volume across time so that all anatomy is lined up as though the person did not move during the entire scan. This is because motion correction corrects for physical movement of the person during the six to 10 minutes they were in the fMRI scan (not to be confused with motion artefacts, which can be from internal movement like cerebrospinal fluid, cardiac, etc.). Temporal filtering is another common preprocessing step that can filter out certain frequencies to reduce the noise in an analysis. For example, a resting state fMRI scan (which will be covered in the section to come) is characterized by low-frequency oscillations. Therefore, you want to filter out high-frequency oscillations (e.g., cardiac rhythms) that could potentially mask the low-frequency oscillations of interest. In this example, this would involve applying a low-pass temporal filter, as the low-frequency oscillations are able to pass through. A similar step can be taken if you are interested in high-frequency oscillations; instead of a low-pass filter, a high-pass filter would be used to eliminate any extremely low-frequency oscillations, such as scanner drift. A band-pass filter, however, is commonly used in resting state preprocessing, which combines both a low-pass and a high-pass filter. A common technique for temporal filtering is to implement a general linear model-Fourier basis set with 2 sine/cosine cycles. Spatial smoothing is another preprocessing step that is common in fMRI analyses where the purpose is to average or smooth a voxel with its neighbors. Smoothing is important because the normalization, which will be discussed momentarily, is not perfect. Smoothing also improves the signal to noise ratio which in turn can enhance the detection rate of a signal (Huettel, 2004). The amount of smoothing can be left up to the experimenter; however, the normal range of
smoothing is 6 to 10 mm full-width half-maximum (Mikl et al., 2008; Worsley, Marrett, Neelin, & Evans, 1996).

Once the preprocessing steps have been completed, it is important to co-register the functional data that was just preprocessed with a 3D high resolution anatomical image. As previously mentioned, functional data does not have high spatial resolution. This is because there must be a balance between temporal and spatial resolution. Because the temporal data is rich in data and unique to fMRI, the actual spatial resolution of the images is sacrificed. To demonstrate, the spatial resolution for functional images is commonly 3 mm x 3 mm x 3 mm, whereas anatomical images are 1 mm x 1 mm x 1 mm. Therefore, in order to better visualize the results, the functional data can be co-registered to a spatially clear anatomical image. Once this co-registration is complete, data can be normalized into a standard space. This ensures all of the data is in the same stereotactic space and comparisons can be made between subjects and grouped analyses can be completed (Huettel, 2004). There are multiple accepted normalization spaces, including Talairach space and Montreal Neurological Institute. The use of normalization allows individual studies to produce common coordinates, allowing for easily comparable data. Although both spaces are valid, I will be using and discussing Talairach space.

*Resting State Functional Magnetic Resonance Imaging*

Functional MRI, up until this point, has been discussed as the ability to use information about neuronal activity as induced by a task to provide information about the brain. Research on resting metabolism has been conducted to reveal that information about neuronal activity can also be obtained while an individual is at rest, or what is referred to as resting state fMRI (P. T. Fox & Raichle, 1986). Specifically, in the seminal study, Fox and Raichle (1986) predicted
greater coupling between blood flow and the metabolic rate of oxygen would exist during a motor task compared to a baseline, rest condition. However, contrary to their hypothesis, they found that there was little difference in the relationship between blood flow and oxygen consumption in the motor task compared to the rest condition. This suggests the brain was never fully at “rest” even during a so-called rest condition. This was the first study to demonstrate that, even at rest, the brain is still active and is similar when performing a particular motor task (P. T. Fox & Raichle, 1986).

More recently, it has been discovered that we can use and rely on the same principle of neuronal activity to obtain data about neuronal connectivity in the brain: functional connectivity. Functional connectivity is defined as areas in the brain with activity that is correlated over time, even if they are not spatially connected. Resting state functional connectivity data is most commonly collected during a resting state fMRI scan. During such a scan, an individual simply lies in the scanner, with their eyes open or closed, depending on the study, and does not perform any particular task. A gradient echo, echo planar imaging (GE-EPI) sequence is commonly used to obtain T2*-weighted functional images that are sensitive to BOLD changes. The resulting data will represent areas of the brain that are functionally connected while the participant is at rest. Functional connectivity data can also be pulled out from task-based functional studies, instead of solely relying on the resting state or baseline conditions. With either method, appropriate functional connectivity data can be acquired.

In resting state literature, patterns of similar neural activity in spatially isolated brain regions have been identified. These regions have temporally coherent neurophysiological activity as assessed by BOLD time points during a restful state and form what are referred to as
functional networks, or resting state networks. Resting state networks are comprised of regions of the brain that activate together over time with oscillations that are characterized as being low-frequency (~0.01 Hz). This similarity in frequency is important because differing types of signal have differing oscillation frequencies (De Martino et al., 2007), which will become clear upon the discussion of independent component analysis (ICA).

There are two different analysis techniques for resting state analyses. ICA is a whole brain, data-driven approach that reveals any significant functional connectivity relationship that exists. However, a region-of-interest (ROI) analysis, which is hypothesis-driven, is also quite common. In a ROI analysis, a predetermined region hypothesized to have some significant pattern of functional connectivity can be identified a priori. This can yield a more significant result as it is not a whole-brain approach and thus does not require as many participants to obtain appropriate power, but it potentially misses significant results that may not have been hypothesized. An ICA is useful for exploratory research with no a priori hypotheses (Huettel, 2004). Clearly, there is a trade-off between the two, but for the purposes of this thesis, an ICA was used due to the data-driven nature and the number of participants in the study.

An ICA is an analysis technique that can identify spatially separate sets of voxels whose activity varies together over time and is maximally distinguishable from that of other sets. Upon further investigation of this correlated activity, regions of the brain that were spatially isolated from one another appeared to have similar patterns of oscillations as one another. The time series activity extracted from the spatially separate areas, or independent components (IC) that are created define the functional connectivity. In order to create ICs, certain information is extracted from the data. In BrainVoyager software (Brain Innovation BV, Maastricht, The Netherlands),
fingerprints are graphical summaries of the information that creates an individual IC, with respect to skewness, kurtosis, spatial entropy, temporal entropy, degree of clustering, one lag auto correlation, and power band width (see Figure 1; figure recreated based on Fig.2 in De Martino et al., 2007). Skewness and kurtosis provide information about the normality of the distribution of voxels, which is typically quite low in a BOLD signal. Spatial entropy is the information about spatial distribution or, in other words, the distribution of values, whereas temporal entropy refers to the information of the time course of a component (i.e., is the signal random, like a BOLD signal or is there a structured time pattern to the signal). Degree of clustering is a ratio that represents the closeness of active voxels. Auto correlation refers to how smooth the timing of a signal is. Finally, the power band width provides information about the frequency of the signal oscillations, all of which can help determine the source of the obtained signal. This information is obtained from the histogram of voxel values, the spatial distribution of the brain, the time-course information, and the power spectrum. According to this fingerprint theory, there are six potential sources of signal, all of which will be differentially represented. If the source of the signal for a certain IC is BOLD, there are certain characteristics that will be evident in the fingerprint: There will be a high degree of clustering, low skewness and kurtosis, high spatial and temporal entropy, high autocorrelation, and frequency oscillations in the 0.05-0.10 Hz range. The benefit of this visual representation of the IC is to allow the researcher to observe the origin of its signal and determine if the signal they observe in an IC is likely from a BOLD signal (De Martino et al., 2007).
Figure 1: Graphical representation of fingerprint.

*Note.* Graphical representation of fingerprint classification in an ICA. Figure recreated based on Fig.2 of De Martino et al., 2007

Both individual level and grouped analysis can be conducted with an ICA approach. At the individual level, the ICA will produce a user-specified number of IC maps of activation within the brain for each subject. This is a major advantage to an ICA because it allows the researcher to dictate the decomposition of data. After this individual level analysis, it is possible to perform a grouped analysis, or what is referred to as a self-organizing group-level ICA (Sog-ICA; Brain Voyager (BV), Maastricht, The Netherlands). In a Sog-ICA, the most similar IC from each individual will be grouped together to create a final set of ICs, which provides the ability to perform group-level analyses.

Another advantage to the ICs that are created in an ICA is the ability to visually inspect what are known as resting state networks. As eluded to earlier, there are spatially isolated areas of the brain that have similar time series patterns; brain regions that are functionally connected.
The first report of resting state fMRI was by Biswal, Yetkin, Haughton, and Hyde (1995). Since this initial discovery of resting state and functional connectivity, there appear to be regions in the brain that consistently demonstrate similar temporal oscillations and form networks. This seminal study by Biswal et al. (1995) found that during a resting condition, BOLD fluctuations in the left sensorimotor cortex were highly correlated with BOLD fluctuations in the contralateral sensorimotor cortex, the supplementary motor area, and the right premotor area (Biswal, Yetkin, Haughton, & Hyde, 1995). These spatially separate regions with similar BOLD signals form what is known as the sensorimotor network, one of the brain resting state networks. In addition to the sensorimotor network, there are many other resting state networks that can be identified. Each network is comprised of key brain regions that distinguish it as a network and these networks can be identified in the majority of individuals. For example, the default mode network (DMN) is one of the most studied resting state networks (Greicius, Krasnow, Reiss, & Menon, 2003; M. E. Raichle et al., 2001; Rosazza & Minati, 2011). This particular network is robust in its activations, making it detectable in most participants. In an ICA, this is advantageous because the DMN is easily identified as an IC at both the individual and group level.
The functional relationship between spatially separate areas of the brain that underlies resting state networks is quite fascinating. These networks appear to be quite consistent across studies, despite differences in data acquisition and analysis techniques (Moussa, Steen, Laurienti, & Hayasaka, 2012). The DMN, as briefly mentioned above, is a common resting state network that can be revealed during periods of rest and is comprised of precuneus, posterior cingulate cortex (PCC), and medial prefrontal cortex (mPFC) activation (see Figure 2; Greicius et al., 2003; M. E. Raichle et al., 2001). The DMN is referred to as a “task-negative” network because it is activated when an individual is not actively involved in a particular task or focused on anything specifically and is relatively deactivated when a demanding task is involved (Greicius et al., 2003). The DMN is the only task-negative network and is anti-correlated with task-positive networks (M. D. Fox et al., 2005). Although activity in this network is common, significant alterations in DMN activity has been linked to unhappiness, hyperactivity, and even psychopathology (Broyd et al., 2009). The DMN is commonly associated with mind wandering (Christoff, Gordon, Smallwood, Smith, & Schooler, 2009; Mason et al., 2007), a mental process in which the mind is not focused on a particular event, but attention leaps from recent to future.
events (Buckner, Andrews-Hanna, & Schacter, 2008). Mind wandering is a common human experience, yet it is associated with lower levels of happiness (Killingsworth & Gilbert, 2010). Research by Brewer and colleagues (2011) found that experienced meditators, who involve focused attention and concentration in their practice had a relatively deactivated DMN compared to healthy controls and that the differences in DMN functional connectivity were consistent with less reports of mind wandering (Brewer et al., 2011). Alterations in DMN functional connectivity have also been linked to hyperactivity, most commonly reported in Attention Deficit Hyperactivity Disorder (ADHD). Attentional lapses are a common symptom of ADHD that have been linked to the inability to suppress the DMN (Weissman, Roberts, Visscher, & Woldorff, 2006). Castellanos and colleagues (2008) investigated this relationship further using BOLD in a resting state fMRI scan and found attenuated anti-correlations between key nodes of the DMN (the precuneus/PCC and mPFC) and weakened anti-correlation between the posterior components of the DMN and the anterior cingulate cortex (ACC), important for the maintenance of attention (Castellanos et al., 2008). This particular finding is interesting as it also introduces the idea that functional connectivity between parts of a network can change or differ between individuals. Finally, psychopathology has also been linked to DMN activity and functional connectivity. Hyperactivity in the precuneus and medial frontal gyrus (MFG) was correlated with positive symptom severity in schizophrenia (Garrity et al., 2007). Zhou and colleagues (2007) argued that this hyperactivity observed in the DMN could contribute to the over-mentalizing and deficit in attentional control that is seen in schizophrenia (Zhou et al., 2007). Studies have also shown aberrant activity and functional connectivity of the DMN in depressed and anxious populations (Broyd et al., 2009). Clearly, the DMN is a widely studied network and has a
proposed function in a variety of modalities, including emotional processing and regulation, cognition and memory, and attention.

In comparison to the DMN, other resting state networks are understudied. The central executive network (CEN) is a task-positive network that is proposed to play a role when performing cognitive tasks and is associated with the control of processing information (Bressler & Menon, 2010; Sridharan, Levitin, & Menon, 2008). The CEN is anti-correlated with task-negative networks; therefore, when the CEN is activated, the DMN is generally deactivated (Sridharan et al., 2008). The most important nodes in the CEN are the bilateral dorsolateral prefrontal cortices (dPFC; see Figure 3; Bressler & Menon, 2010), but parietal regions related to attention (Shomstein, 2012) and dual tasking (Hartley, Jonides, & Sylvester, 2011) are also involved. Because of its importance to the attentional system, the CEN contributes to many higher-order cognitive functions such as working memory, planning, and decision making (Koechlin & Summerfield, 2007; Menon, 2011; Miller & Cohen, 2001). There has been some work looking at aberrant activity within the CEN as it relates to psychiatric conditions such as schizophrenia (Meda et al., 2012; Seidman et al., 2006; Woodward, Rogers, & Heckers, 2011); however, the results have been mixed. What is consistent within these studies is the function of the CEN and its relation to cognitive tasks.
The salience network (SN) is another task-positive resting state network with the insula and the ACC as the key brain structures (see Figure 4; Bressler & Menon, 2010; Menon, 2015b). The SN plays a role in the allocation of attention and integration of information (Menon, 2015b). The SN is usually activated in concert with the CEN (Menon & Uddin, 2010) and it has recently been proposed that the SN acts as a “switch” between the activation of the DMN and CEN (Menon, 2015b; Sridharan et al., 2008). Specifically, the anterior insula has been described as the hub of the SN and works to integrate sensory, cognitive, and emotional information (Menon & Uddin, 2010). Although limited work has examined the effects of abnormal SN activity or functional connectivity, some studies have revealed implications associated with hyperactivity in the anterior insula. Heightened right insular activity was found to be associated with increased auditory verbal hallucinations in schizophrenia (Sommer et al., 2008). In contrast, hypoactivity of the right insula was associated with negative social processing in a group of individuals with autism spectrum disorders (Di Martino et al., 2009). With the support of the literature that has been reviewed, it is abundantly clear how the DMN, CEN, and SN are associated with every day processes and functioning and that there is some interaction between these networks.
Mindfulness

Humans are structured to be very vigilant, yet many individuals go through life without being completely aware of their day-to-day experiences (Paulson, Davidson, Jha, & Kabat-Zinn, 2013). Mindfulness is defined as purposeful attention to moment-to-moment experiences with an accepting and nonjudgmental stance (Brown & Ryan, 2003; Kabat-Zinn, 1994a, 2003a). The definition of mindfulness has two key components; (1) attending to the present moment-to-moment experience and (2) adopting a nonjudgmental stance about your experience (Kabat-Zinn, 1994a). Attending to the present moment allows an individual to slow down and better understand and appreciate their current experiences, instead of living life on auto-pilot or worrying about the future (Kabat-Zinn, 1994a). Judgment is pervasive in everyday life and can become dominating and harmful. For example, if when trying to focus on the breath, such as in a Mindfulness Based Stress Reduction (MBSR; which will be described below) course, you find yourself saying, “I am not good at this,” or “When will this be over,” judgment is present. Mindfulness does not remove the presence of judgment in our lives, but it allows one to
recognize judgment when it is present and take a more impartial stance towards the particular issue (Kabat-Zinn, 1994a). Engaging in mindfulness does not mean absolute adherence to these two components. The importance of mindfulness is to commit to the practice and understand the underlying principles and practically apply this to the real world, whatever that may mean to any particular individual.

Although mindfulness is defined as a state of being, it can be trained through various mindfulness-based interventions or measured with standardized questionnaires. MBSR is one of the most common empirically-supported mindfulness-based interventions (Kabat-Zinn, 1991). It is predicated on systematic training of mindfulness techniques rooted in Buddhism. Rather than being a passive experience, MBSR is cultivated actively through rigorous training to address physical, psychological, and psychosomatic variables (Kabat-Zinn, 1991). A MBSR program is typically 8 weeks long, with 2.5 hour weekly sessions and homework exercises and practice. This mindfulness approach consists of exercises in order to reduce judgment and enhance awareness to moment-to-moment experiences.

Since its inception in 1979, MBSR has been implemented in various contexts. A recent systematic review that included 115 randomized control trials found that compared to waitlist control conditions, MBSR or other mindfulness-based cognitive therapies improved various health symptoms including depression, anxiety, stress, quality of life, and physical functioning (Gotink et al., 2015). This review included over 8,500 individuals suffering from various conditions, but interestingly repeatedly revealed similar results. For example, in cancer populations, individuals enrolled in a mindfulness-based intervention reported improvements in depressive and anxious symptoms, stress, and quality of life compared to the control groups.
The amount of time spent meditating was associated with improvements in mood and the number of MBSR sessions attended was related to greater reduction of stress, suggesting that the benefits of mindfulness are time-related (Ott, Norris, & Bauer-Wu, 2006). Interestingly, this suggests that the benefits of mindfulness can be time-dependent, with increased benefits stemming from increased mindfulness practice. MBSR has also been implemented in chronic pain populations where chronic headache patients in the experimental group reported improvements in pain intensity, fatigue, and quality of life (Bakhshani, Amirani, Amirifard, & Shahrakipoor, 2016) and chronic back pain patients reported less pain catastrophizing (Turner et al., 2016). Burgeoning research on the effectiveness of MBSR or mindfulness-based interventions for cardiovascular patients exists, with preliminary results demonstrating improvements in depression, anxiety, and stress (Abbott et al., 2014). Beyond physical conditions, mindfulness-based interventions have also been successful in treating psychiatric and mental disorders. Conditions such as depression (Piet & Hougaard, 2011), anxiety (Chen et al., 2012), and schizophrenia (Davis & Kurzban, 2012) reveal similar reductions in depression and anxiety scores and a lessened presence of distressing thoughts as compared to their respective control conditions. This is of interest because it illuminates the breadth of MBSR and other mindfulness-based interventions and the success of such interventions on various aspects of human life.

It has become clear that mindfulness can be trained through various mindfulness-based interventions. However, mindfulness can also be assessed as an innate quality or human tendency. Trait mindfulness is said to reflect an individual’s natural mindfulness tendency, which can be viewed as a stable personality-like characteristic or trait (Brown & Ryan, 2003). Trait mindfulness still encompasses the core components such as attending to the current experience
and having focused attention (Baer, Smith, & Allen, 2004) and having acceptance to the present moment (Brown & Ryan, 2003), it just is assessed as an enduring dispositional trait. There are numerous standardized questionnaires that exist that assess trait mindfulness. The Mindful Attention and Awareness Scale (MAAS; Brown & Ryan, 2003) is a 15-item unidimensional instrument that assesses an individual’s attention to or awareness of experiences across various domains (i.e., cognitive, emotional, physical, etc.) in daily life. Individuals rank their responses on a 6-point Likert scale from 1 (almost always) to 6 (almost never) where higher total scores reflect a higher degree of mindfulness. The MAAS is frequently utilized in research to assess trait mindfulness and it has demonstrated good internal consistency and test re-test reliability (Brown & Ryan, 2003). The Kentucky Inventory of Mindfulness Skills (Baer et al., 2004) was developed in 2004 and divided mindfulness into four factors: (1) observing, (2) describing, (3) act with awareness, and (4) accept without judgment. This was revised in 2006 and was renamed the Five Facet Mindfulness Questionnaire (Baer, Smith, Hopkins, Krietemeyer, & Toney, 2006) to include five facets of mindfulness: (1) non-reactivity, (2) observe, (3) act with awareness, (4) describe, and (5) nonjudgment. This 39-item self-report mindfulness instrument has acceptance reliability and internal consistency for all five factors (Christopher, Neuser, Michael, & Baitmangalkar, 2012) and has also been implemented in research contexts to assess mindfulness and mindfulness qualities in daily life.

Since its growing popularity, mindfulness has been assessed in a variety of contexts to reveal its relationship with psychological improvements. A Swedish population-based sample examined the relationship between trait mindfulness and psychological functioning in a healthy community sample (Branstrom, Duncan, & Moskowitz, 2011). It was found that mindfulness was highly related to general well-being and perceived health and that a higher score of
mindfulness may actually moderate the effect of stress on overall well-being (Branstrom et al., 2011). These results suggest trait mindfulness alone may have a positive influence on psychological functioning. In an attempt to demonstrate the ability to cultivate mindfulness and acquire the associated benefits, Shapiro and colleagues (2008) randomized university students into one of two 8-week mindfulness conditions or a waitlist control group (Shapiro, Oman, Thoresen, Plante, & Flinders, 2008). Following the intervention, both mindfulness groups revealed increases in mindfulness scores, as assessed by the MAAS, compared to the waitlist control group. Relatedly, the observed increases in mindfulness were found to mediate reductions in overall stress and rumination. This paper demonstrates not only the ability to cultivate mindfulness, but that the benefits associated with mindfulness can be attained after a very short period of time. Although not all of the literature has been reviewed, similar patterns of reductions of stress or anxiety and enhancement of general well-being exist in the literature.

Moving from the psychological benefits associated with mindfulness, neuroimaging investigations of mindfulness have looked at the physiological changes that accompany mindfulness. There is a range in the literature about particular outcome measures, such as tracking differences in gray matter (GM) and neural activity. Such variability in neural activity or structure is observed partly because of the measure of mindfulness that is used, including standardized questionnaires, mindfulness-based interventions, or even comparing experienced and novice meditators. One of the first and most highly referenced papers on the relationship between mindfulness meditation experience and cortical thickness is by Lazar et al. (2005). Compared to healthy matched controls, experienced meditators showed thicker PFC and right anterior insula with group-differences in the PFC thickness being most robust in older participants (Lazar et al., 2005). The researchers provided preliminary evidence that the
plasticity of the cortex can be associated with meditation experience. Since the publication of this paper, numerous cortical and sub-cortical investigations have been conducted to further understand the structural correlates of mindfulness. Holzel and colleagues have published papers in this area but instead used voxel-based morphometry (VBM) patterns to examine the changes in GM. In their 2011 publication, a whole-brain GM analysis revealed GM increases in the left hippocampus, PCC, temporo-parietal junction, and cerebellum in participants in the MBSR compared to the control condition (Holzel et al., 2011). More recently, Lu and colleagues (2014) investigated the VBM structural correlates of the MAAS and found greater GM volume in the right hippocampus/amygdala and bilateral ACC but less GM in the bilateral PCC in individuals with high trait mindfulness scores compared to individuals with low trait mindfulness scores (Lu et al., 2014). Interestingly, a contradictory finding about the GM volume in the PCC has been revealed (Holzel et al., 2011; Lu et al., 2014), yet both authors discussed their respective findings in terms of enhanced involvement of self-referential thought. Perhaps the discordant findings are the result of the differing measure of mindfulness used in the study (e.g., MBSR participants versus a measure of trait mindfulness); however, it is not possible to draw a conclusion here. In just reviewing three papers, there appears to be consistency in brain structure as it relates to mindfulness in that more mindfulness or experience is associated with brain regions involved in attention, emotional regulation, and self-referential thought (Holzel et al., 2011; Lazar et al., 2005; Lu et al., 2014).

In addition to structural studies, research has examined a variety of task-based functional changes that are associated with mindfulness. As previously mentioned, mindfulness and mindfulness-based interventions have commonly been implemented as treatment strategies for anxious and depressive symptomology. In line with this belief, Taylor and colleagues (2011)
sought to understand the neural mechanisms that tie emotional responses and mindfulness together (Taylor et al., 2011). Compared to novice meditators, experienced meditators were able to attenuate the perceived emotional stimuli and this was reflected in a deactivation of key nodes of the DMN (mPFC and PCC). In contrast, novice meditators revealed down-regulation of the amygdala during the emotional processing. These data suggest experienced meditation can enhance emotional stability through key components of mindfulness, such as acceptance and presence, rather than influencing affective brain systems, such as the amygdala (Taylor et al., 2011). In an affect labeling task, high MAAS scores (indicating high trait mindfulness) were associated with greater PFC activation and lessened amygdala activity compared to a gender labeling task (Creswell, Way, Eisenberger, & Lieberman, 2007). The negative relationship observed between the PFC and amygdala was only present when scores on the MAAS were high, which suggests that mindfulness may attenuate negative affect by enhancing executive function or regulation (Creswell et al., 2007).

As it has become evident, structural MRI and fMRI are able to detect neurological changes that are associated with mindfulness. Whether it is a structural or a task-based functional study, a wealth of information relating to key cortical and subcortical structures and human functioning can be learned. A relatively new contribution to the mindfulness literature is how it relates to functional connectivity and resting state networks in the brain. Obtaining such information would provide a greater understanding of how mindfulness influences our physiology.

**Resting State Networks and Mindfulness**
Considering resting state functional connectivity analyses is relatively new to the literature, there are a finite number of papers that have been published on mindfulness and resting state network functional connectivity to date. Out of what has been published, the majority of these papers have focused solely on the DMN (Brewer et al., 2011; Jang et al., 2011; Taylor et al., 2013; Wang et al., 2014). Within these publications, differing measures of mindfulness have been used. For example, Jang and colleagues (2011) compared experienced and novice meditators to determine the differences in functional connectivity that would be observed within the DMN. They found that compared to novices, experienced meditators demonstrated increased functional connectivity in the mPFC within the DMN (Jang et al., 2011).

In contrast, Taylor and colleagues (2013) found that in general, novice meditators had increased functional connectivity between key nodes of the DMN, such as the mPFC and PCC (Taylor et al., 2013). These opposing results could be due to the differing ROIs that were used in the analyses, but it demonstrates the lack of consistency in the literature. Instead of looking at the neural correlates of mindfulness in experienced meditators, Wang and colleagues (2014) examined the alterations in DMN associated with the MAAS. In this paper, the researchers used the key nodes of the DMN as seed regions and investigated the functional connectivity between these nodes using graph-theory. Their results revealed that higher scores of trait mindfulness were associated with weakened functional connectivity between the thalamus and the PCC. In a post-hoc analysis, the thalamus appeared to be the significant brain region associated with the decrease in functional connectivity. The authors concluded that the thalamus could be the switch between mindfulness and mind-wandering (Wang et al., 2014).

More recently, a paper was published that looked at the functional connectivity patterns in the DMN, SN, and CEN. Doll and colleagues (2015) not only wanted to examine the
relationship between mindfulness and resting state networks in addition to the DMN, but they also wanted to look at the relationship between these networks. In their study, an ICA was employed to observe the intrinsic functional connectivity with mindfulness scores (A. Doll, Holzel, Boucard, Wohlschläger, & Sorg, 2015). This study combined the use of a brief mindfulness intervention, the MAAS, and the Freiburg Mindfulness Inventory (FMI; Walach, 2006) to assess mindfulness. Results revealed that there was a significant negative correlation between the FMI and inter-intrinsic functional connectivity (i.e., the functional connectivity between resting state networks) between the insula of the SN and the posterior ventral DMN. The authors concluded that perhaps this negative relationship between the DMN and SN with respect to the FMI could represent a less evaluative stance, which falls nicely in line with the acceptance portion of the FMI (Walach, 2006). A similar negative relationship between scores on the MAAS and the intrinsic functional connectivity of the anterior and posterior DMN was observed (A. Doll et al., 2015). Because the MAAS is focused on measuring attention and awareness of the present moment, lower functional connectivity in regions such as the PCC would make sense as this region has been shown to have reduced functional connectivity during focus on the present (Garrison et al., 2013). To further support these results, the researchers performed the same analyses on the visual network to serve as a control network and no significant results were found. This is one of the first papers to examine how mindfulness influences functional connectivity in multiple resting state networks. Data suggest that changes in patterns of functional connectivity in central brain networks are linked to mindfulness, specifically in regards to the nonjudgment and attention to the present moment. As these are still relatively new findings, it is essential for further work to be conducted to either corroborate or contrast what has been presented here.
Chapter II: Rationale and Objectives

Statement of Problem

The purpose of this thesis is to contribute to the understanding of the resting state neural correlates of mindfulness. Because this is a relatively new area of research, there has not been consistency with the methodology that has been used, in terms of both the measure of mindfulness and the actual analyses performed. It is important to either support what has been published to date or provide a new perspective to develop a more comprehensive understanding of the neural correlates of mindfulness.

Rationale and Aims

**Rationale:** It is unknown what the neural correlates of the MAAS are in the DMN, SN, and CEN. Previous fMRI studies have either focused solely on the DMN or used other measures in addition to the MAAS to understand the relationship between mindfulness and functional connectivity. Because the DMN, SN, and CEN all have relevance to the definition of mindfulness, it is important to investigate how patterns of functional connectivity in these networks can be altered depending on an individual’s level of mindfulness.

**Aim:** This study aims to measure how levels of trait mindfulness, as assessed by the MAAS, influence the patterns of functional connectivity within three of the brain’s resting state networks. This work can elucidate if the DMN, SN, and CEN can be identified within the data and determine the neural representation of a well-known and validated questionnaire that is concerned with attention and awareness aspects of mindfulness.
Hypotheses

I hypothesize that mindfulness will alter the patterns of functional connectivity within the DMN, SN, and CEN. Specifically, I predict that high scores on the MAAS will be associated with an overall suppression of key nodes of the DMN, which logically follows the underlying principles of mindfulness (i.e., less DMN activity which represents less mind wandering and more focus on the present moment). I also anticipate high MAAS scores to be associated with enhanced functional connectivity in both the SN and CEN because both have functions associated with attention and awareness.

Chapter III: Manuscript
Resting state network functional connectivity patterns associated with the Mindful Attention and Awareness Scale

Under review at *Mindfulness*
Abstract

Mindfulness refers to attending to moment-to-moment experiences with acceptance and no judgment. Mindfulness can be examined at two different levels of analysis: as a transient moment-to-moment state of being (state mindfulness), or as a more permanent personality characteristic (trait mindfulness). The Mindful Attention and Awareness Scale (MAAS) is particularly sensitive to trait mindfulness; it is proposed to measure the attention component of mindfulness. The purpose of this study was to test this hypothesis by identifying the neural correlates of the MAAS in three resting state networks related to attention—the default mode network (DMN), the salience network (SN), and the central executive network (CEN). Thirty-two healthy university students naïve to mindfulness completed the MAAS and later underwent a resting state functional magnetic resonance imaging scan. Resting state data were analyzed using an independent component analysis; the scores from the MAAS were co-varied to the connectivity maps in an analysis of co-variance. The results indicate the presence of inter-network functioning connectivity. For example, the precuneus had decreased functional connectivity within both the SN and CEN, even though it is a key component of the DMN. Similar cross-network patterns involving a decrease in functional connectivity of the insula within the DMN and of the posterior cingulate cortex in the SN were also observed. These results suggest that the neural correlates of the MAAS are consistent with attention-related alterations in network functional connectivity.

Keywords: trait mindfulness, MAAS, fMRI, resting state, functional connectivity
Introduction

Mindfulness is defined as purposeful attention to moment-to-moment experiences with an accepting and nonjudgmental stance (Brown & Ryan, 2003; Kabat-Zinn, 1994b, 2003b). Mindfulness originates from Eastern traditions grounded in Buddhist meditative practice (Kabat-Zinn, 1994b; Thera, 2014); however, in more recent years, mindfulness has been introduced into Western cultures in a more secular form. Various mindfulness-based programs exist to teach individuals the skills and techniques required to practice mindfulness. Indeed, it has been implemented as a non-invasive training course or treatment for various healthy (Goodman & Schorling, 2012) and patient populations, including anxious (Goldin, Ramel, & Gross, 2009) and depressed populations (Hofmann, Sawyer, Witt, & Oh, 2010), as well as experimentally induced (Bilevicius, Kolesar, & Kornelsen, 2016) and chronic pain (Kabat-Zinn, Lipworth, & Burney, 1985; la Cour & Petersen, 2015; Reiner, Tibi, & Lipsitz, 2013).

Mindfulness can be further classified into two categories: state and trait mindfulness. State mindfulness is a measure of how mindful someone is in the present moment and reflects a more transient, moment-to-moment experience of mindfulness (Tanay & Bernstein, 2013). Trait mindfulness, on the other hand, is defined as an individual’s natural or innate mindfulness tendency, which has been perceived as a stable, permanent characteristic (Brown & Ryan, 2003). The construct of trait mindfulness is multifaceted, including the ability to attend to current experiences and be able to describe them, the ability to have focused and sustained attention (Baer et al., 2004), and the ability to have continuous openness and receptive awareness to these experiences (Brown & Ryan, 2003).

One validated and commonly used measure of trait mindfulness is the Mindful Attention and Awareness Scale (MAAS; Brown & Ryan, 2003). The MAAS is proposed to be a one-
dimensional model that measures the attention component of mindfulness, but not the nonjudgmental component (Brown & Ryan, 2003). It is this ongoing, sustained attention that underlies the enduring quality associated with trait mindfulness, as assessed by the MAAS.

Functional neuroimaging has allowed researchers to delineate the neural substrates of many of the characteristics related to trait mindfulness. Indeed, cortical and subcortical regions of the brain have been linked with attentional, emotional regulation, and sensory integration functions that make up the multifaceted trait of mindfulness (Creswell et al., 2007; Ives-Deliperi, Solms, & Meintjes, 2011). For example, a task-based study by Creswell and colleagues (Creswell et al., 2007) revealed that the MAAS was associated with greater activity in the medial prefrontal cortex (mPFC). They concluded that the negative relationship observed between mPFC and amygdala activation was due to the increased attention involved in their affect-labelling task. This reveals that the MAAS is able to tap into the attentional component of mindfulness and suggests that focused attention is important in trait mindfulness. The processing of emotion has been proposed to be associated with the subcortical anterior cingulate cortex (ACC; van Veen & Carter, 2002). Ives-Deliperi and colleagues (Ives-Deliperi et al., 2011) revealed participants who underwent a mindfulness intervention and scored high on the Five-Facet Mindfulness Questionnaire (FFMQ; Baer et al., 2006) had a reduction in ACC activity. It is important to note that unlike the MAAS, the FFMQ assesses additional components of trait mindfulness such as nonjudgment and non-reaction. The decreased ACC activity was discussed as an indication of reduced emotionality, revealing that FFMQ scores are associated with enhanced emotional control. Finally, sensory awareness and integration is commonly associated with insular activity (Craig, 2009). The same study by Ives-Deliperi and colleagues (Ives-Deliperi et al., 2011) revealed that higher mindfulness, as assessed by the FFMQ, was associated
with depressed insula activity. Ives-Deliperi and colleagues also revealed that the posterior cingulate cortex (PCC) was the only region to be associated with increased activity. The researchers justified this activity because of the focus on the present and attentional awareness that is associated with mindfulness. In other words, if an individual is more mindful, activity in the PCC would be expected to be higher in order to inhibit the retrieval of memories and focus more on the here and now, which is associated with PCC activity (Ryan et al., 2001). The activity found in this study is in line with focused and sustained attention that is associated with mindfulness, allowing an individual to attend to what is relevant in the present moment and respond appropriately, and not emotionally. This is an interesting result found with the FFMQ; however, to our knowledge, the same attention-related physiological changes have yet to be revealed with the MAAS as the measure of trait mindfulness.

Although the highlighted brain activity related to mindfulness appears to be quite dispersed, the same regions have been identified as being key nodes in some of the brain’s resting state networks. Therefore, an examination of resting state networks could provide important insights regarding the neural substrates of mindfulness. Resting state networks are comprised of brain structures with activity that is highly correlated over a period of time when the brain is at rest; what is referred to as functional connectivity. The best-known resting-state network is the default mode network (DMN), a network comprising the mPFC, PCC, and precuneus (Raichle, 2015; Marcus E. Raichle et al., 2001). The DMN is functionally active when an individual is mind wandering, which is essentially the opposite of mindfulness (Beckmann, DeLuca, Devlin, & Smith, 2005; M. D. Fox & Raichle, 2007).

Previous studies have identified the neural correlates of trait mindfulness within the DMN. For example, Wang and colleagues (Wang et al., 2014) used a region-of-interest (ROI)
analysis to identify the role of the DMN in relation to trait mindfulness and found weakened functional connectivity between the thalamus and PCC. In a post-hoc graph-based analysis, they found that all of the graph-based relationships for the significant negative relationship of the DMN and trait mindfulness were predictive for the thalamus, not the PCC. Another study that examined the relationship between the DMN and trait mindfulness in older adults found a positive relationship between the functional connectivity between the dorsal PCC and the precuneus and trait mindfulness (Shaurya Prakash, De Leon, Klatt, Malarkey, & Patterson, 2013). Finally, a study by Kong and colleagues (Kong, Wang, Song, & Liu, 2016) reported that trait mindfulness was positively correlated with the right insula, left parahippocampal gyrus, and left orbitofrontal cortex (OFC) and was negatively correlated with the right inferior frontal gyrus (IFG). These researchers also found that functional connectivity in the OFC and IFG were predictive of well-being; however, both were mediated by trait mindfulness, suggesting that there is a relationship between mindfulness and well-being. These studies reveal a lack of consistency in the literature in terms of the directionality of the relationship between the DMN and trait mindfulness, and underscore the focus of the DMN in resting state trait mindfulness research and the opportunity to examine multiple networks that have been less studied in the literature.

In the current study, we examined the relationship between trait mindfulness and the DMN as well as two other resting state networks, the central executive network (CEN) and the salience network (SN). The CEN is characterized by functional connectivity between groups of neurons in the dorsolateral PFC (dLPFC) and the posterior parietal cortex and is associated with the control of processing of information (Seeley et al., 2007). This function is critical in mindfulness as an individual aims to be present and open, which in turn allows him or her to tune out irrelevant or unimportant information. The SN has been defined by activity within the insula
and ACC (Menon, 2015a) and is important for the integration of sensory information (Seeley et al., 2007). It has recently been proposed to be a “switch” between the activation of the DMN and the CEN (Goulden et al., 2014; Menon, 2015a). In mindfulness, detecting and attending to important, salient information is referred to as purposeful attention, which can be underscored by the function of the SN. Given that the functions of the SN and CEN both have clear relevance to mindfulness, an examination of how the functional connectivity of these networks relates to trait mindfulness will provide novel information about the neural substrates of this personality characteristic. Examining how functional connectivity is influenced by MAAS scores will help corroborate the view that the MAAS is sensitive to the attentional components of mindfulness. Based on previous literature, high MAAS scores would be correlated with the interoceptive portion of mindfulness, which would be reflected as decreased functional connectivity in key nodes of the DMN (Brewer et al., 2011; Josipovic, 2014). Awareness would be anticipated to be correlated with high MAAS scores, which would relate to increased functional connectivity in the two main nodes of the SN, the ACC (Manna et al., 2010) and the insula (Hasenkamp & Barsalou, 2012). This is consistent with the awareness function of this network (Menon, 2015a). Finally, the focused attention component of the MAAS should be associated with enhanced cognitive processing. This would be expected to manifest as increased functional connectivity of frontal regions of the brain, such as the PFC, that make up the CEN (Hasenkamp, Wilson-Mendenhall, Duncan, & Barsalou, 2012), as focused attention is a key component measured by the MAAS and involved with the PFC.

**Methods**

**Participants**
Thirty-two students enrolled in Introductory Psychology at The University of Winnipeg and who were naïve to mindfulness participated in the study (10 males, 22 females; mean age = 18.16; SD = 1.08; age range 17-22 years). The study was approved by the University of Winnipeg Research Ethics Board and the National Research Council Ethics Board, all participants completed Magnetic Resonance Safety screening prior to scanning, and written informed consent was obtained from all participants prior to participation. Participants received a $25 honorarium for their participation.

**Psychological Measures**

During a session in the Fall and prior to the scanning dates, participants completed the MAAS (Brown & Ryan, 2003). By definition, the MAAS is proposed to measure one of the two key components of mindfulness: attention. This 15-item questionnaire includes statements such as “I rush through activities without being really attentive to them” and “I find myself doing things without paying attention.” Participants independently rated items on a 6-point Likert scale from 1 (Almost Always) to 6 (Almost Never). Items on the MAAS are reverse scored; higher scores indicate high trait mindfulness. The MAAS has been demonstrated to have good internal consistency (α = .85) and test re-test reliability (r = .81) (Brown & Ryan, 2003).

**Study Paradigm**

A 3D high resolution anatomical MRI was acquired for all participants after the initial localizer. Following the anatomical scan, a 7-minute resting state scan was run to obtain the functional data. Participants were instructed to lay still with their eyes closed without falling asleep for the entire scanning session. All participants reported to have remained awake during the scanning session.

**Data Acquisition**
Data were collected using a 3T Siemens TRIO MRI scanner (Siemens, Erlangen, Germany). For co-registration of functional data, high resolution T1-weighted gradient-echo images were acquired using an MP-RAGE sequence (1-mm slice thickness, 0 gap, 176 slices, TR/TE = 1900/2.2 ms, in plane resolution .94 × .94 mm, 256 × 256 matrix, field of view [FOV] 240 mm x 240 mm).

The functional MRI component of the study was collected using conventional BOLD imaging techniques. Resting state fMRI data was acquired with a whole brain echo planar imaging (EPI) sequence. This 7-minute scan consisted of 140 volumes and was acquired using the following parameters: 3-mm slice thickness, 0 gap, 42 slices, TR/TE = 3000/30 msec, flip angle = 90°, 64 × 64 matrix, FOV 240 mm x 240 mm.

**Data analysis**

Imaging data preprocessing and statistical analyses were performed with BrainVoyager QX 2.8 software (Brain Innovation BV, Maastricht, The Netherlands). The preprocessing of functional data began with slice scan correction using cubic spline to correct for the interleaved order the data was collected. Preprocessing continued with a trilinear/sync interpolation 3D motion correction. This output provides an estimate for movement in any of the 3 translations and 3 rotations that are then regressed out from the data before further analyses. An absolute value of 2 mm was the threshold for movement in any of the 6 directions and the output confirmed that movement was adequately corrected for. Finally, a general linear model-Fourier transformation temporal filter with 2 sine/cosine cycles and spatial smoothing with an 8 mm full-width half-maximum Gaussian filter were applied. The high resolution anatomical data was manually spatially normalized by warping the data into the standardized Talairach space.
Following the normalization, the anatomical data were co-registered to the corrected, preprocessed functional data.

Single-subject independent component analysis (ICA) was carried out with the fast ICA algorithm (Hyvarinen & Oja, 2000) for all 32 participants to reveal fluctuations in correlated neural activity. During this initial step, 20 independent components (IC) for all 32 subjects were extracted from the data. To perform a grouped analysis, a group-level ICA was completed using the self-organizing group ICA (Sog-ICA; Esposito et al., 2005) plugin. In this step, the most similar ICs for all 32 participants were clustered at the group level, resulting in a final set of 20 ICs. These 20 components were visually inspected to identify the DMN, SN, and CEN and these networks were verified by comparing Talairach coordinates to those reported in previous studies.

To determine the relationship between trait mindfulness and the functional connectivity in the brain, individual scores from the MAAS were entered as co-variates in an analysis of covariance (ANCOVA). MAAS scores were co-varied with the three resting state networks ICs (i.e., DMN, SN, and CEN) individually, resulting in brain maps that represent trait mindfulness contributions to the neural representation of mindfulness, within these networks. The resulting cluster maps were corrected with a Monte Carlo cluster threshold estimator correction plugin at 1000 iterations and evaluated at $p = .05$. Finally, these cluster maps were converted to volumes of interest (VOIs), which provided output including the number of active voxels and the probability value of the observed clusters. This output was then entered into Talairach-Daemon software (http://www.talairach.org/daemon.html) which provided the anatomical name of the peak and center of gravity of the clusters and the Brodmann areas (BA), if applicable.

Results
Psychological Measures

The MAAS was scored according to its standardized method. We recorded each individual score, with higher scores representing higher trait mindfulness. These scores were later used in the ANCOVA (Mean = 3.95; SD = 0.56; range = 2.73-5.07).

Identification of Resting State Networks

The results from the Sog-ICA indicate that the resting state networks were identified in the data before they were co-varied with mindfulness scores (see Table 1). The DMN was identified within a single component, with key nodes such as the posterior cingulate, medial prefrontal, and middle temporal cortices identified in the coordinates for the cluster. The SN was also identified within a single component, including a temporal parahippocampal cluster that included the insula, and the anterior cingulate, both of which are the key nodes of the SN. The CEN, on the other hand, was isolated as two separate components; the right CEN (rCEN) and the left CEN (lCEN), where both hallmark parietal and frontal cortices were identified.

Table 1. Talairach coordinates of resting state networks.

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>Gyrus</th>
<th>BA</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limbic</td>
<td>Left</td>
<td>Cingulate</td>
<td>31</td>
<td>-3</td>
<td>-40</td>
<td>31</td>
<td>45602</td>
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<tr>
<td>Region</td>
<td>Hemisphere</td>
<td>Region</td>
<td>X</td>
<td>Y</td>
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<tr>
<td>Frontal</td>
<td>Left</td>
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Anterior Right Culmen 12 -37 -5 26471

**Posterior** Right Declive 42 -88 -17 383

Right Inferior semi- lunar 30 -70 -41 2138

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a. Peak nearest gray matter coordinates for the resting state networks.

b. Abbreviations: Brodmann area (BA); default mode network (DMN); salience network (SN); right central executive network (rCEN); left central executive network (lCEN).

c. No gray matter found at this coordinate using +/- 5 mm.

d. Brain region label for +/- 5 mm from the listed Talairach coordinate.

**Default Mode Network Functional Connectivity**

Following the identification of each resting state network, scores on the MAAS were co-varied within the four networks’ identified components to examine the contribution of mindfulness. Table 2 outlines the functionally connected brain regions, BAs, Talairach coordinates of the peak voxel within each cluster, cluster sizes, and corresponding $r$ and $p$-values for the four resting state networks co-varied with scores on the MAAS. Once the scores were co-varied for each of the networks’ components, the observed clusters revealed the significant contribution of each network’s key nodes and that of other networks’ nodes (i.e., cross-connectivity). In the DMN, the largest and most significant clusters were found in the bilateral medial frontal gyrus (MFG; BA 32, BA 10), left superior temporal gyrus (STG; BA 38), and the
left insula. The medial frontal and superior temporal gyri make up the key components of the DMN, while the insula is a major component of the SN (see Figure 1), demonstrating the presence of between network functional connectivity, or cross-network connectivity. Increased functional connectivity was observed in the right parahippocampal gyrus (BA 36), followed by the left caudate and right MFG (BA 10) indicating that a higher score on the MAAS was associated with greater functional connectivity in these regions. Weakened functional connectivity was noted in the left MFG (BA 32), and in left insula (BA 13) which demonstrates that higher levels of trait mindfulness resulted in decreased functional connectivity in BA 32 and 13.

![Fig. 1](image)

**Fig. 1** Functional connectivity co-varied with mindfulness scores in the DMN on a Talairach brain using a cluster threshold of 20 voxels (N = 32), as corrected for multiple comparisons, using Monte Carlo simulations at 1000 iterations. Displayed are the brain regions with increased functional connectivity depicted with orange voxels and regions with decreased functional connectivity depicted with blue voxels.
Table 2. Talairach coordinates of trait mindfulness functional connectivity of resting state networks.

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**ICEN**

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\[a.\] Peak nearest gray matter coordinates of resting state networks co-varied with MAAS scores.

\[b.\] Abbreviations: Brodmann area (BA); default mode network (DMN); salience network (SN); right central executive network (rCEN); left central executive network (ICEN).

**Salience Network Functional Connectivity**

In the SN, the right cuneus cluster (BA 18) was the largest and most significant region that was observed to decrease in functional connectivity. This cluster encompasses the precuneus, which is characteristic of the DMN, and was observed to decrease in connectivity.
This result reveals that higher scores on the MAAS resulted in a decrease in functional connectivity in a key component of the DMN, again suggesting the presence of cross-network connectivity. Other areas, such as the left ACC (BA 32), left insula (BA 13) were also highly significant, with increased connectivity associated with higher scores of trait mindfulness observed in both the ACC and the insula.

![Functional Connectivity](image)

**Fig. 2** Functional connectivity co-varied with mindfulness scores in the SN on a Talairach brain using a cluster threshold of 20 voxels (N = 32) as corrected for multiple comparisons, using Monte Carlo simulations at 1000 iterations. Displayed are the brain regions with increased functional connectivity depicted with orange voxels and regions with decreased functional connectivity depicted with blue voxels.

**Central Executive Network Functional Connectivity**

The Sog-ICA identified two separate CEN components, the left CEN and the right CEN. These components were run through separate ANCOVAs. The rCEN had significant clusters in the right middle frontal gyrus (BA 10), left sub-gyral nucleus, and right precentral gyrus. The
right middle frontal gyrus (BA 6), left STG, and the left precuneus were significant clusters found in the lCEN (see Figure 3). Again, the precuneus involvement reveals a pattern of cross-network connectivity observed in the lCEN. Increases in functional connectivity were found in the right middle frontal gyrus in both the right and left CEN, which demonstrates that high trait mindfulness is associated to this increase in functional connectivity. Similar reductions in the left cuneus and precuneus were found in the right and left CEN respectfully. It is interesting to note that key components of the DMN network appear to be functionally connected within both the right and left CEN.

Fig. 3 Functional connectivity co-varied with mindfulness scores in the (A) rCEN and (B) lCEN on a Talairach brain using a cluster threshold of 20 voxels (N = 32) as corrected for multiple comparisons, using Monte Carlo simulations at 1000 iterations. Displayed are the brain regions
with increased functional connectivity depicted with orange voxels and regions with decreased functional connectivity depicted with blue voxels.

**Discussion**

The present study revealed that a higher score on the MAAS, indicating a higher degree of trait, or intrinsic mindfulness, is associated with changes in network functional connectivity. In the DMN, significant decreases of functional connectivity were observed in the left middle and left inferior frontal gyri. The SN revealed significant increases of functional connectivity in the ACC and the left insula. Both the right and left CEN demonstrated increased functional connectivity in the right middle frontal gyrus. These data suggest that the neural components of the MAAS are in line with brain regions that are involved in attention to moment-to-moment experiences, rather than the nonjudgment component of mindfulness.

The DMN is a resting state network that has been associated with activity in brain regions, such as the medial frontal and superior temporal gyri, the PCC and the precuneus (Buckner et al., 2008). In the current study, decreased functional connectivity was observed in key nodes of the DMN. Mind wandering, often defined as the inability to remain focused on a single topic (Buckner et al., 2008; Smallwood & Schooler, 2006) has been observed to characterize the DMN. Mindfulness has been associated with presence and focused attention (Brown & Ryan, 2003; Kabat-Zinn, 2003b) which is contrary to mind wandering. Therefore, differences in the functional connectivity of the DMN, particularly in the left MFG and left STG, likely contribute to the cognitive and emotional characteristics associated with trait mindfulness.

The SN has also been associated with mindfulness, but in terms of the integration of sensory information (Seeley et al., 2007). It has recently been proposed to play a role in the switching of activation between the DMN and CEN (Goulden et al., 2014). Results from the
current study reveal increased functional connectivity in the left ACC and decreased connectivity in the right precuneus. This suggests that high trait mindfulness is related to increased functional connectivity in the SN and decreased functional connectivity in the DMN. In other words, high trait mindfulness is associated with a tendency to have greater attentional control as evidenced by the increased functional connectivity in the ACC. Therefore, the altered patterns of functional connectivity in the ACC and precuneus are important for the switch between mind wandering and increased attention to sensations and experiences.

In the current study, the CEN was divided into two separate components, the rCEN and the lCEN. Interestingly, the patterns of functional connectivity within the rCEN and lCEN were quite similar. Specifically, a decrease in functional connectivity in the cuneus in the rCEN and precuneus in the lCEN was observed. An increase in functional connectivity in the middle frontal gyrus was found in both the rCEN and lCEN. Other studies have shown only a lCEN effect in mindfulness (Anselm Doll, Hölzel, Boucard, Wohlschläger, & Sorg, 2015; Hasenkamp & Barsalou, 2012); however, Hasenkamp and colleagues (2012) suggested that the increased functional connectivity observed between the right and left dLPFC in more experienced meditators allows for improved executive functioning and specifically attentional processing due to the bilateral nature of the connectivity. The bilateral effect observed here provides a novel finding about the potential relationship between mindfulness and executive functioning/attentional abilities. Future studies linking resting-state functional connectivity with behavioural assessments of executive functioning and attentional processing would help clarify this point.

It is worth noting the relationship of functional connectivity between various resting state networks, or what is referred to as cross-network connectivity. Cross-network connectivity is
commonly observed when key nodes of one network appear within a separate network (i.e., if the PCC emerged in a map of the SN; Hemington, Wu, Kucyi, Inman, & Davis, 2015). In recent literature, the appearance of cross-network connectivity has been linked to pathologies or neurological conditions (Hemington et al., 2015; Whitfield-Gabrieli & Ford, 2012). For example, abnormal cross-network connectivity has been observed in schizophrenia and major depressive disorder (MDD; Whitfield-Gabrieli & Ford, 2012); the DMN and CEN are less anti-correlated in the former and the DMN connectivity spreads to encompass the ACC in the SN in the latter.

Additional cross-network connectivity work has examined the abnormal functional connectivity in chronic pain populations. For example, a recent paper by Hemington and colleagues (Hemington et al., 2015) used resting state fMRI to investigate the disrupted cross-network functional connectivity in chronic pain patients. A post-hoc analysis revealed the PCC was the driver of the significant interaction and concluded the PCC is the “hub” for the altered network interaction. Similar patterns of cross-network connectivity were observed in the current data, including cross-network connectivity between the DMN and SN and the DMN and CEN. The PCC is known to play a role in the retrieval of episodic memories (Maddock, Garrett, & Buonocore, 2001). However, when an individual is mindful, they are focused on the present, which does not entail the recollection of memories. Moreover, the decreased connectivity of the PCC in the SN observed in the current study suggests that decreased connectivity of the PCC is associated with high trait mindfulness.

It is worth mentioning the inclusion of white matter in the significant clusters in the results from the current study. Although both Table 1 and Table 2 report the results as the anatomical region for the grey matter nearest to the cluster’s peak coordinates, the clusters extend into white matter regions, as evident in all three figures (see Figures 1, 2, and 3). The
ability of fMRI to detect (or report detected) white matter has been controversial, but it has more recently become a more accepted practice (Gawryluk, Mazerolle, & D'Arcy, 2014). Even though white matter analyses were not part of the current study, the results, in context with the current literature, provides insight on the capability of using fMRI to detect changes in white matter. Further, although it is a standard option to include a gray matter mask in the processing of fMRI data, we did not restrict our data in this way. The true variation is white matter detection among fMRI studies is difficult to know, given that many results are presented following use of a gray matter mask.

The present study is limited by the lack of quantitative measures of the cross-network functional connectivity. The use of the Sog-ICA for producing the functional connectivity maps allowed all significant changes to emerge, rather than focusing solely on the connectivity of pre-selected seed regions. The original hypotheses surrounded the functional connectivity changes within each network; however, this whole-brain, data-driven approach may be the reason why we obtained unanticipated cross-network functional connectivity results. As a result, the current paper can only report quantitative measures for functional connectivity changes within each network and explain observed patterns of between network functional connectivity changes. However, our study can provide insight into which ROIs should be selected for future seed-based resting state network studies.

In conclusion, our study demonstrates that scores on the MAAS are associated with specific patterns of functional connectivity in the DMN, SN, and CEN. High trait mindfulness showed an overall reduction in the functional connectivity in key components of the DMN and an increase of the ACC in the SN. Surprisingly, trait mindfulness is associated with a large amount of cross-network functional connectivity, which was not anticipated at the outset of this
study. Future projects should quantitatively examine the cross-network functional connectivity of trait mindfulness in order to develop a better understanding of the neural underpinnings of this phenomenon. Attaining a deeper understanding of mindfulness, specifically the communication between brain regions and resting state networks associated with increased mindfulness, may allow researchers to identify patient populations that have alterations in these areas. This in turn could enhance the likelihood of success of mindfulness-based interventions for such patients by identifying ideal candidates.

**Compliance with Ethical Standards:** This study has been approved by our institutional ethics committees and has been performed in accordance to the outlined ethical standards. All participants provided written, informed consent prior to their participation in the study.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent:** Informed consent was obtained from all individual participants included in the study.
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Chapter IV: Brief Discussion

The goal of this thesis was to contribute to the understanding of the neural underpinnings of trait mindfulness. To achieve this, we used a resting state fMRI study to investigate the neural correlates of the MAAS, a common measure of trait mindfulness. The results reveal that the DMN, SN, and CEN were all detectable in the data and that higher levels of mindfulness were associated with alterations in the patterns of functional connectivity within these networks. There is controversy that exists about the use of the MAAS in measuring trait mindfulness (Medvedev et al., 2016; Van Dam, Earleywine, & Borders, 2010). The main criticism is that the MAAS does not provide information to discriminate between levels of mindfulness, specifically with regards to the nonjudgment component of mindfulness. However, the creators of the MAAS outline that their scale focuses on the attentional and awareness component of mindfulness (Brown & Ryan, 2003). The use of the MAAS could be viewed as a criticism, but the conclusions that have been drawn from the data have been summarized to only reflect the attentional aspect of mindfulness. These data are still important, not only to further our understanding of the neural correlates of mindfulness, but to corroborate other publications. Our data appears to fit in with the existing resting state literature that utilizes the MAAS. As previously mentioned, the DMN is the network of focus in the literature and it has been shown to decrease in functional connectivity when correlated with scores on the MAAS (A. Doll et al., 2015; Wang et al., 2014). We found similar DMN reductions, providing further support for the resting state neural correlates of trait mindfulness within the DMN.

This thesis not only corroborates current literature, but it also provides novel resting state contributions. In our opinion, networks other than the DMN have been underrepresented in the
literature. Here, we examined how trait mindfulness influenced patterns of functional connectivity in other networks that have functions associated with mindfulness to provide a basis for furthering our overall understanding of resting state network functional connectivity. For example, our results demonstrated that higher scores on the MAAS were correlated with increased functional connectivity within key nodes of the SN and decreased functional connectivity within the DMN, similar to the findings by Doll and colleagues (A. Doll et al., 2015). These results could reflect the “switching” role of the insula between the activation and deactivation of the CEN and DMN, respectively. However, going beyond this proposed role of the insula, the ACC and insula both play a role in attending to and incorporating salience information (Menon, 2015b). Logically, with high scores on the MAAS, which reflect greater attention and awareness to the present moment, we would expect to see increased connectivity between these regions. Future studies should look to replicate this work in order to have more confidence in interpreting what these and similar findings mean.

It is important to note that no causal statements can be drawn from this data. The analysis of covariance (ANCOVA) approach we used is correlational in nature. Therefore, the results reflect changes in functional connectivity patterns, but no definitive cause-effect relationships. That being said, this thesis serves as a foundation for studies that want to further understand the association between mindfulness and brain functional connectivity.

Chapter V: Future Directions

This thesis provided insight into the resting state neural correlates of mindfulness with a correlational approach. A simple replication of the current study would be a beneficial future study. As described above, the inconsistency of results could be the result of various mindfulness
measures or analysis techniques that are used in each individual study. A study that follows exactly the methodology described here would be beneficial to validate the current results. Future studies should also look to implement a longitudinal (pre-post) resting state fMRI study accompanying a mindfulness intervention and examine both the white and gray matter alterations that follow from such an intervention. The alterations in functional connectivity could then be confidently drawn as a causal relationship and we could develop a more succinct understanding of the neural architecture that underlies a mindfulness-based intervention.

Ultimately, mindfulness is an ever-growing phenomenon that is beginning to be applied to various patient populations. Any future studies that can aid in furthering our understanding of the neural correlates of mindfulness are useful. We understand the basic functions of the brain’s resting state networks, but we do not yet fully understand how their patterns of connectivity change with mindfulness. Resting state physiology research can be greatly advanced because we can develop further insights into the functional relationships of networks and inter-network connectivity patterns that have not received as much attention in the literature.

Chapter VI: Conclusion

Resting state fMRI has been successful in assessing patterns of functional connectivity within networks of the brain. It has been determined that resting state fMRI can detect changes in these patterns when correlated with scores from a mindfulness questionnaire. Although the DMN and its relation to mindfulness has been explored in the literature, research is lacking in the understanding of how mindfulness alters the functional connectivity in the SN and CEN, and the cross-network connectivity between these three networks. From this work, we have enhanced the overall understanding of resting state brain networks, specifically with the changes associated
with the DMN, SN, and CEN, using an ICA technique. The overall goal of contributing to the advancement of knowledge about resting state functional connectivity of mindfulness was achieved.


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