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Changes in Default Mode Network Connectivity in the Months Following a Motor Vehicle Collision

by

Andrew S. Cotton

Submitted to the Graduate Faculty as Partial fulfillment of the requirements for Masters of Science in Biomedical Sciences in Medical Physics

__________________________________________
Dr. Michael Dennis, Committee Chair

__________________________________________
Dr. John Wall, Committee Member

__________________________________________
Dr. Xin Wang, Committee Member

__________________________________________
Dr. Patricia Komuniecki, Dean
College of Graduate Studies

The University of Toledo

August 2013
An Abstract of

Changes in Default Mode Network Connectivity in the Months Following a Motor Vehicle Collision

by

Andrew S. Cotton

Submitted to the Graduate Faculty as Partial fulfillment of the requirements for Master of Science in Biomedical Science in Medical Physics

The University of Toledo

August 2013

This study investigates stress-related changes in the connectivity to the posterior cingulate cortex, the central node in the default mode network, in the survivors of Motor Vehicle Collisions (MVCs). Thirty-two subjects underwent Functional Magnetic Resonance Imaging (fMRI) resting-state scans two weeks following their MVCs. A subset of seventeen subjects completed an additional resting-state scan three months later. Stress symptoms were assessed with the Posttraumatic Stress Disorder Checklist (PCL) stressor version at each time point. Group difference analyses and correlation analyses between functional connectivity maps and PCL scores using SPM 5, fMRI analysis software, yielded significant results in the inferior parietal cortex/visual cortex, the hippocampi, the lateral temporal cortices, the anterior cingulate cortex, the amygdalae, and the dorsolateral prefrontal cortex. The results suggest that there was increased functional connectivity to limbic structures during the acute stress period when stress symptoms were high. This may reflect increased priming of those brain regions in response to acute stress. The connectivity decreased for subjects whose stress symptoms decreased three months later. Furthermore, correlations in the left inferior parietal
cortex/visual cortex and left hippocampus indicate that there was a change in the way information was processed in the brain, consistent with a change from outwardly focused attention to inwardly focused attention as stress symptoms subsided.
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1 Introduction

1.1 Aim of Study

1.1.1 Purpose

Although numerous studies have examined the functional connectivity in the default mode network (DMN) for subjects with acute traumatic stress and for subjects with chronic traumatic stress (Bluhm, Williamson et al. 2009), no studies to our knowledge have investigated how the DMN's functional connectivity changes between the weeks and months following a trauma in relation to reported stress symptoms. Functional connectivity itself provides a measure of the synchronization of spontaneous fluctuations in different brain regions. Researchers believe that the fluctuations in the DMN prime the brain for internal mentation. Understanding how stress affects the DMN's functional connectivity, as a result, may facilitate the development of cognitive techniques that aid in the psychological recovery from a trauma. We therefore investigated the DMN's functional connectivity at two time points for subjects who had been involved in motor vehicle collisions (MVCs). In particular, we acquired resting-state functional magnetic resonance imaging (fMRI) scans and Posttraumatic Stress Disorder Checklist (PCL) surveys two weeks and three months after the subjects' MVCs. By correlating the activity in the posterior cingulate cortex (PCC), a core node in the DMN, with the activity in the rest of the brain, we calculated the functional connectivity of the DMN.
1.1.2 Introduction to the Default Mode Network

The DMN has been described by Van Dijk as an intrinsic connectivity network (Van Dijk, Hedden et al. 2010). It thus, by definition, consists of brain regions which have intrinsic white matter connections, which share common functions, and which exhibit synchronized oscillatory activity. The latter may be observed by obtaining information about metabolic activity via functional imaging. The oscillations serve as a basis for investigating functional connectivity, which is defined as "the temporal correlation of a neurophysiological index measured in different brain areas" (Friston, Frith et al. 1993). Correlation analyses of the oscillatory activity between different brain regions revealed by blood oxygen level dependent (BOLD) imaging allow researchers to quantify connections. Greater positive correlations (higher connectivity) are interpreted as indicators of parallel processing of related information. Some researchers have hypothesized that the oscillations serve as a means to temporally bind information (Engel, Fries et al. 2001). This has been investigated extensively in the visual cortex, in which different features of the visual field are processed in separate regions of the brain (Engel, Konig et al. 1992). However, more prevalent interpretations include that the oscillations reflect the previous use of brain regions in concert and that the oscillations prime brain regions for future use (Fox and Raichle 2007). It has been well established by previous BOLD imaging studies that the DMN regions exhibit oscillatory activity in the frequency range of .1-.01Hz (van den Heuvel and Pol 2010). In addition, researchers have identified two sub networks of the DMN in which the oscillatory activity is 180° out of phase (Uddin, Kelly et al. 2009). Based on their opposing functions, the sub networks have been termed the task negative (TN) network and the task positive (TP) network (Sonuga-Barke and Castellanos 2007). The TN network, which includes the medial
prefrontal cortex (mPFC), the anterior cingulate cortex (ACC), the dorsolateral prefrontal cortex (dLPFC), the PCC, the bilateral hippocampi and parahippocampi, the lateral temporal cortices (LTCs), and the bilateral inferior parietal cortices (IPCs)/visual cortices, exhibits decreased metabolic activity when an individual engages in goal directed activity that requires focused attention. One should note, however, that the characteristic oscillations remain; their intensity decreases. In contrast, the TP network exhibits increased metabolic activity when an individual engages in tasks. Studies of the role of the DMN in psychiatric disorders have primarily focused on the TN network (Broyd, Demanuele et al. 2009). In fact, many researchers restrict the definition of the DMN to the TN component. We adopt the same convention in this study: the DMN from this point forward will always refer to the TN DMN.

### 1.1.3 Hypotheses

In order to assess changes in the DMN, resting-state scans were acquired for MVC subjects two weeks and three months after their MVCs. In such scans, subjects were asked to relax and let their minds wander freely. Correlations between the functional connectivity to the PCC and the Posttraumatic Stress Disorder Checklist (PCL), a self-administered psychometric survey used to evaluate stress symptoms, were utilized to assess the change in the DMN and emotion regulation regions in response to stress over time.

Two weeks following a trauma, we believe the DMN regions are primed to allow an individual to respond to future threats. This will be reflected in increased connectivity to the PCC. The mPFC is activated when an individual plans for the future and when an individual attempts to down-regulate emotions (Spreng and Grady 2010). We believe that the mPFC will be primed to prepare for possible future threats after an MVC. In addition,
in healthy individuals, it will aid in down-regulating negative emotions that lead to
distraction. The ACC also plays an important role in down-regulating emotions.
Activation of the ACC down-regulates the fear-related response in the amygdalae
(Lanius, Vermetten et al. 2010). We believe that the ACC will be primed to down-
regulate negative emotions. The hippocampi are activated during episodic memory
retrieval. A common traumatic stress symptom is intrusive recollection of the trauma
(McNally 1998). We believe that this symptom, during the acute stress phase, will be
associated with increased priming of the hippocampi. Lastly, we believe that the priming
of the IPCs/visual cortices will increase following a trauma. These regions are activated
when an individual passively monitors his or her environment (Hahn, Ross et al. 2007)
and may be associated with the hyper-vigilance symptoms of stress. This results in
increased PCC connectivity. Three months later, when the initial stress has passed, the
priming of the DMN brain regions will decrease and the PCC connectivity will decrease
as a result.

We therefore formed two hypotheses which we could use to investigate the above
views. First, we hypothesized that there would be a statistically significant decrease in
PCC connectivity to DMN brain regions between the two-week and three-month scans.
We investigated this hypothesis with single sample T-tests on the two-week PCC
connectivity maps, the three-month PCC connectivity maps, and the change in
connectivity maps for the subjects who recovered from the MVCs. Second, we
hypothesized that the functional connectivity to the DMN brain regions would be
positively correlated with stress scores. We investigated this hypothesis with correlations
between the two-week PCC connectivity and the initial stress scores, between the three-
month connectivity and final stress scores, and the change in PCC connectivity and the change in stress scores.

1.1.4 Overview of MRI Physics, Stress, and the Default Mode Network Literature

The interpretation of this study's results requires an understanding of concepts from three fields: diagnostic medical physics, psychology, and functional neuroscience. Medical physics allows one to understand how MRI images are obtained and what information those images provide. In particular, the physics section of the introduction (section 1.2) discusses how high magnetic fields change the subatomic properties of matter and how those properties may be manipulated by magnetic field gradients and radiofrequency (RF) pulses to obtain MRI images. Once the basic principles of MRI have been established, I describe the properties of echo planar imaging—the pulse sequence most frequently used in fMRI studies and used in this study. The physics section also describes how the physical properties of the hemodynamic response, a physiological result of neural metabolism, are used to obtain information about ongoing cognitive processes. The psychology section (section 1.3) of the introduction discusses the symptoms of stress and how the symptoms relate to human physiology. For example, the psychology section describes the diagnostic criteria for acute stress disorder and PTSD. The relationship between stress symptoms and changes in the activation of different brain regions are described in the next two sections (sections 1.4 and 1.5). The neuroscience section discusses the properties of the brain regions identified as being part of the DMN and of the brain regions in the limbic system which are most commonly referenced in the stress related literature. It summarizes findings from animal studies, anatomical dissections, lesion studies, and fMRI research. The last section of the introduction, section 1.5, discusses the history of the DMN, addressing how it was discovered, the
techniques used to study it, and recent research into its role in psychiatric disorders. Section 1.5 also summarizes recent studies investigating the effects of stress on the DMN.

1.2 Physics

The physical properties of subatomic particles ultimately allow us to acquire images in MRI. The general MRI physics section discusses how magnetic fields affect the spins of such particles. It discusses how magnetic field strength affects spin frequency and how RF pulses may be used to flip the orientations of spinning particles in a field. Manipulating these properties allows one to obtain an image of the body. The next section discusses echo planar images and its imaging parameters. It is a unique technique given how rapidly it allows one to obtain images. However, it uses lower resolution than most scans and therefore is limited in its use. The final section discusses how the hemodynamic response is observed using BOLD imaging.

1.2.1 General MRI Physics

In order to understand fMRI, one must first understand the physics of magnetic resonance. Charged particles possess intrinsic spins, which are represented by spin vectors. The direction that the spin vectors point is determined by the right hand rule of classical physics. MRI, in particular, utilizes the properties of the spinning proton in hydrogen in order to form images. When an object is placed in a uniform magnetic field, the protons in hydrogen atoms align parallel and anti-parallel to the magnetic field lines. However, the alignment is not perfect: the proton spin vectors actually precess around the magnetic field lines (Bushberg 2012).
The alignment of the particles can be explained in part by classical physics, in which spinning charged particles are approximated by tiny loops of current. Electrodynamics stipulates that moving charges, such as those in a current, experience a force when placed in a magnetic field. The forces simultaneously acting on a small loop of current create a torque. The rotation that results from the torque aligns the loop's central axis with the magnetic field. Note that the right hand rule of electrodynamics determines the direction that the current travels around the loop.

However, the classical model does not entirely account for the behavior of spinning particles: quantum mechanical principles must be introduced to explain some effects. First, due to quantum exclusion rules, spin vectors cannot align perfectly with the magnetic field. The size of the angle that is formed between the magnetic field line passing through the center of a particle and that particle's spin vector is inversely related to the strength of the magnetic field. However, the particle's precessional frequency is directly proportional to the magnetic field strength. The constant that relates the two is called the gyromagnetic ratio $\gamma$. The precessional frequency of a hydrogen proton in a magnetic field, furthermore, is called hydrogen's Larmor frequency, $f_0$, given by:

$$f_0 = \frac{\gamma}{2\pi} B_0$$

In addition, according to quantum mechanical rules, a spinning charge in a magnetic field has only two energy states. In the lower energy state, a particle's spin vector precesses about an axis parallel to the magnetic field lines. In the higher energy state, it precesses antiparallel. The energy difference between the two states for a proton
in a 1.5T magnetic field (a common field strength for a medical MRI scanner) is equivalent to the energy of a RF photon. In fact the frequency of the photon is equal to that of the Larmor frequency of the proton.

Most textbooks refer to the sum of the proton spin vectors for an infinitesimal volume as the spin, or isochromate. Due to thermal fluctuations, some protons are always in the higher energy state. In a 1.5T magnetic field, 1,000,004 protons precess parallel for every 1,000,000 protons that precess antiparallel. As a result, the spin points in the direction of the applied magnetic field. When the protons are excited by radio waves that match the transition energy between their two states, more protons are rotated antiparallel. The cumulative effect is the rotation of the spin towards its opposite pole. The duration and polarization of the pulse determines the time and power, respectively, required to flip the spin vector by a certain angle. The polarization of the applied RF pulse also determines the phase of the hydrogen protons in an excited volume.

The magnetic properties of a material to be scanned most often are described in terms of two quantities, the \( T_1 \) and \( T_2 \) relaxation time constants. The \( T_1 \) relaxation time constant describes the time required for the component of the spin parallel to the magnetic field lines, \( M_z \) (also called the longitudinal component), to return to 63% of its equilibrium magnitude \( M_0 \). If \( M_0 \) is the longitudinal value of the vector at time \( t_0 \), then the magnitude at time \( t \) is defined by the below equation.

\[
M_z(t) = M_0 \left( 1 - e^{-t/T_1} \right)
\]
The number of possible interactions between spins and a material’s vibration states
determines the $T_1$ magnitude. Materials that have a large number of vibration states with
frequencies similar to the Larmor frequency have small $T_1$ values.

In contrast, the $T_2$ constant describes the time required for a spin to dephase due
to spin-spin interactions. Dephasing occurs when protons in an infinitesimal volume
precess at different speeds, causing them to lose synchronization. As a result, the length
of the transverse spin component—the component of a spin vector that is projected on the
plane perpendicular to the longitudinal component—decreases over time. The dephasing
itself is caused by tiny differences in the local magnetic field strength, which alter the
precessional speeds of protons. Since spinning charges create tiny local variations in the
magnetic field, interactions between protons (the spin-spin interaction) cause dephasing.
However, the $T_2$ of a material cannot be measured directly due to the complicating effect
of inhomogeneities in the main magnetic field, which also alters the precessional
frequencies of protons. The combined effect of the spin-spin interactions and the main
field inhomogeneities is reflected in a $T_2^*$ time constant. The change in the transverse
component over time can be modeled by the following equation.

\[ M_{xy}(t) = M_0 e^{-t/T_2^*} \]

Directing radio waves at a material in a uniform magnetic field deposits energy in
its lattice. In order to restore thermodynamic equilibrium, the energy is reemitted in the
form of additional RF photons. Some of the photons are absorbed by the material lattice,
while other photons are radiated outward. When the radiated photons have the same
phase, which occurs when the material's hydrogen protons precess in synchrony, they add constructively and create a signal that is detectable by an antenna. It is this property that allows an MRI scanner to collect information about a scanned object and construct an image. One should note, however, that as the spins dephase, the signal intensity decreases.

An MRI scanner uses high intensity magnetic fields in conjunction with radio wave transmitters and receivers in order to reconstruct slices of an object. Most hospitals utilize superconducting magnets with magnetic field strengths of 1.5 to 3T in order to image patients. Typically, MRI systems utilize super conducting Nb-Ti coils with circulating current in order to create the high intensity magnetic fields. The coils are contained in the scanner's doughnut shaped gantry. Due to their solenodal arrangement, the magnetic field parallels the internal axis of the gantry. In addition, the scanner contains gradient coils that create linearly increasing magnetic fields along three axes (the z axis parallels the gantry axis and the y axis parallels the direction of gravity). Applying the gradients in conjunction with RF pulses allows the scanner to acquire an image.

For general purpose MRI imaging, three types of magnetic gradients are used to encode spin information: the slice select gradient, the phase encode gradient, and the frequency encode gradient. Typically, these correspond with the x, y, and z gradients respectively. In order to select a slice for imaging in the human body, coils create a linear magnetic field gradient. The increasing magnetic field causes the precession rate of the spins to increase in the same direction. Thus, when a limited bandwidth radio wave is applied, only a plane of spins whose precession frequencies correspond to those of the radio waves are excited. The radio waves flip the spins away from equilibrium. For
example, a $\pi/2$ pulse flips the net magnetization vector 90° into the plane perpendicular to the equilibrium vector. The phase encode gradient, applied after the slice select gradient, causes the excited spins to precess at different rates. It is applied only briefly and then removed, causing the spins to return to their original spin frequency. As a result, the spin phases vary linearly along the axis of the phase encode gradient. At that point, however, the spins have begun to dephase. The slice select gradient is therefore reapplied along with a $\pi$ RF pulse. This causes the spins to rephase, generating a detectable RF signal. The frequency encode gradient is then applied in order to increase the rate of precession of the hydrogen protons along another axis. Thus, the radio waves radiated by the protons vary as a function of position. The resulting RF signal provides information about the magnitudes and positions of the spins in the selected slice and may be used to create an image when combined with additional data.

In order to reconstruct an image of the selected slice, the signal detected from the receive antennae must be processed. This involves transforming the signal to the spin rotating frame of reference and sampling the data. The result is a single line of data in k-space (a data matrix used in Fourier image reconstruction). In order to reconstruct the image, a sufficient number of lines in k-space must be collected. A Fourier transform of the acquired data matrix generates a slice image.

Pulse sequences, such as spin echo, are often described in terms of two quantities: echo time (TE) and repetition time (TR). In the case of spin echo, the TE is two times the time between the $\pi/2$ excitation pulse and the $\pi$ rephasing pulse and refers to the time between the initial excitation pulse and the RF signal caused by rephasing. In addition, the TR refers to the time before the repetition of the sequence. For some sequences, the
TR may refer to the time required to collect a single line in k-space whereas, for other sequences, the TR may refer to the time required to acquire an entire image slice (McRobbie 2003).

1.2.2 Echo Planar Imaging

Echo planar imaging is a fast imaging technique that is used in BOLD studies. It typically uses a TR on the order of 1 to 3s. After the initial $\pi/2$ radio frequency pulse, the phase encode gradient is applied at regular intervals to increment the phase of the spins. Multiple echoes are created by continuously switching between positive and negative lobes of the readout gradient in synchrony with the phase encode gradient. The inverting read out gradient causes the spins to rephase, restoring the signal for each echo. The final image acquired is $T_2^*$ weighted due to the fact that gradient mediated phase reversals do not compensate for magnetic field inhomogeneities. A single pulse sequence acquires all of the k-space lines for a single slice. Typically, an entire slice is acquired in approximately 100ms. However, because echo planar sequences frequently utilize multi-slice acquisition, requiring stacked pulse sequences, an entire volume may be acquired every 3s (Howseman, Grootoonk et al. 1999). The effective TE of an echo planar sequence refers to the time between the initial excitation pulse and the echo time at which the origin of k-space is encoded for a single slice (Poustchi-Amin, Mirowitz et al. 2001). It should be noted that, in an effort to acquire as many volumes as possible with as much signal per voxel as possible, the dimensions of an echo planar slice is typically 64 by 64 with voxel dimensions of 3mm by 3mm by 5mm (Poustchi-Amin, Mirowitz et al. 2001; Buxton 2002).
1.2.3 Blood Oxygen Level Dependent Imaging

Hemoglobin is the complex protein responsible for transporting oxygen in the blood stream. It consists of four amino acid strands which are each bound to a single iron atom. It is the iron atoms themselves that facilitate the binding of oxygen to hemoglobin. The oxygenated form of hemoglobin has four receptor sites for oxygen. When erythrocytes containing hemoglobin are exposed to bicarbonate ion, the oxygen is released into the blood plasma. From there, it diffuses through capillary walls to neurons requiring oxygen. As the oxygen is released from the hemoglobin, the oxygenation state of iron changes. Oxyhemoglobin has paired electrons, whereas deoxyhemoglobin has unpaired electrons. The unpaired electrons, like the protons in the nuclei of hydrogen atoms, are spinning particles. Thus, when hemoglobin is placed in a strong uniform magnetic field, the unpaired electrons create small changes in the local magnetic field.

BOLD imaging utilizes the magnetic properties of hemoglobin in order to study the activation of brain regions in response to different tasks or conditions. After the activation of action potentials in neurons, their metabolism increases in order to support the Na+ and K+ protein pumps that repolarize their membranes. This results in the release of carbon dioxide into the blood stream, which is converted to bicarbonate ion for transport to the lungs. Several mechanisms, including relaxation of smooth muscle in vessels in response to decreased blood oxygen and feedback from vessel introceptors, cause the vessels to dilate in response to the changes. This results in increased oxygenated blood flow into regions with high neural metabolic activity. This hemodynamic response over compensates for the initial drop in blood oxygenation that results from the activation of the brain regions. The influx greatly exceeds the oxygen requirements caused by the activation, leading to a decrease in the magnetic susceptibility
of the region. This causes the intensity of the RF signal from the region to increase. The hemodynamic response is typically delayed between one and two seconds after the metabolic activation and lasts from two to four seconds (Buxton, Uludag et al. 2004).

1.3 The Stress Response and Trauma Related Stress Disorders

The human stress response is an evolutionary physiological and behavioral response which allows an individual to react to danger. A person exposed to threatening stimuli will naturally experience fear, activating neural and endocrine mechanisms that alter cognitive processes and the body's hemostasis. If the threat is of low intensity and frequency, then the stress response subsides within a matter of minutes or hours depending on the stress mechanisms activated. However, if the threat is of high intensity and/or frequency, then the stress response may activate cognitive and behavioral symptoms which last for days. The symptoms furthermore may have a profound impact on an individual's ability to function socially and occupationally. Individuals, in such cases, may be diagnosed with acute stress disorder. If the symptoms become chronic (they last for more than a month), then an individual may be diagnosed with PTSD. Ultimately, an understanding of stress symptoms directs the interpretation of changes in DMN connectivity over time that correlate with stress measures.

1.3.1 Stress Response

In their book, *The Clinical Guide to the Treatment of the Human Stress Response* George Everly and Jeffrey Lating outline the neural and physiological progression of events that lead to the human stress response (Lating 2012). According to their model, the activation of the human stress response may be divided into three phases: cognitive appraisal, cognitive affective integration, and neurological triggering. Information about
stimuli is initially collected from sensory modalities and is relayed to the limbic system. During cognitive appraisal, higher order processing centers such as the mPFC and the ACC process information concerning stimuli. These regions appraise stimuli and classify them as either stressors or non-stressors. The process of classification is complex and involves innate biology, personality factors, and prior learning. These factors influence the generation of affect, which "colors" the cognitive process. Affect generation, furthermore, is influenced by the current affective state. For example, an individual in a stressed state is more likely to appraise ambiguous stimuli as threatening.

If the cognitive appraisal and affect integration leads to a stimulus being classified as a stressor, then a number of neurological mechanisms may trigger events that lead to the stress response. The mechanisms may be divided into three groups: the neural mechanisms, the neuroendocrine mechanisms, and the endocrine mechanisms. They are listed in order of increasing duration of effect.

The neural mechanisms directly stimulate the sympathetic nervous system through the hypothalamus and the skeletal muscular system. Organ innervation by the sympathetic nervous system results in increased heart rate, heart contractibility, constriction of skin and visceral vessels, dilation of skeletal muscle vessels, dilation of pupils, dilation of lung bronchi, glycogenolysis, and inhibited digestion. Innervation of the skeletal muscles, on the other hand, results in increased muscle tone in preparation for action.

Activation of the neuroendocrine system leads to fight or flight response and is facilitated by activation of the amygdalae. Efferent fiber tracts pass from the amygdala to the hypothalamus and eventually to the adrenal gland. Stimulation of the adrenal complex
leads to the secretion of the catecholamines norepinephrine and epinephrine. These produce the same physiological effects as the sympathetic nervous system, but for a longer duration.

Research suggests that four primary endocrine glands are activated in response to stress: the adrenal cortices, the anterior pituitary, the posterior pituitary, and the thyroid. Of the four, the adrenal cortices are the most extensively studied in stress disorders. In response to a stressor, the hippocampus activates the hypothalamus, which releases corticotrophin-releasing factor. This stimulates the pituitary to release adrenocorticotropic hormone. The adrenal cortex receives the hormone via the circulation, releasing glucocorticoids such as cortisol. Again, glucocorticoids such as cortisol produce the same effect on human physiology as the sympathetic nervous system. However, prolonged secretion of cortisol leads to decreased immune system function and feelings of helplessness.

The activation of the stress response disrupts homeostasis in order to prepare the body to respond to a threat. The removal of external stressors usually terminates the stress response and encourages the return to homeostasis. However, repeated exposure to stress in addition to certain risk factors may result in damage to the mechanisms that facilitate the return to homeostasis, leading to stress-related illnesses and psychological disorders. This is especially true of the hippocampus which is damaged by cortisol.

1.3.2 Acute Stress Disorder

Acute stress disorder may be diagnosed for an individual in the immediate aftermath of a trauma. According to the DSM IV TR (American Psychiatric Association. and American Psychiatric Association. Task Force on DSM-IV. 2000), a person must meet eight criteria in order to be diagnosed with acute stress disorder. Criterion A
specifies the etiology. Individuals must have experienced or witnessed a traumatic event in which they or others faced the possibility of death or bodily harm, which caused them to feel fear or horror. Criteria B through E describe the symptom categories of acute stress disorder: dissociation, re-experiencing, avoidance, and hyper-arousal. The dissociative symptoms are more pronounced in acute stress disorder than PTSD. An individual must have at least three of five listed dissociative symptoms in DSM IV in order to be diagnosed with acute stress disorder. Some dissociative symptoms include emotional numbing, reduced awareness of one's surroundings, and dissociative amnesia. The other symptoms criteria are more general and do not require a specific subset of criteria for a diagnosis. Re-experiencing symptoms may include intrusive thoughts pertaining to the trauma and nightmares. Avoidance symptoms may include avoiding situations, conversations, or thoughts that serve as reminders of the trauma. In addition, the hyper-arousal symptoms may include irritability, hyper-vigilance, and an exaggerated startled response. Criterion F specifies that the symptoms must cause significant impairment to the ability of an individual to function socially and occupationally. Furthermore, criterion G specifies that the symptoms must occur for a minimum of two days and last for a maximum of four weeks. If the symptoms last for more than four weeks, then a diagnosis of PTSD is considered. Lastly, criterion H specifies that the symptoms cannot occur as a result of other factors such as substance abuse or a medical disorder. For this study, all subjects exhibited some acute stress symptoms as determined by the PCL.

1.3.3 Posttraumatic Stress Disorder

Posttraumatic stress disorder as the name implies is a chronic anxiety disorder which occurs in response to a trauma. The trauma to which individuals are exposed may
be classified as type I or type II (Thomaes, Dorrepaal et al. 2009). A type I trauma is a single non-interpersonal event that threatens the physical wellbeing of an individual. People who survive major earthquakes, for example, have been exposed to a type I trauma. Furthermore, MVCs, which the subjects in this study experienced, would be classified as a type I trauma. On the other hand, a type II trauma refers to a series of interpersonal events that threatens an individual's physical wellbeing. Repeated physical abuse as a child is an example of type II trauma. Individuals exposed to type II trauma are more likely to develop PTSD than those who experienced a type I trauma. In addition, even if those individuals do not develop PTSD in response to the trauma, they are more likely to develop it in response to subsequent traumas.

Similar to acute stress disorder, individuals must satisfy seven criteria in order to be diagnosed with PTSD. Again, criterion A specifies that, in order to be diagnosed with PTSD, individuals must have experienced or witnessed a traumatic event in which they or others faced the possibility of death or bodily harm, which caused them to feel fear or horror. Criteria B through D define symptoms of PTSD. There are three categories: re-experiencing, avoidance, and hyper-arousal. In order for an individual to be diagnosed with PTSD, he or she must have at least one of five re-experiencing symptom, which may include intrusive recollection of the trauma, reoccurring nightmares pertaining to the trauma, feeling as if the event were reoccurring, feeling psychological distress in response to reminders of the event, and physiological reactivity to reminders of the event. For a patient to be diagnosed with PTSD, he or she must also have at least three of seven avoidance symptoms. These may include avoiding thoughts that serve as reminders of the trauma, avoiding situations that remind one of the trauma, decreased ability to recall the
trauma, decreased interest in significant activities, feeling detached from others, having a restricted range of affect, and having a "sense of a foreshortened future." It should be noted that some researchers debate whether the inability to recall aspects of a trauma should be considered an avoidance symptom since intrinsic physiological changes in response to a traumatic event may inhibit memory (Layton and Krikorian 2002). An individual must also have at least two of five arousal symptoms in order to be diagnosed with PTSD. A patient may have difficulty falling asleep, irritability, problems concentrating, hyper-vigilance, and an exaggerated startle response. Criterion E requires that symptoms must persist for at least one month following the onset of a trauma. Before that time the disorder is classified as acute stress disorder. PTSD may also be classified as simple or complex (Thomaes, Dorrepaal et al. 2009). In simple or classic PTSD there is greater emphasis on the hyper-arousal and avoidance symptoms. In complex PTSD, on the other hand, there are more dissociative symptoms and re-experiencing symptoms. The memory of the trauma itself may be increasingly impaired. Complex PTSD most often occurs as a result of type II trauma.

1.4 Review of the Anatomy and Functional Characteristics of Regions of Importance in the Default Mode Network and Stress

This section describes the properties of the brain regions investigated in this study. Subsections address details such as how brain regions normally function in different situations and how the brain regions function when an individual is stressed. The information provides a context in which this study's results may be interpreted.

1.4.1 Prefrontal Cortex

The PFC has some of the most diverse functions of all of the brain regions. Generally, the size of the PFC in species is directly related to the complexity of their
social interactions. It, therefore, supports a number of social related processing tasks. For example, fMRI studies suggest that it supports theory of mind, self-referential thought, and social planning (Spreng and Grady 2010).

Previous fMRI studies have tied the dlPFCs with working memory. They also play an important role in learning new tasks. In *Wandering Minds*, Mason and Norton describe their study in which subjects were asked to perform verbal reasoning tasks over several scanning sessions (Mason, Norton et al. 2007). After training subjects in the task over four sessions, Mason scanned the subjects and administered psychometric surveys to determine the extent of daydreaming while subjects performed old and novel tasks. The propensity to daydream was positively correlated with activation in DMN regions. As a result, the connectivity to the mPFC increased. The connectivity correlated with psychometric instruments in which subjects reported how often they daydreamed during their scans.

There have been a number of other studies relating changes in the DMN with psychiatric pathologies. For example, it has been demonstrated that individuals with depression have increased activation of the mPFC when assessing negatively-valenced words (Anand, Li et al. 2005). Researchers have hypothesized that this result reflects the fact that the mPFC plays a strong role in social cognition and self-referential processing (Lemogne, Mayberg et al. 2010). One of the key characteristics of depression is uncontrolled rumination. An individual will review and analyze the significance of past memories, drawing negative conclusions which result in negative emotions. The mPFC supports such functions.
The DMN has also been analyzed in individuals who practice mindfulness meditation (Taylor, Daneault et al. 2013). In this type of meditation, individuals are trained to focus their awareness on their senses. Moreover, meditators are taught to view their thoughts as transient phenomena, which should be observed but not elaborated upon. Proponents of meditation believe that this is an ideal state in which individuals are more aware of the present moment and worry less about the future. Research suggests that experienced meditators exhibit decreased connectivity to the mPFC, the brain region which supports self-awareness and planning for the future.

Ultimately, interpretation of PCC connectivity to the mPFC is difficult. Decreased connectivity has been found in mental disorders. Furthermore, increased PCC connectivity to mPFC is associated with the rumination of depression as well as learning to perform a new task. Thus, when interpreting connectivity results to the PFC, it is important to analyze the subject pool and context in which the measurement is being made.

1.4.2 Anterior Cingulate Cortex

The ACC plays a key role in regulating emotions, and some authors classify it as part of the limbic system. It is frequently divided into the rostral and dorsal divisions (Etkin, Egner et al. 2006). The rostral division is activated in certain types of emotional processing, whereas the dorsal division is activated during analytic processing. In research into stress disorders, investigators have studied the role of the rostral division in emotion dysregulation (Kim, Chey et al. 2008). One experimental tool which is frequently employed to activate the ACC is a Stroop test. In such a test, individuals must identify a word or picture that satisfies a certain criteria while ignoring other details. The emotional counting Stroop test, for instance, requires subjects to count the number of
words that appear on a screen. Some groups of words may have neutral connotations, such as table or lamp, whereas other words may have negative connotations, such as explosion or body bag. Individuals with PTSD have greater activation in the ACC relative to controls when viewing trauma related words (Shin, Whalen et al. 2001). In general, PTSD subjects demonstrate greater ACC activation when viewing words with negative connotations. The degree of ACC activation is positively correlated with the time required to perform a Stroop task.

In addition, fMRI imaging studies indicate that the ACC plays a role in different autonomic and somatic processes (Brodal 2004). Electrical stimulation of the ACC, for example, causes changes in respiration, heart rate, and blood pressure variation. It also causes pupil dilation. Somatic effects of electrical stimulation include changes in muscle tone and movement inhibition. The latter may be related to the fear freezing response. This may be important for patients who have dissociative PTSD (Lanius, Vermetten et al. 2010). For example, PTSD subjects who dissociate when hearing a narrative description of their traumas demonstrate increased activation of their ACCs and decreased activation of their amygdalae.

1.4.3 Posterior Cingulate Cortex

The PCC was used as the seed for the analysis. It is located posterior to the corpus collosum and anterior to the precuneus. Studies have demonstrated that this region is activated during the recall of episodic memories (Wagner, Shannon et al. 2005). In fact it is believed to form a part of a sub-network of the DMN which supports episodic memory retrieval (Buckner, Andrews-Hanna et al. 2008). Connectivity studies, furthermore have identified it as a major hub in the DMN. Partial correlation analyses indicate that it influences all regions in the DMN except the right medial temporal lobe (the
hippocampal complex) (Fransson and Marrelec 2008). It is the only region in the DMN to influence so many other regions. Studies also suggest that the PCC contributes to processing information concerning the visual field. In particular, it is activated by stimuli in the peripheral visual fields and supports visual reorienting to such stimuli (Hahn, Ross et al. 2007).

1.4.4 Inferior Parietal Cortices/Visual Cortices

The IPC/visual cortices serve as major nodes in the DMN. In particular, research indicates that they are involved in passively monitoring features of the visual field. The regions are activated when an individual is in a resting-state and are deactivated when an individual focuses his or her visual attention on a particular stimulus (Shulman, Fiez et al. 1997). However, the regions remain active when a person uses his or her entire visual field to search for a stimulus. In Hahn's visual field study (Hahn, Ross et al. 2007), the activity in the left angular gyrus, which is located at the boundary between the parietal lobe and temporal lobe, was negatively correlated with the response time to uncued targets. The author thus concluded that the IPCs and the PCC are responsible for supporting the passive monitoring of the environment. It has also been noted that patients with damage to their IPCS cannot perceive their visual field as a whole: This is called Blatant's Syndrome (Buckner, Andrews-Hanna et al. 2008). Electrode measurements of the neuronal activity in the cortices of monkeys supports that claim. When the monkeys viewed a flashing pattern in a localized region, the electrical activity in the IPCs decreased. However, when the monkeys viewed a large test pattern that filled their entire visual fields, the activity increased, suggesting that the regions integrate information from the entire field of view (Raichle, MacLeod et al. 2001).
1.4.5 Lateral Temporal Cortices

The lateral temporal cortices of the DMN are associative regions of the visual system. As such, they do not receive sensory nerve fibers and their efferent fibers do not connect to the subcortical motor nuclei (Brodal 2004). They are, in particular, involved in higher level processing of visual stimuli. For example, the regions are activated when an individual is asked to identify facial expressions (Gazzaniga 2000). Thus, the regions are believed to be involved in feature analysis of the environment. Electrical stimulation of the regions during surgery causes individuals to experience dream-like hallucinations as they relive episodes of their lives. Nerve tracts to DMN regions such as the hippocampi and PCC may underlie this phenomenon.

1.4.6 Hippocampi

The hippocampi have been shown to play a strong role in the encoding of declarative memory. Episodic memory, one type of declarative memory which involves the memory of episodes in an individuals life, has been shown to be impaired after bilateral ablation of the hippocampi. In particular, individuals can have pronounced anterograde amnesia as well as retrograde amnesia for a year to several years prior to the damage. It is believed that the former amnesia results from the fact that the hippocampi can no longer participate in the encoding of episodic memories. However, long-term memories are eventually encoded in the neocortex, a process which is believed to require about a year (Henke 2010). Thus, some retrograde amnesia occurs during the encoding process due to the fact that some of the memories are encoded in the hippocampi and have not yet been incorporated into the neocortex. It is believed that the encoding of new memories is facilitated by long-term potentiation. This refers to the increased efficacy of synaptic connections due to repeated excitation. It has been demonstrated that short-term
stress inhibits long-term potentiation in the hippocampi (Kim and Diamond 2002). Moreover, excessive exposure to stress hormones has an indirect neurotoxic effect, preventing the neurons from recycling glutamate (Sapolsky 2000). Excess extracellular glutamate triggers protein channels to open that allow a rapid influx of Ca2+, often resulting in cell death (Choi 1994).

It is possible that the fluctuations in the DMN itself contribute to the memory retrieval process that is characteristic of daydreaming, thereby strengthening memory traces (Vincent, Snyder et al. 2006). There are also important differences between the left and right hippocampi. The left hippocampus demonstrates greater activation during the encoding of verbal and semantic memory than the right hippocampus. Verbal memory consists of a knowledge of words and their meanings whereas semantic memory consists of a knowledge of facts about the world. Episodic memory, which refers to a knowledge of past events and consists primarily of visual memories, is supported by both hippocampi. However, the encoding of emotionally valenced episodic memories preferentially activates the right hippocampus (Gerdes, Wieser et al. 2010). In addition, contextual memory refers to a knowledge of the features of a particular location and their arrangement. Both hippocampi support contextual encoding, though the right hippocampus demonstrates greater BOLD activation. A much broader category of memory is autobiographical memory, which consists of information about one's self. It includes episodic, semantic, and contextual memory. Due to its semantic component, the encoding and retrieval of autobiographical memories occurs primarily through the left hippocampus (Cabeza and St Jacques 2007).
1.4.7 Amygdalae

The amygdalae are major structures in the limbic system which are responsible for connecting emotions to stimuli. They consist of two major nuclei: the corticomedial and basolateral nuclei. The former process information pertaining to taste and pain. The latter, on the other hand, are involved in emotional and sensory processing. Stimulation or damage to the amygdalae may result in a number of physiological and affect related effects. Damage to the amygdalae in monkeys causes a decrease in aggressive behavior. In humans, damage to the amygdalae impairs the fear response, preventing the activation of the fight or flight physiological mechanisms. Thus, although a person may feel cognitive anxiety when confronted with a threatening stimulus, all physiological signs remain normal. Damage to the amygdalae also hampers an individual’s ability to recognize facial expressions, especially fear. Electrical stimulation of the basolateral nuclei causes animal subjects to search for external threats. Strong stimulation of the basolateral nuclei induces fear or rage. In humans, stimulation of the amygdalae during surgery induces anxiety and/or hallucinations. It has been demonstrated, furthermore, that damage to the amygdalae impairs emotional learning, particularly reward/punishment conditioning (Cabeza and St Jacques 2007).

When viewing emotional faces, especially faces showing anger or fear, subjects demonstrate amygdalae activation (Liberzon and Martis 2006). Furthermore, the right amygdala is preferentially activated when individuals recall emotional pictures (Dolcos, LaBar et al. 2005). In addition, studies have indicated that the amygdalae also play a role in fear extinction (Rauch, Shin et al. 2006).
1.5 History of the Default Mode Network

Raichle first coined the term default mode in his paper "A default mode of brain function" (Raichle, MacLeod et al. 2001). He hypothesized that there is a default pattern of brain activation when an individual is at rest. Moreover, he asserted that despite differences in cerebral blood flow and the cerebral metabolic rate of oxygen across the brain, the brain has a uniform oxygen extraction factor (OEF) in the default mode. By imaging three groups of subjects, Raichle confirmed that the OEF was uniform across the brain for the eyes open rest condition. In order to discuss the brain activity that is characteristic of the default mode of brain function, Raichle reviewed studies that identified regions which were deactivated when subjects performed tasks. He believed that such regions supported the functions of the default mode of brain function. In particular, he discussed Shulman's meta-analysis of PET studies, which identified regions in the DMN (Shulman, Fiez et al. 1997). He suggested that the precuneus and PCC facilitate the passive monitoring of the environment. To support this claim, he referenced a study discussed previously in which the cortical activity of the precuneus was monitored in monkeys using electrodes (Baker, Petersen et al. 1981). In addition, Raichle posited that the mPFC supports the integration of emotional information into higher order cognitive processes. He argued, therefore, that the mPFC, PCC, and precuneus act in concert to support monitoring of a person's environment and state.

The functional connectivity of brain regions was first studied using BOLD imaging by Biswal (Biswal, Yetkin et al. 1995). He studied the resting-state oscillations in the motor cortices. On the other hand, BOLD functional connectivity of the DMN regions was first investigated by Greicius (Greicius, Krasnow et al. 2003). He attempted to provide additional support for the default mode hypothesis presented by Raichlie. In
particular, he sought to find indications of interactivity between the brain regions which were identified as being deactivated when subjects engaged in attention demanding tasks.

Research into the properties of the DMN indicates that the oscillations continue to occur in some stages of unconsciousness. For example, research indicates that the oscillations continue during the initial phases of sleep and light sedation (Larson-Prior, Zempel et al. 2009). DMN oscillations subside, however, during deep sleep (Horovitz, Braun et al. 2009). Furthermore, oscillations occur in patients in vegetative states, but not in patients who are brain dead (Boly, Tshibanda et al. 2009). These characteristics support the interpretation that the DMN serves as a priming system which allows an individual to immediately process information upon waking.

It is also interesting to note that the DMN evolves as a person ages. In infancy, the anterior and posterior portions of the DMN do not oscillate in synch. Furthermore, less of the brain is recruited by the two networks in children than adults. This is especially true in the prefrontal cortex. Children, in particular, do not have as much activation in the dLPFC, regions which have been noted to be responsible for working memory. This perhaps reflects the limited ability of children to plan for future events in response to current stimuli (Fair, Cohen et al. 2008). The development of synchrony between the mPFC and posterior components has been suggested to underlie the development of a self-identity. Episodic memory recall and encoding is integrated with social cognition. However, researchers have hypothesized that early life abuse may disrupt the development of the DMN (Daniels, Frewen et al. 2011).

In older individuals, the extent of DMN activation decreases, especially in the PCC. Furthermore, there is also a gradual decrease in connectivity that occurs between
the posterior and anterior portions of the DMN. Researchers have hypothesized that this may occur as a result of the degradation of white matter tracks with aging and may be associated with natural cognitive decline (Andrews-Hanna, Snyder et al. 2007).

In his paper "Spontaneous attentional fluctuations in impaired states and pathological conditions: A neurobiological hypothesis," Sonuga-Barke hypothesized that the DMN might be altered in certain pathologies such as attention deficit disorder (Sonuga-Barke and Castellanos 2007). In particular, he argued that the DMN might be activated at inappropriate times. The activation disrupts attention and changes an individual's focus. He supports his claim with the fact that task performance is hindered when DMN regions are activated.

However, other researchers have noted that decreased connectivity to the mPFC may also reflect pathology. One of the hallmarks of PTSD, for instance, is the loss of the ability to regulate emotions. Because the mPFC provides top-down regulation of emotions, Ochsner hypothesized that it should be desegregated from the DMN in PTSD (Ochsner and Gross 2005). This is supported by studies of the survivors of childhood abuse, who demonstrate decreased connectivity between anterior DMN regions, such as the mPFC, and posterior DMN regions. This may underlie some of the problems PTSD patients have with regulating episodic memory retrieval and stress responses.

Investigators have argued that repeated abuse interrupts the development of the DMN. As a result, individuals with PTSD have DMNs that more closely resemble the DMNs in children.

One feature of PTSD is the disruption of memory retrieval. While most PTSD patients readily recall details relating to their traumas, they have impaired memories for
non-trauma related material (McNally 1998). In addition, researchers have noted that individuals with PTSD have difficulty delineating the events of their traumas, which suggests that the autobiographical memory retrieval was disrupted. Impaired autobiographical memory, however, can extend beyond the trauma. Furthermore, individuals with PTSD have overgeneralized autobiographical memory. This is believed to occur because the memory retrieval process itself is disrupted. Autobiographical memory retrieval is believed to occur in phases: more general memories are retrieved first, followed by more specific details. In individuals with PTSD, the memory recall process is interrupted before the second phase. Individuals with PTSD also have very pronounced deficits in verbal memory. This may be due to the fact that many individuals who develop PTSD experienced childhood trauma. The extent of childhood trauma negatively correlates with the size of the left hippocampus, suggesting that it is damaged by excessive stress exposure (Sapolsky 2000).

Studies have also examined the effects of abnormal connectivity between different brain regions in the DMN. For example, autistic individuals have been demonstrated to have almost no PCC connectivity to the mPFC. This is believed to underlie the fact that such individuals typically have impaired social abilities and a disrupted sense of self. As mentioned before, the mPFC plays a strong role in self-referential thought and social cognition, thus supporting the listed functions. Individuals with Alzheimer's show a similar lack of connectivity to the mPFC, perhaps underlying their similar set of symptoms (Wermke, Sorg et al. 2008).

Recently, Lanius at the University of Western Ontario carried out a MVC study similar to the present study. She recruited 15 subjects who had been involved in major
motor vehicle collisions. Nine subjects were scanned approximately six weeks after their MVCs, while six subjects were scanned 12 weeks after their MVCs. All subjects were assessed for PTSD using CAPs 6 weeks and 12 weeks after their scans. A correlation analysis of the CAPs scores with the PCC connectivity at the time of the subjects' scans revealed positive correlations with the ACC. She also performed correlation analyses with the PCC connectivity at six weeks and the CAPS scores acquired 12 months after the subject MVCs. PCC connectivity to the right amygdala was positively correlated with the CAPs scores. She suggested that the greater connectivity to the ACC for the concurrent scans and CAPs may have reflected subject awareness of symptomology. However, she did not speculate as to the cause of the correlation between the PCC connectivity to the right amygdala and the 12-week CAPs scores (Lanius, Bluhm et al. 2010).

An additional PTSD functional connectivity study was conducted in China following an 8.0 magnitude earthquake (Lui, Huang et al. 2009). Subjects were scanned within 25 days of the earthquake. Comparing the resting-state scans between 44 earthquake survivors and 32 controls, they determined that there was decreased connectivity between limbic regions such as the amygdalae and ACC as well as decreased connectivity between DMN regions such as the mPFC and PCC. They concluded that the findings were consistent with current PTSD literature. In particular, they suggested that the decreased connectivity between the limbic regions might reflect decreased emotion regulation in response to stress.

Ultimately, the last two articles present findings which are important for predicting the possible patterns of connectivity during the acute and chronic stress
periods. Lanius's MVC paper suggests that there will be increased PCC connectivity to the ACC that is correlated with stress symptoms. Awareness of symptoms possibly lead to greater priming of the ACC in order to down-regulate emotions. We expect to see a similar result during the acute stress period. On the other hand, Lui's paper suggests that there may be decreased PCC connectivity to the mPFC during the acute stress period. However, this analysis did not examine the relationship between stress symptoms and PCC connectivity. It is possible that the trauma was much more severe in the earthquake study than the MVC study, leading to a pattern of brain activation that is consistent with hyper-arousal symptoms and decreased emotion regulation (Lanius, Vermetten et al. 2010). This differs from the expected PCC connectivity for individuals who eventually recover from stress and is more consistent with Ochsner's findings in the victims of childhood abuse.
2 Materials

In order to perform functional connectivity analyses, we acquired resting-state MRI scans as well as respiratory and heart rate data. The material section describes the hardware and software utilized to acquire and process the data. The hardware consisted of the MRI scanner, the physiological acquisition devices, the paradigm computer, the goggle system, and the interface devices (cables and optical isolator). Software used to analyze the data included SPM, SPM add-ons, and SPSS. The data processing pipeline is described and discussed in the methods section.

2.1 Equipment

Our equipment allowed us to accomplish three tasks: acquire raw fMRI and physiological data, synchronize data acquisition, and display images to subjects during scanning. The MRI scanner, the respiratory strap, and the pulsox monitor allowed us to acquire fMRI, respiratory rate, and heart rate data respectively. The paradigm computer allowed us to synchronize the data and generate the images viewed by the subjects. The goggle system allowed subjects to view the generated images. The subject preparation and scanning procedure are discussed in the methods section.

2.1.1 Scanner

Subjects were imaged with a three-tesla GE Signa MRI scanner. Physiological data, which included breathing and heart rate measurements, was acquired using a pulsox and respiratory strap. The devices directly interfaced with the MRI scanner. The
physiological data was displayed on the MRI control console and outputted through a serial cable to the paradigm computer on which we collected and processed data. In addition, whenever a slice was acquired by the scanner, a +5V pulse was generated in a BNC cable. This pulse was used to synchronize our data acquisition with the start of each resting-state scan.

### 2.1.2 Paradigm Computer

A Dell desktop PC with 3 GHz and Intel(R) Core(TM) Quad CPU was used to run Matlab and PowerPoint, receive data from the MRI scanner, and interface with the goggle system. It was equipped with two monitors: one monitor displayed the experimenter workspace while the other monitor displayed the image the subject viewed through the goggles. The serial cord carrying the physiological data packets interfaced directly to a serial port. However, the BNC cable was not connected directly to the computer. In order to prevent electronic feedback into the scanner, the BNC was connected to an optical isolator, which used a light emitting diode and sensor to transmit a signal in one direction between electrically isolated circuits. Voltage into the input circuit caused the diode to alight. When the sensor detected the light, it generated a corresponding voltage pulse. The output of the optical isolator was interfaced with a serial cable, which connected to the paradigm computer.

### 2.1.3 Goggle System

During the resting-state scan, the subjects viewed a white fixation cross-hair on a black background using a pair of NordicNeuroLab goggles. The goggle system consisted of three components: the computer interface module, the goggle interface module, and the goggles themselves. The computer interface module received power from an external source and connected directly to the computer via a serial port. This module was also
connected to the goggle interface module via a fiber optic cable. The former module was situated on the cart housing the computer during subject scanning while the latter module was positioned inside the scanner room away from the scanner itself (the module contained ferromagnetic material). The fiber optic cable traveled through a hole in the MRI room's copper RF shield which prevented unwanted interference with the MRI receiver coils. The goggles connected to the goggle interface module via an insulated cord and were mounted on the MRI head coil.

2.2 Software

Our software enabled us to process the imaging and physiological data, perform statistical analyses, and display our results on brain templates. We utilized SPM to perform our primary data processing and analysis. The add-ons xjView, Marsbar, and VBM allowed us to perform advanced analyses discussed below. SPSS allowed us to perform statistical tests on survey data.

2.2.1 SPM

Image analyses were performed using SPM, a Matlab software package developed by The Wellcome Trust Centre for Neuroimaging at the University College of London. It consisted of a number of tools for brain imaging analysis including fMRI functions for preprocessing, first level analysis, and second level analysis. Preprocessing steps include realignment, slice-timing correction, coregistration, normalization and smoothing. The functions are discussed in greater detail in sections 3.8-3.10.

2.2.2 SPM Add-Ons: xjView, Marsbar2.0, and VBM

SPM add-ons xjView and Marsbar2.0 were used for advanced visualization of results and advanced region of interest analysis, respectively. Xjview includes several brain atlases that allow clusters of significance to be analyzed. In particular, it specifies
the number of voxels within each cluster, the peak intensity voxel, template regions that intersect the clusters, and the number of cluster voxels that intersect the template regions. Marsbar provides tools for constructing ROIs and extracting data from SPM results. For instance, Marsbar allows users to create spherical ROIs, define ROIs based on brain anatomical templates, define ROIs based on imaging results, and perform set operations on the ROIs. Mean image intensity values can also be extracted for ROIs. VBM—an acronym for voxel based morphology—is an SPM add-on which provides additional segmentation tools. It uses probability maps obtained from prior imaging studies to assess whether voxels with particular intensities should be classified as gray matter, white matter, or cerebral spinal fluid, and was used to extract white matter, grey matter, and cerebral spinal fluid masks (Ashburner and Friston 2000).

2.2.3 SPSS

SPSS 19 was used to analyze survey data. Maintained and licensed by IBM, it provided the tools used to perform our study's statistical tests, including Pearson correlations and paired and unpaired T-tests.
3 Methods

3.1 Recruitment

Adult patients who were involved in MVCs were recruited from the University of Toledo Medical Center Emergency Department. Subjects were at least 18 years of age and had not experienced any injuries which would prevent them from undergoing an MRI scan. Patients with mild concussions and/or memory loss were eligible. The recruiters explained the nature of the study to the patients, and asked whether they would be willing to participate. All participating subjects signed a consent form approved by the University of Toledo Institutional Review Board (IRB) and completed an initial survey packet containing a number of psychometric instruments, including the PCL. These provided a measure of the subjects' initial stress symptoms.

3.2 Timeline of Subject Activities

Subjects who participated in the study were asked to undergo two MRI scans. The first was scheduled within three weeks after the MVC, while the second was scheduled three to four months after the initial scan. Subjects also received survey packets on a monthly basis after the MVC, which included the PCL survey. For the purpose of our study, only the initial and final surveys were evaluated. In addition, the subjects were administered a CAPS interview by one of two psychology students three months following the MVCs. The CAPS was given on the same day as the second fMRI scan for most subjects.
3.3 Positioning Subjects in Scanner

After a brief training session, each subject was brought into the MRI scanner room. The technician provided the subject with earplugs and requested that the subject lie on the scanner table. A respiratory strap, which had previously been placed on the table, was then fastened around his or her waist. A standard 8-channel head coil was positioned over each subject's head. The goggles, which mounted on the head coil, were positioned over the subject's eyes. The subject was then asked to position and focus the goggles so that he or she could clearly read displayed text. When the subject finished adjusting the eyepieces, the MRI technologist placed a pulsox on the subject's finger to record cardiac activity throughout the scan. The subject was also given an emergency squeeze ball, which could activate an alarm if the subject felt unable to continue. The subject was then positioned in the MRI. During the resting-state scan, the subject was required to fixate on a white crosshair on a black background without focusing on any train of thought. This replicated the conditions described by Raichle for optimal activation of the DMN.

3.4 Stress Related Surveys

3.4.1 Posttraumatic Stress Disorder Checklist

The Posttraumatic Stress Disorder Checklist (PCL) is a psychometric instrument that assesses the symptomatic criteria for PTSD as designated by DSM IV. There are three versions of the scale: military, civilian, and specific versions (McDonald and Calhoun 2010). The lattermost, which was utilized for this analysis, addresses symptoms that arise from a specific traumatic event—an MVC in this case. Questions 1-5 evaluate diagnostic criteria B, or intrusive recollection, for PTSD. For example, question one asks whether the subject experiences "Repeated, disturbing memories, thoughts, or images of the stressful experience." The second group of questions (6-12) evaluate criteria C, or
avoidance behavior and numbing sensations. Question 7 asks whether the subject is "Avoiding activities or situations because they reminded you of the stressful experience." Questions 13 through 17 evaluate criteria D, or hyper-arousal. For example, question 16 asks whether the subject feels as if he or she is "Being super alert or watchful or on guard." For all 17 questions, the subjects respond on a Likert scale from 1 (Not at all) to 5 (Extremely).

3.4.2 Clinician Administered Posttraumatic Stress Disorder Scale

The Clinician Administered Posttraumatic Stress Disorder Scale (CAPS) was administered approximately three months after the subjects were involved in collisions. The scale was developed at the National Center for PTSD. It consists of a series of questions which assess the severity and frequency of PTSD symptoms, the impact of the symptoms on social and occupational functioning, improvement of PTSD symptoms since the last CAPS evaluation, the response validity, and the overall PTSD severity (Weathers, Ruscio et al. 1999). Subjects were diagnosed with PTSD if they had one criteria B symptom (re-experiencing), three criteria C symptoms (avoidance/numbing), and two criteria D symptoms (hyper-arousal). The total score for each subcategory reflected the intensity and the frequency of the symptoms.

3.5 MRI Scans

3.5.1 Localizer

A three-plane localizer scan was obtained after a subject was situated in the scanner. The acquired volume was used to align the slices of future scans. The technologist aligned the slices in order to provide complete coverage of the cerebral cortex. Sagittal planes were aligned parallel to the hemispheric midline. If possible, the
technologist also attempted to align the axial planes parallel to the top of the corpus collosum.

3.5.2 Resting-State fMRI

The initial resting-state scan utilized an eight minute EPI/GR protocol. A total of 34 slices for 240 phases were obtained (TR=2000ms, TE=30ms, matrix=64 × 64, voxel dimensions=3.75 × 3.75 × 3.5 mm).

3.5.3 Spoiled Gradient Echo

A high-resolution anatomical image was obtained with a 3D spoiled gradient (SPGR) scan. The scan lasted 11 minutes and 15 seconds, recording 164 axial slices (TR=7.9 ms, TE=3ms, TI=650 ms, matrix=252 × 248, voxel dimensions=1 × 1 × 1mm).

3.5.4 Overlay

An additional T1 weighted scan was obtained to overlay the fMRI scan to the SPGR. The scan lasted two minutes, recording 34 slices (TR=250, TE=3.6, flip angle=90°, matrix=256 × 256, voxel dimensions= 1 × 1 × 3.5 mm).

3.6 System Check

After the subject was positioned in the scanner, we confirmed that the paradigm computer was receiving physiological data. The scanner transmitted the data via a standard serial cable in packets consisting of six 16 bit integers. The first integer was unsigned and served as a counter variable which was incremented for each packet; when the counter reached its maximum value it would restart at the minimum value. The second and third integers received data from additional cardiac leads which were not used in our setup. The fourth and fifth integers consisted of the pulsox and respiratory data respectively. We used Matlab to receive and record the data. To insure that the computer did in fact receive the data, incoming packets from the communication port were
recorded for 10 seconds. The integers from the individual packets were then rebinned and plotted in six independent graphs. If graphs 3 and 4, corresponding to the third and fourth unsigned integers in the packet, displayed the cardiac and respiratory activity respectively, then it was determined that the computer was receiving data.

3.7 Rebinning Data

In some instances, Matlab, which reads incoming serial data in individual bytes, parsed the incoming packets incorrectly. Sometimes data would start recording mid-packet. In fact, in some cases, the data would record mid-integer. To correct this problem, Matlab scripts were written to remove the first incomplete packet and reparse the integer values.

3.8 Image Processing

3.8.1 Introduction to Processing

Processing of MRI imaging data was performed in five steps: physiological correction, preprocessing, region of interest (ROI) building, segmentation, and nuisance filtration. Each processing step was performed by either a bash or Matlab script. The physiological data removed influences of cardiac and respiratory activity. The preprocessing script normalized, coregistered, and smoothed the functional images and anatomical images. The ROI building script defined the PCC ROI which would be used in the connectivity analysis. The segmentation analysis defined the white matter, grey matter, and CSF masks that would be used in the next scripts. The nuisance script modified the white matter and cerebral spinal fluid masks to correct for subject motion. The segmentation realignment script realigned those masks with the SPGR anatomical image.
3.8.2 Physiological Correction

During the initial phase of the processing, we ran the data through a script provided by Dr. Scott Peltier at the University of Michigan. The script performed a convolution operation on the respiratory and cardiac data, removing noise. The processed data were entered as regressors to remove physiological artifact from the fMRI data. The script also performed a realignment operation. Although we instructed the subjects to remain still during their scans, unintentional movement on the order of millimeters was normal. The realignment operation used the first brain volume acquired as a template, translating and rotating each successive brain volume into alignment with the original. In addition, the program performed a slice timing correction operation in order to correct for the fact that the voxels in separate slices were acquired at different times. Since the voxel intensities vary as a function of time, an interpolation operation is used to estimate the voxel intensity values for a common time point over the entire volume.

3.8.3 Preprocessing

The preprocessing script performed the co-registration, normalization, and smoothing operations. During co-registration, two volumetric images are aligned to one another using anatomical landmarks. The preprocessing script first co-registered the overlay to the SPGR. The fMRI resting-state scans were then co-registered to the overlay. In addition, the resting-state scans were normalized to the T1 weighted image included in SPM5's canonical template folder. The brain was aligned with the standard Montreal Neurological Institute (MNI) brain space, a template defined by averaging the brain images of a large collection of healthy subjects (Chau and McIntosh 2005). It should be noted that the template image had not been skull stripped. The normalization process warps the volumes in the resting-state scan through rotations, translations, and resizing.
operations in order to align them with the template. In the last step of the preprocessing script, the resting-state data was smoothed with a Gaussian kernel defined with a full width at half maximum of 8mm in the x, y, and z directions.

3.8.4 Region of Interest Building

One subject included in the analysis was selected as a template to provide the coordinates for the ROI. Selecting the subject at random, the ROI script called the ROI Builder function in SPM5 to create a 10mm radius sphere centered at MNI coordinate (0, -56, 20). The latter coordinate corresponded with the location of the PCC in standard anatomical space, and was chosen based on Lanius's prior work (Lanius, Bluhm et al. 2010). The output image consisted of the spherical mask centered at the coordinate in the subject's space.

3.8.5 Segmentation

Each SPGR image was additionally used to create white matter, grey matter, and cerebral spinal fluid masks. Segmentation was performed using VBM. These masks were refined in the nuisance-filtering step and combined with the spherical ROI in the first-level processing script in order to define the seeds for the functional connectivity analyses.

3.8.6 Nuisance Filtering

The nuisance filtering steps refined the white matter and the cerebral spinal fluid masks by applying more stringent processing conditions than VBM. In particular, the script selected all of those voxels which had voxel intensities greater than .85 and which had twenty neighboring voxels. All those voxels which did not have 20 neighbors were excluded from the map. The script then refined the map again to determine which voxels still had 20 neighbors. Some of the voxels which had 20 voxels during the first check no
longer satisfied that condition because some of their neighbors had been removed. For the cerebral spinal fluid masks, on the other hand, the script only required that each voxel have eight neighbors during the second step. The required number of neighbors was smaller because there were fewer voxels in the cerebral spinal fluid map in general. These scripts essentially reduced the number of voxels in the boundaries of the masks. The complement of the union of the cerebral spinal fluid mask and the white matter mask was used to create the grey matter mask that was used in the first level analysis.

3.9 First Level Analysis

The nuisance images, the grey matter mask, the overlay, and the fMRI images for each subject were entered into the first-level analysis script. The time course for each voxel was passed through a bandpass filter for the frequency range .1Hz to .01Hz to prevent spurious signals from influencing the analysis. The script extracted the mean time course for the intersection of the PCC ROI with the grey matter map for each of the subjects. A correlation analysis was performed with the extracted time courses and the respective fMRI scans. This yielded maps in which each voxel intensity value represented a Pearson correlation coefficient. In order to improve the range of values represented, the Pearson correlation coefficients were entered into a Fisher-Z transformation, which mapped the values from $-\infty$ to $+\infty$. The resulting maps indicated the PCC functional connectivity, or the degree to which activity in individual voxels correlated with activity in the PCC grey matter ROIs.

3.10 Second Level Analysis

Statistical tests were performed to study changes in PCC connectivity over time. These tests allowed us to visualize the DMN in the initial stage after the MVC and in the final stage after the recovery from the initial stress. In addition, the tests allowed us to
quantify the changes between scans. Correlation analyses allowed us to examine the relationships between stress symptoms and connectivity.

3.10.1 Single Sample T-Tests

The initial and final connectivity maps for the subjects were each entered into a single sample T-test to identify regions in which the connectivity, as represented by the voxel z-score values, differed significantly from zero. The voxel intensity values in the two generated maps represented the T-values. This allowed us to visualize the DMN and perform a preliminary subjective analysis at each time-point.

3.10.2 Difference Maps

In order to perform regression analyses on the change in PCC connectivity over time, we needed to calculate the difference between the connectivity maps acquired at two weeks and three months. Each connectivity difference map was created using SPM's imageCalc utility to perform the voxel-wise subtraction of the initial PCC z-score map from the final PCC z-score map. The maps represented the change in PCC functional connectivity over three months.

3.10.3 Single Sample T-test of Difference Maps

A single sample T-test of the difference maps was used to quantitatively analyze the differences between the initial and final connectivity maps for those subjects who completed both scans while using age and gender as covariates. This analysis would not have been possible using a simple paired T-test. The voxels in the resulting map represented the T-scores for the difference between the two maps. By selecting a threshold of significance, brain regions in which the connectivity changed significantly could be visualized. In addition, reports were generated for the clusters of significant difference using xjView. This allowed us to use the standard Anatomical Automatic
Labeling (aal) template to label each cluster for analysis (Tzourio-Mazoyer, Landeau et al. 2002).
4 Results

The results section covers the basic subject statistics as well as the image analyses. The statistics, most importantly, revealed how our sample's stress symptoms varied over time. The image analyses revealed how the DMN changed over time. In order to address our hypotheses, we performed two types of analyses on the PCC connectivity maps: T-tests and whole brain correlation analyses. The T-tests allowed us to visualize the group DMN at two weeks and three months. A single sample T-test on the difference maps, furthermore, allowed us to visualize the significant changes over time. The T-tests investigated the first hypothesis that there would be a decrease in PCC connectivity to the other DMN regions between two weeks and three months. Furthermore, we performed whole brain correlation analyses to identify additional correlations occurring in the DMN and thereby investigate our second hypothesis that PCC connectivity was correlated with stress measures. The significance of each of the correlations is briefly addressed in each of the result subsections; they are explored in greater detail in the discussion section.

4.1 Sample Statistics and Survey Results

A total of 34 subjects completed the initial scan without a diagnosis of PTSD three months later. However, two subjects could not be included in the connectivity analysis due to problems with their physiological data: one subject's data was overwritten and another subject's physiological data was of poor quality. Thus, 32 subjects (17 f, 15 m) were included in the subject pool for the initial time-point analysis. The data for the
subjects is displayed in Table A.1. The mean (±std) subject age was 35(±11) years. Subjects completed their first scan a mean (±std) of 9(±5) days after their MVCs. The mean (±std) PCL score for the initial survey was 37(±14). The survey was completed a mean (±std) of 8(±5) days after the MVC for 28 of the 32 subjects; the survey completion dates were not recorded for 4 subjects.

A total of 18 subjects completed the second scan and were not diagnosed with PTSD. One subject, whose initial physiological data was overwritten, was not included in the final scan analysis. The final group thus consisted of 17 subjects (11 f, 6 m). The mean (±std) initial PCL score for that group was 36(±12), whereas the final mean (±std) PCL score was 27(±11). These subjects completed the survey a mean (±std) of 9(±5) days after their MVCs. The subjects completed their first scans a mean (±std) of 9(±5) days after their MVCs and their second scans 113(±26) days after their MVCs. They completed their final surveys 107(±14) days after their MVCs. A paired T-test revealed a statistically significant difference (p<.001) between the initial and the final PCL scores for the 17 subjects. The change in PCL scores over time for the 17 subjects is displayed in Figure 4-1. Researchers frequently use a PCL score of 44 as the cutoff for predicting PTSD in civilian samples, though some researchers advocate using a cutoff score as low as 30 (McDonald and Calhoun 2010). The mean initial PCL score for our sample was below the most common cutoff, but not below the minimum. On the other hand, the mean final PCL score for the sample was below the minimum cutoff. This suggests that the subjects, as a group, recovered from the stress of the trauma over three months.
4.2 Single Sample T-Tests of Functional Connectivity Maps

The Single T-tests at the two week and three month time point allowed us to observe the qualitative change in PCC connectivity over time. By performing a T-test on the difference maps, we were able to identify changes in the DMN which were statistically significant.

4.2.1 Single Sample T-Test for the Three-Week Posterior Cingulate Cortex Connectivity Map for 32 Subjects, Correcting for Age and Gender

The DMN was identified using a PCC seed to detect correlations across the whole brain. We entered the connectivity maps for the 32 subjects who completed the initial scan and satisfied the scanning criteria into a single sample T-test, correcting for age and gender.
gender. Positive and negative clusters, having a minimum of 5 voxels, were identified using a false detection rate correction and a p-value of less than .05.

Clusters with significant positive T-values were identified in the bilateral IPC/visual cortical regions, parahippocampi/hippocampi, LTCs, dIPFC, mPFC, and ACC. The DMN regions we observed were consistent with those previously identified regions in the literature. These regions are displayed in the axial slices of Figure 4-2.

Figure 4-2: Axial slices for the single sample T-test of the 2-week PCC connectivity maps. The color bar at the left indicates the range of positive T-values and the text in top left-hand corners of the images indicate the MNI coordinate of the slice. The arrow in image A points to the right dlPFC. The topmost arrow in image B points to the mPFC while the bottommost arrow points to the right IPC/visual cortex. The topmost arrow in image C points to the left LTC while the bottommost arrow points to the right parahippocampus/hippocampus.
4.2.2 Single Sample T-Test for the Three-Month Posterior Cingulate Connectivity Map for 17 Subjects, Correcting for Age and Gender

The significant PCC functional connectivity at three months was also identified for the 17 subjects who successfully completed the second exam. The z-maps for the individual subjects were entered into a single sample T-test analysis in SPM 5, correcting for age and gender. Positive and negative clusters, having a minimum of 5 voxels were identified using a false detection rate correction with a p-value of .05.

The analysis yielded the DMN regions, shown in Figure 4-3, including the PCC (the seed), bilateral IPC/visual cortical regions, parahippocampi/hippocampi, left LTCs, and mPFC. However, the clusters no longer encompassed the right LTC, the dlPFC, and the ACC. Furthermore, the extent of the connectivity to the remaining DMN regions appeared to decrease. These results appeared to be consistent with our first hypothesis that connectivity of the DMN would decrease over time.
In order to investigate the change in the PCC functional connectivity between the initial and final scan, we performed a single sample T-test on the connectivity difference maps, entering age and gender as covariates. Only the 17 subjects who completed both the initial and final scans were included in the analysis. Significant clusters having a minimum of five voxels (k=5) were identified with an uncorrected threshold of p=.001. The image results are displayed in Figure 4.4, and are outlined in Table A.2.

4.2.3 Single T-Test for the Posterior Cingulate Cortex Connectivity Difference Map for 17 Subjects, Correcting for Age and Gender

Figure 4-3: Axial slices (the same MNI slice coordinates as Figure 4-2) for the single sample T-test of the 3-month PCC connectivity maps. The color bar at the left indicates the range of positive T-values and the text in the top left-hand corners of the images indicate the MNI coordinate of the slice. Image A displays the axial slice for MNI z=40; the dlPFC is not present. The arrow in image B points to the left IPC/visual cortex. In image C, the top left arrow points to the left MTC, the bottom left arrow points to the right parahippocampus/hippocampus, and the top right arrow points to the ventral mPFC.
Significant decreases in PCC connectivity (regions with negative z-score differences) were identified in the dLPFC, the ACC, and the left IPC/visual cortex. These results provided additional support that the connectivity of the DMN regions decreased in the months following a trauma. In the discussion, we will address the significance of the changes as they pertain to stress symptoms and clusters in the ACC, PFC, and left IPC/visual cortex.

4.3 Results for the Whole Brain Correlation Analyses

The correlation analyses identified relationships between PCC connectivity and PCL scores. They allowed us to test the second hypothesis that connectivity of DMN regions at two weeks and three months is related to reported stress. Moreover, we investigated whether the change in PCC connectivity over the three months was correlated with changes in stress scores. That analysis allowed us to study the dynamic nature of the DMN and identify stress related changes that were not identified in the
single time point analyses due to natural variability in the DMN connectivity. The analyses ultimately revealed significant correlations in the left IPC/visual cortex, the hippocampi, and the right amygdala.

4.3.1 The Whole Brain Correlation between the Two-Week Posterior Cingulate Cortex Connectivity and the Initial Posttraumatic Stress Disorder Checklist Scores for 32 Subjects, Correcting for Age and Gender

A whole brain correlation was performed for the two-week PCC functional connectivity maps with the initial PCL scores. This investigated the relationship between priming of the DMN regions immediately after the accident and facilitated the investigation of hypothesis two. A total of 32 subjects completed the first scan within three weeks after their accidents; they all completed the initial PCL survey.

The analysis yielded 11 significant correlation clusters (two positive) in aal-defined regions. The results are outlined in Table A.3. However, none of the correlations occurred in the hypothesized regions. This may be due to the high variability of the PCC connectivity between the subjects. The change in connectivity over time in relation to changes in stress symptoms may give a better indication of how connectivity is affected by exposure to a trauma.

4.3.2 The Whole Brain Correlation Between the Three-Month Posterior Cingulate Cortex Connectivity and the Final Posttraumatic Stress Disorder Checklist Scores for 17 Subjects, Correcting for Age and Gender

A whole brain correlation analysis was performed for the three-month PCC connectivity maps and the final PCL survey scores in order to examine the relationship of residual stress on DMN connectivity three months after MVC. Negative correlation clusters identify regions where the individuals with higher stress scores at three months had less PCC connectivity. Because we studied a healthy sample, we hypothesized that
there would be positive correlations in the hippocampi, ACC, mPFC, and the IPCs/visual cortices. A total of 17 subjects, all of who completed the final scan and survey, were included in the analysis. The image results are displayed in Figure 4-5 and are outlined in Table A.4.

A total of 12 significant correlation clusters (seven positive) were identified in aal defined regions. A significant negative correlation was identified in the right hippocampus, an important stress and DMN related region. This correlation suggests that individuals with increased PCC connectivity to the right hippocampus had decreased stress symptoms. The right hippocampus in particular is activated during the recall of contextual and visual information (Addis, Moscovitch et al. 2004). Contextual or visual reminders of a stressful event often trigger stress symptoms themselves (Liberzon, Taylor et al. 1999). However, individuals who have undergone a trauma often have disrupted memories, characterized by impaired memory retrieval for information unrelated to the trauma and intrusive memories for information pertaining to the trauma. Perhaps increased connectivity to the right hippocampus is indicative of normal memory function.
4.3.3 The Whole Brain Correlation between the Change in Posterior Cingulate Cortex Connectivity and the Change in Posttraumatic Stress Disorder Checklist Scores for 17 Subjects, Correcting for Age and Gender

In order to assess the relationship between the change in DMN connectivity and the change in acute stress symptoms, a correlation analysis was performed between the connectivity difference maps and the change in PCL scores. A total of 17 subjects completed both resting-state scans and were included in the analysis; all 17 subjects completed the initial and final PCL surveys. Positive correlations indicated that decreases in stress corresponded with decreases in the regional z-scores whereas negative correlations indicated that decreases in stress corresponded with increases in regional z-scores. The results are displayed in Figure 4-6 and are outlined in Table A.5.

The whole brain correlation analysis identified six clusters (three positive) with voxels in aal-defined regions. A significant positive correlation was identified in the left IPC/visual cortex. On the other hand, significant negative correlations were found in the left hippocampus and the right amygdala. The positive correlation in the left IPC was consistent with our second hypothesis. During the acute stress period, we believe priming...
of the left IPC increases to facilitate the passive monitoring of the visual field. This is related to the stress symptom hyper-vigilance. When stress symptoms decreased, the connectivity to that region decreased. However, the negative correlation in the left hippocampus was unexpected. The left hippocampus plays an important role in the encoding and retrieval of autobiographical memories. We believe that autobiographical memory function decreases during the acute stress phase when an individual devotes more internal resources to monitoring the environment. This is reflected in decreased connectivity to the left hippocampus. When stress symptoms subside three months later, connectivity to the left hippocampus increases, resulting in the observed correlation.

While our hypotheses did not address connectivity to the amygdalae, we believe that the negative correlation in the right amygdala is significant. It suggests that the activity in the right amygdala was down-regulated during the acute stress period when symptoms were high. When the symptoms decreased three months later, the priming of the right amygdala increased.
Figure 4-6: These images display the correlations for the change in PCC connectivity and the change in PCL scores. The red and blue color bars indicate the range of positive and negative T-values, respectively. The red arrow in image A (sagittal slice, MNI x=-21) points to a negative correlation in the left hippocampus. The red arrow in image B (sagittal slice, MNI x=35) points to a negative correlation in the right amygdala. The red arrow in image C (axial slice, MNI z=28) points to a positive correlation in the left IPC/visual cortex.
5 Discussion

This section addresses the significance of the results in terms of the hypotheses and the stress literature. The discussion subsections are arranged according to brain region. Correlations differed between the left and right hemisphere, reflecting the lateralization of function. Two significant findings were observed for the hippocampi, one for the amygdalae, one for the ACC, one for the mPFC, and two for the IPC/visual cortex. The results suggest that there was a change in the way information was processed in the brain in the weeks following the MVC. Specifically, it appears that regions for monitoring external stimuli, such as the IPC/visual cortices were activated while regions such as the hippocampus were deactivated. Furthermore, the results suggest that there was greater priming of brain regions associated with emotion regulation during the acute stress period.

5.1 Hippocampi

There were two significant results for the hippocampi: the negative correlation between the change in PCC connectivity and the change in PCL scores in the left hippocampus and the negative correlation between the three-month PCC connectivity and the final PCL scores in the right hippocampus. These results were contrary to our second hypothesis.

The negative correlation between the change in PCC connectivity and the change in PCL scores may indicate a change in memory encoding and retrieval when an individual is in a stressed state. Subjects whose stress symptoms decreased experienced an increase in PCC connectivity to the left hippocampus. It might be argued that the
individuals who recovered from stress experienced a change in the way they process information. When individuals enter a stressed state, they become increasingly watchful, or hyper-vigilant (Bracha 2004). This may have provided the evolutionary benefit of being more aware of threats following a recent dangerous experience. Increased outwardly directed attention decreases the need for self-referential (autobiographical) memory encoding. Instead visual episodic memory and contextual memory, which provides a knowledge of threat cues for the future, becomes more important. Since autobiographical memory is preferentially encoded in the left hemisphere, where verbal analytic thought provides the foundation for semantic memory, the PCC connectivity to the left hippocampus decreases following the MVC. However, when an individual's stress subsides, the PCC connectivity to the left hippocampus again increases. This leads to the correlation between the change in connectivity and the change in symptoms. The high degree of correspondence between the anatomical boundaries of the hippocampus and the cluster of significance suggests that this correlation is a very strong and important result.

The negative correlation between the three-month PCC connectivity and the final PCL scores in the right hippocampus indicates that those subjects with increased connectivity to the right hippocampus experienced lower stress symptoms. The PCC connectivity to the right hippocampus thus may be a factor that relates to coping with long-term stress symptoms. The right hippocampus, in particular, plays a strong role in the encoding of contextual visual information associated with episodic memory (Burgess, Maguire et al. 2002). It should be noted that one of the defining characteristics of trauma related stress disorders is the disruption of episodic memory encoding for the period following the trauma (Layton and Krikorian 2002). However, some argue that poorer
episodic memory encoding prior to stress exposure is a risk factor for developing symptoms and not caused by the trauma itself (McNally 1998). Perhaps, individuals with superior episodic memory encoding are better at acquiring new memories, which can serve as the focus of internal mentation. In addition, because the right hippocampus plays a stronger role than the left in the recall of emotional episodic memories (McNally 1998), it might be argued that individuals with increased connectivity to the right hippocampus are better at processing emotional memories. It has, furthermore, been noted that individuals with PTSD employ cognitive avoidance strategies that prevent them from emotionally processing the memories relating to their traumas (McNally 1998). This has been suggested to contribute to the maintenance of PTSD symptoms (Ehlers and Steil 1995). Better ability to process emotional memories, as reflected by increased connectivity, may thus be a factor for stress resilience.

5.2 Anterior Cingulate Cortex

Between the three-week and three month scans, there was a statistically significant decrease in PCC connectivity with the ACC. This result was consistent with our first hypothesis. Functional neuroimaging studies of the ACC have indicated that it plays a key role in resolving conflicts between competing cognitive processes (Shin, Whalen et al. 2001). It exhibits increased activation, for example, when an individual performs a type of Stroop test in which he or she must ignore emotionally valenced words. Furthermore, studies show that the ACC demonstrates increased activation when a person attempts to suppress a negative memory (Butler and James 2010). This may be important for actively forgetting details of a trauma during the acute stress period in order to plan for possible future threats. In the case of MVCs, threats could include resulting medical, financial, and/or legal problems. Ultimately, we believe that the ACC plays an
important role in regulating stress during the acute stress period. Although stress symptoms may be high, awareness of the symptoms in healthy individuals may activate the ACC to down-regulate and manage emotions. This is consistent with Lanius's interpretation of her correlation in the ACC between PCC connectivity and concurrent CAPS scores (Lanius, Bluhm et al. 2010). As subjects recovered three months later, as indicated by their decrease PCL scores overtime, the ACC connectivity decreased.

It is possible that some of the change in connectivity was due to familiarity with the repeated scanning procedure. Studies have shown that there is increased activation of the ACC when an individual first attempts a task (Petersen, van Mier et al. 1998). In the future, we will need to compare our results to those of a control group to rule out this effect.

5.3 Inferior Parietal Cortex/Visual Cortex

Two significant results were observed for the IPC/visual cortical regions of the DMN: there were significant decreases in PCC connectivity between the two-week and three-month scans, and there was a positive correlation between the change in PCC connectivity and the change in PCL scores. These results were consistent with our predictions for hypotheses one and two.

Perhaps in the two-week period immediately following the MVC, the increased PCC connectivity to the left IPC reflected the symptoms of increased watchfulness or vigilance. The IPCs themselves play important roles in integrating sensory and cognitive information as well as in passively monitoring the environment (Spreng and Grady 2010). The fact that similar correlations were not observed in the right hemisphere may reflect competition between functions performed by the left IPC and other brain regions. The left hemisphere is well known for being associated with verbal and semantic
processes. It is possible that such processes inhibit connectivity to the left IPC when an individual is in a non-stressed state. It has been noted, for example, that verbal working memory tasks significantly hinder visual attention to detail in the right visual field more than in the left (Hellige and Cox 1976). In contrast, when an individual is in a stressed state, processes that support the monitoring of the environment predominate, leading to increased connectivity to the left IPC. Attention to threat cues following a dangerous situation would have provided an evolutionary advantage. This may also reflect the brain's healthy response to threats. Individuals who have dissociative symptoms, such as decreased awareness of surroundings, immediately after a trauma are more likely to develop severe PTSD symptoms. (Marshall and Schell 2002). Ultimately, as stress subsided, monitoring the external environment became less of a priority. This led to the symptom correlated decrease in PCC connectivity to the IPC/visual cortex.

5.4 Prefrontal Cortex

The primary result for the PFC related to the change in connectivity between the two-week and three-month scans. In the initial scan, there were more regions of significant connectivity in the mPFC and the dIPFC. The former region has been identified as being involved in a number of higher order cognitive processes including self-referential thought and the top-down regulation of emotions (Gusnard, Akbudak et al. 2001). It is possible that the mPFC was primed during the acute stress period in order to down-regulate negative emotions following the MVC. This would have facilitated the ability to cope with stress and respond to additional threats after the trauma. In addition, the dIPFCs are frequently associated with working memory tasks (Greicius, Krasnow et al. 2003) and serve as a means for exchanging information with the attention networks. It is possible that the dIPFCs were primed after the MVC to facilitate communication
between the DMN and the attention networks. This would have allowed individuals to change attention strategies based on the internal mentation.

When subjects returned three months later for their second resting-state scan, the PCC connectivity to the mPFC and dIPFCs significantly decreased. We believe the connectivity changes reflected decreased priming of those regions. The mPFC was no longer primed to down-regulate emotions, and the dIPFCs were no longer primed to facilitate information exchange. However, one must also consider the possibility that scanning familiarity promoted the changes. The connectivity to the mPFC could have decreased due to decreased subject self-consciousness during the second scan. In other words, because the subjects were familiar with the procedure, they were less likely to use self-monitoring and emotion regulation in order to relax in the scanner. Because the dIPFCs demonstrate the greatest connectivity variation of all the DMN brain regions (Shehzad, Kelly et al. 2009), the changes in those regions may have been due to chance. Furthermore, the working memory regions in the dIPFCs may not have been primed during the second scan due to the fact that the scanning procedure had been committed to intermediate memory.

5.5 Amygdala

A significant negative correlation was present for the right amygdala for the whole brain analysis between the change in PCC connectivity and the change in PCL scores. This indicated that connectivity to the right amygdala increased for the average subject whose stress scores decreased over time. This may be related to the down-regulation of the right amygdala by the ACC during the acute stress period. Lanius noted that individuals with dissociative symptoms have greater activation of the ACC and decreased activation of the amygdalae (Lanius, Vermetten et al. 2010). In addition, the
fact that our PCC connectivity to the right amygdala was correlated with the change in symptoms is arguably consistent with Lanius's research in which PCC connectivity to the right amygdala predicted CAPS scores 12 weeks following a MVC (Lanius, Bluhm et al. 2010). In other words, those subjects who were better able to down-regulate activation of the right amygdala in the acute stress period were better able to cope with symptoms in the long-term. As stress symptoms subsided and connectivity to the emotion regulation centers of the brain decreased at three months, the connectivity to the right amygdala increased.

While both amygdalae play important roles in processing emotion, researchers have observed greater excitability in the right amygdala in PTSD patients (Hamann 2001). Furthermore, because the spontaneous fluctuations in the DMN have been linked to the recall of episodic memories (Mason, Norton et al. 2007), connectivity to the amygdala may reflect a tendency to experience emotion in response to internal mentation. The connectivity correlations may reflect the actual emotional content of the thoughts or intrinsic patterns of information processing in the brain. It is conceivable that experiencing the emotion could lead to symptoms of hyper-arousal. It is therefore advantageous for an individual to be able to down-regulate activity in the amygdala in the acute trauma period in order to plan for future threats.
6 Conclusion

Ultimately, the results may be summarized in terms of their relationship to monitoring the visual field, emotion regulation, and memory function. Between two weeks and three months, there was a decrease in PCC connectivity to the left IPC/visual cortex. This change was positively correlated with the decrease in stress symptoms. Thus, the results were consistent with our first and second hypotheses. During the acute stress period, we believe the left IPC was primed to monitor the visual field. We believe that the decrease in connectivity over three months corresponded with a decrease in hyper-vigilance symptoms and outwardly directed attention. Between two weeks and three months, furthermore, there were also decreases in connectivity to emotion regulation centers such as the ACC and mPFC. This was consistent with our first hypothesis. We believe the high connectivity to those regions in the acute stress period aided individuals with coping with negative emotions elicited by a trauma. In addition, changes in memory function may have been associated with the correlations in the left hippocampus and the right hippocampus. The PCC connectivity to the left hippocampus increased as stress symptoms decreased, perhaps reflecting an increase in autobiographical memory encoding and recall. The negative correlation in the right hippocampus at three months following the MVC suggests that higher connectivity to the hippocampi, in general, is related to better stress coping.
References


Appendix A: Result Tables

Table A.1: This displays the statistics for the 32 subjects who completed the initial scan. The columns, from left to right, identify the identification number, gender, initial PCL score, final PCL score, and change in PCL scores for each of the subjects.

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Table A.2: This displays the properties of the clusters for the single sample T-test on the PCC difference maps. The columns, from right to left, identify the MNI coordinates of the peak voxel in a cluster, the total number of voxels in each cluster, the minimum or maximum value of the peak voxel, the aal regions in the cluster, and the number of voxels associated with each aal region.

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Table A.3: This displays the properties of the correlation clusters for the whole brain analysis between the two week PCC connectivity and the initial PCL scores. The columns, from right to left, identify the MNI coordinates of the peak voxel in a cluster, the total number of voxels in each cluster, the minimum or maximum value of the peak voxel, the aal regions in the cluster, and the number of voxels associated with each aal region.

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<th>Sub Voxels</th>
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Table A.4: This displays the properties of the correlation clusters for the whole brain analysis between the three-month PCC connectivity and the initial PCL scores. The columns, from right to left, identify the MNI coordinates of the peak voxel in a cluster, the total number of voxels in each cluster, the minimum or maximum value of the peak voxel, the aal regions in the cluster, and the number of voxels associated with each aal region.

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<th>Coordinates</th>
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Table A.5: This displays the properties of the correlation clusters for the whole brain analysis between change in PCC connectivity and the change in PCL scores. The columns, from right to left, identify the MNI coordinates of the peak voxel in a cluster, the total number of voxels in each cluster, the minimum or maximum value of the peak voxel, the aal regions in the cluster, and the number of voxels associated with each aal region.

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