MAGNETIC RESONANCE IMAGING OF DEPRESSED PATIENTS

SPECTROSCOPIC & FUNCTIONAL MAGNETIC RESONANCE IMAGING OF FIRST AND MULTIPLE EPISODE DEPRESSED PATIENTS

By

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ABSTRACT

Major Depressive Disorder (MDD) is a common affective disorder associated with persistent states of negative mood and selective cognitive impairments. Fronto-temporal dysregulation in MDD patients is thought to contribute to the symptoms seen in these patients.

Based on prior evidence of structural and functional alterations in the hippocampus (Hc) and prefrontal cortex (PFC) in MDD patients, we were interested in examining the changes in cerebral function that underlie the cognitive dysfunction seen in two different MDD populations. We studied psychotropically naïve depressed patients experiencing their first treated episode (FTE) of depression, MDD patients who had experienced multiple past treated episodes (MTE) of MDD and healthy controls.

Two functional magnetic resonance imaging studies (fMRI) were conducted. The first study used an Hc dependent process dissociation task to examine Hc activation during recollection memory. The second fMRI study examined the activation in the PFC during reward and punishment conditions of a reversal-learning paradigm. Finally, we conducted magnetic resonance spectroscopy scans to measure levels of metabolites indicative of neuronal and glial cell integrity in the Hc of depressed patients and controls.

We observed differing results across all three studies in our FTE and MTE depression groups. Our studies examining the Hc suggest that MTE patients have decreased activation in this region as well as corresponding memory errors during recollection memory. Additionally, these patients have smaller Hc volume and signs of increased neuronal membrane turnover. Conversely, our FTE patients displayed

heightened Hc activation without memory deficits. Moreover, FTE patients had signs of increased glial cell density in the Hc without volumetric differences in this region. Our examination of reward processing revealed several health-to-illness gradients of activation in areas as the nucleus accumbens, anterior cingulate and ventral prefrontal cortices during the processing of rewards and punishers.

These findings suggest that several regions in the brain may be sensitive to the impact of disease burden and repeated episodes of MDD. In the Hc, first treatment patients may engage in compensatory processes during the early stages of illness that are attenuated with repeated episodes of illness. Moreover, reward processing may be affected in the early course of the disorder, however with a protracted course of illness these regional alterations in activation become more pronounced.

PREFACE

Each of the three scientific manuscripts included in this thesis are multiply authored. As such, the Guide for the Preparation of Theses of McMaster University mandates that I clearly outline my contribution to each of these works, and indicate when the work was conducted.

1) Milne, A., MacQueen, G.M., Yucel, K., Soreni, N. & Hall, G.B.C. (2009) Hippocampal metabolic abnormalities at first onset and with recurrent episodes of major depressive disorder: A proton magnetic resonance spectroscopy study. *NeuorImage*, 47(1): 36-41.

This work was conducted over the period of September 2005 – October 2008. I was responsible for conducting the experiments, completing the spectroscopy and statistical analysis. I also prepared the manuscript. Dr. Yucel analyzed the hippocampal volume data. Dr. Soreni assisted in technical aspects of the statistical analysis. Dr. Hall and Dr. MacQueen developed the study design and protocol prior to my arrival at McMaster. All authors assisted in editing the manuscript.

2) Milne, A.M.B, Hall, G.B.C., MacQueen, G.M. Altered hippocampal activation in response to recollection memory trials in Major Depressive Disorder: An fMRI Study. In preparation for submission to Nature Neuroscience.

This work was conducted over the period of February 2006 – October 2009. I assisted Dr. Hall in the computer programming of the task. I was responsible for conducting all of the experiments, completing the statistical analysis of the data and preparation of the manuscript. Dr. Hall oversaw and contributed to the analyses. Dr. Hall

and Dr. MacQueen developed the study design and protocol prior to my arrival at McMaster and assisted in editing the manuscript.

3) Hall, G.B.C., Milne, A.M. & MacQueen, G.M. An fMRI study of reward circuitry in patients with minimal or extensive past history of major depression. Submitted to Neuropsychopharmacology, October, 2009.

This work was conducted over the period of February 2006 – March 2009. I assisted Dr. Hall in the computer programming of the task. I was responsible for conducting all of the experiments and conducting the statistical analysis of the data. I also co-wrote the manuscript with Dr. Hall. Dr. Hall oversaw and contributed to the analyses. Dr. Hall and Dr. MacQueen developed the study design and protocol prior to my arrival at McMaster. Additionally, Dr. MacQueen assisted in editing the manuscript.

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LIST OF ABBREVIATIONS

1-H MRS	Proton Magnetic Resonance Spectroscopy
ACC	Anterior Cingulate Cortex
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BDI	Beck Depression Inventory
BOLD	Blood Oxygen Level Dependent
BRS	Brain Reward System
Cho	Choline
DLPFC	Dorsolateral Prefrontal Cortex
DSM IV	Diagnostic and Statistical Manual for Mental Disorders IV
FC	Frontal Cortex
FTE	First Treated Episode
FWHM	Full Width Half Maximum
GABA	Gamma-aminobutyric Acid
Gln	Glutamine
Glu	Glutamate
GLX	Combined Glutamate & Glutamine Peak
HC	Healthy Controls
Hc	Hippocampus
HPA	Hypothalamic-pituitary-adrenal
HRSD	Hamilton Rating Scale for Depression
fMRI	Functional Magnetic Resonance Imaging
MDD	Major Depressive Disorder
MPFC	Medial Prefrontal Cortex
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MTE	Multiple Treated Episode
NAA	N-acetyl-aspartate
NAc	Nucleus Accumbens
NMV	Net Magnetic Vector
OFC	Orbital Frontal Cortex
PFC	Prefrontal Cortex
RF	Radio Frequency
ROI	Region of Interest
SCID	Structured Clinical Interview for DSM - IV
TE	Echo Time
TR	Repetition Time
VMPFC	Ventral Medial Prefrontal Cortex

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CHAPTER 1:

INTRODUCTION

Major Depressive Disorder

Major Depressive Disorder (MDD) is one of the most common mental health disorders affecting people today. According to the World Health Organization, approximately 121 million people worldwide are affected by MDD. Moreover, MDD is the single leading cause of disability (World Health Organization, 2000). Health Canada estimates that 7.9-8.6% of adult Canadians will experience symptoms of MDD at some point in their lives (Government of Canada, 2006). It is estimated that the mental illness cost approximately 33 billion dollars in Canada annually, with depression topping the list of costs (Institute of Health Economics, 2008). MDD often follows a chronic cyclical course associated with social and occupational disability, comorbitity with other psychiatric disorders and suicide (Lepine, 2001). Although the impact of MDD on the individual and society are great, the exact neural underpinnings associated with the symptoms remain unknown. Numerous studies have identified changes in several brain regions including fronto-temporal dysregulation. These changes may reflect the cognitive dysfunction and mood dysregulation associated with MDD (Elliott, 2002). A greater understanding of these neurological changes in depression is necessary to improve clinical outcomes. In-vivo neuroimaging, with the use of Magnetic Resonance Imaging (MRI) technology, allows for both the identification of changes in brain activation during cognitive tasks and the quantification of brain metabolites reflecting various neuronal parameters. MRI research in MDD may help to provide a more comprehensive understanding of the disorder, and provide predictors of treatment outcome.

Characteristics of Major Depressive Disorder:

MDD is defined as a state of sadness or despair of such severity that it disrupts an individual's social and/or daily functioning. Although MDD represents a change in mood and usually a lack of interest or pleasure, it often times affects other aspects of a person's life. MDD often involves changes in eating, sleeping, weight and energy levels (American Psychiatric Association, 1994). The clinical course of MDD often follows a longitudinal course with periods of varying illness severity (Judd & Akiskal, 2000). It has also been noted that following a first episode of depression approximately 70% of MDD patients go on to develop future episodes, highlighting the often chronic nature of this disorder. These subsequent episodes often occur after a shorter period of remission therefore leading to increased cycling between periods of illness and remission (Post, 1992).

Patients with MDD often show a range of cognitive impairments. Studies have shown that cognitive deficits in MDD include decreased attention, and deficits in executive functioning and memory, although these results have not been consistently reported (Marvel, 2004). Executive functions encompass a range of cognitive processes including higher-level thinking and decision-making. Frontal lobe tasks, such as planning, execution and cognitive flexibility are often affected in MDD patients. Various neuropsychological tasks, such as the Wisconsin Card Sorting Task and the Colour-Word test (Stroop), have been employed to assess executive functioning. Studies have shown that MDD patients perform worse on these two tests compared to normal controls

(Rogers et al., 2004; Lampe et al., 2004). Various neuropsychological test batteries have shown that psychomotor performance, verbal learning, selective attention, planning, set-shifting and inhibitory control are affected in illness and persist during remission (Weiland-Fiedler et al., 2004; Lampe et al., 2004; Neu, et al., 2005; Paelecke-Habermann et al., 2005; Paradiso et al., 1997; Reppermund et al., 2007).

Memory is another area of cognitive function affected in MDD. A meta-analysis has shown that MDD diagnosis has a large negative effect on recollection memory (Zakzanis et al., 1998). Recollection memory is the conscious recall of specific facts. In contrast, habit memory describes memory for unconscious skills (Zubenko et al., 1990). Patients with MDD show deficits in recollection but not habit memory (Deuschle et al., 2004; MacQueen et al., 2003), although findings of intact habit memory are sometimes mixed (see Campbell et al., 2004).

These findings of cognitive and memory deficits in MDD parallel neuroanatomical abnormalities in the prefrontal cortex (PFC) and limbic structures that have been identified through various neuroimaging techniques. Reciprocal connections between limbic structures and the neocortex have shown inverse activation during periods of sadness in MDD, suggesting that these connections could mediate the relationship between emotional processing and attention (Mayberg et al., 1999). It is now thought that depression is the result of an intrinsic failure in the extended limbic emotion

system, which prevents the normal maintenance of emotional homeostasis during periods of stress (Mayberg, 2003).

One of the most consistent findings in depression neuroimaging studies is an inverse relationship between depression severity and frontal lobe metabolism and blood flow (Mayberg, 1997). Various changes in the FC have been noted in MDD including, increased activity in the medial prefrontal cortex (MPFC) and anterior cingulate cortex (ACC) (Harvey et al., 2005); decreased absolute regional cerebral glucose metabolism in the right prefrontal cortex (Kimbrell et al., 2002) smaller frontal lobe white matter volumes (Steingard et al., 2002); and altered functional connectivity in prefrontal and ACC regions (Vasic et al., 2009).

Additionally, abnormalities in the limbic system have been documented in areas associated with emotion, social cognition and homeostatic regulation (Drevets, 2001; Damasio, 1999). Decreases in amygdala and hippocampal (Hc) volume have been widely, though inconsistently, reported in MDD (Rosso et al., 2004). Recent meta-analyses have shown an overall significant reduction in Hc volume (Campbell et al., 2004; Videbech & Ravnkilde, 2004; McKinnon et al., 2009). When addressing the consistency of these findings, all three studies point out the importance of subject heterogeneity and illness duration in particular, as a confounding factor in the results to date.

Various observations from behavioural and neuroimaging studies have highlighted the frontal-temporal dysregulation present in MDD. In addition, the protracted, cyclical nature of MDD symptoms been well documented. However, the relationship between when these abnormalities occur in the time course of MDD is not well understood. It is unclear whether these abnormalities are present at illness onset or if they develop as a result of illness duration.

Overview of Magnetic Resonance Imaging

As previously outlined, a number of volumetric and functional brain changes have been identified in MDD. Improvements in Magnetic Resonance Imaging (MRI) have greatly increased the understanding of neural changes that occur across different psychiatric disorders. This non-invasive in-vivo imaging tool allows for the characterization of subtle alterations in regional volumetric, functional and metabolic changes between populations. Moreover, because of its excellent spatial resolution and soft tissue discrimination, MRI is viewed as an excellent tool for imaging brain tissue.

Images obtained during MRI scans are generated with the use of a strong magnetic field and the application of a radio frequency (RF) pulse. A number of atoms in the body, including hydrogen, carbon, nitrogen, oxygen, fluorine, sodium and phosphorus, possess the quantum mechanical property of spin because their nuclei contain a different number of neutrons and protons (McRobbie et al., 2003). The spin

property along with the charge of these atoms induces a magnetic moment, which allows the atoms act like small magnets. It is these magnetic moments that are utilized by the magnetic field to generate a signal (Westerbrook et al., 2005).

Hydrogen is the most abundant atom in the body due to its presence in water and fat stores. Moreover, hydrogen contains only one proton, which produces a relatively large magnetic moment (Rinck, 2001). Therefore, hydrogen is the most clinically useful atom for MRI. Under normal circumstances, the magnetic moments of hydrogen atoms are randomly orientated. However, when they are placed in a strong magnetic field, such as that used for MRI, an abundance of the nuclei align in the direction of the magnetic field (B_0) (See Figure 1), creating a net magnetic vector (NMV) of the hydrogen atoms in the direction of B_0 (Westerbrook et al., 2005). Once the hydrogen nuclei are under the influence of B_0, they begin a new type of movement – precession. Precession is a circular movement following a path around B_0, the speed of which is governed by the precessional frequency (See Figure 2). The precessional frequency is unique to each molecule at each magnetic field strength (Rajan, 1998).

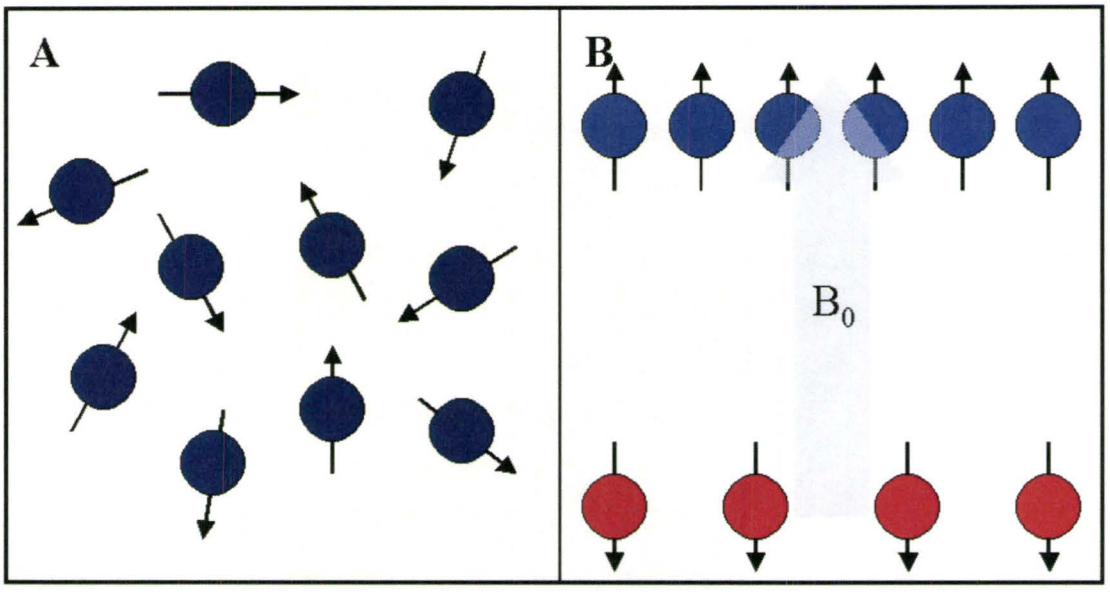

Figure 1: Magnetic field influences on hydrogen atoms. In the absence of a magnetic field (A) the magnetic moments of hydrogen atoms are randomly orientated. When a strong magnetic field is applied (B), the majority of hydrogen atoms (shown in light blue) align in the direction of the magnetic field (B_0). A few of the hydrogen atoms align anti-parallel (illustrated in red) to B_0.

If additional energy is applied to a molecule at its exact precessional frequency, the molecule will absorb this energy and begin to resonate. Resonance is achieved in MRI scanning with the application of a radio frequency (RF) pulse delivering energy that matches the precessional frequency of hydrogen (Westerbrook et al., 2005). The RF pulse causes more hydrogen nuclei to align anti-parallel to B_0; a higher energy state. This movement results in a change of orientation for the NMV (Weishaupt et al., 2003).

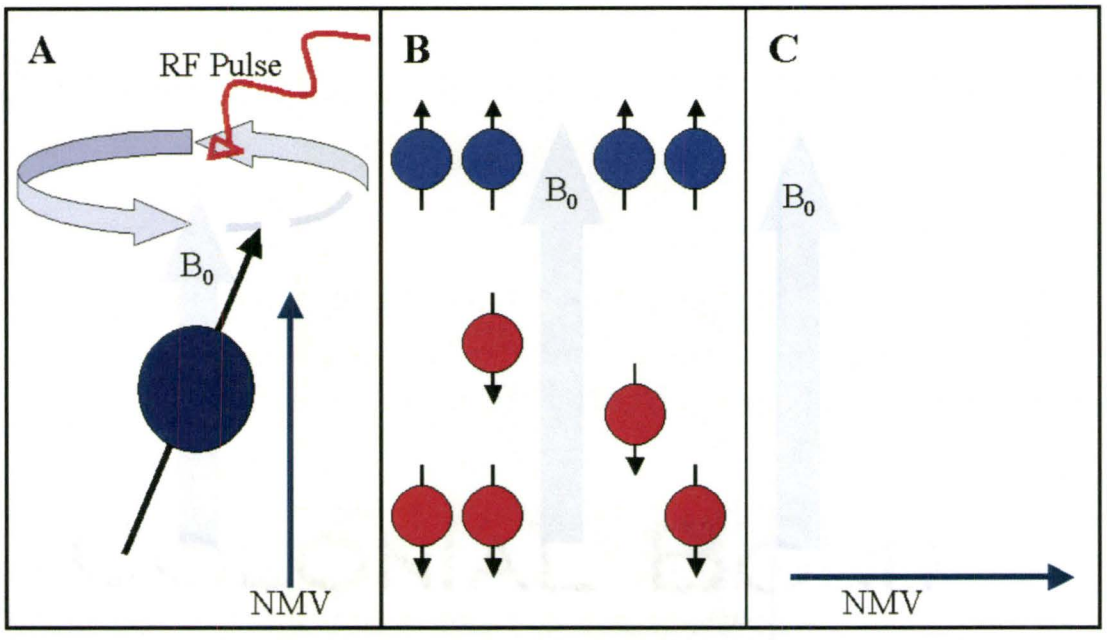

Figure 2: Transition of the net magnetic vector of hydrogen atoms. In the presence of B_0 and an RF pulse, the majority of magnetic moments of hydrogen atoms precess in the direction of B_0 (A). This creates a net magnetic vector of these magnet moments in the direction of B_0. After the application of an RF pulse, more hydrogen atoms are converted to the higher energy, anti-parallel orientation (B). This moves the net magnetic vector out of alignment of B_0 (C).

An MR signal is produced when the NMV of the hydrogen atoms moves across the receiver coil at a 90° angle. RF pulses are only applied for short periods of time. After the NMV of hydrogen has been moved out of alignment of B_0, the RF pulse is switched off. With the additional energy source removed, the hydrogen atoms release their absorbed energy as they realign with B_0. This energy release is the signal that is detected at the coil, and is the basis of the raw MR signal (Markisz, et al., 1996).

A number of factors affect the type of image generated during MRI. Different pulse sequences are used in MRI that determine when the RF pulse is turned on and off. The pulse sequences are determined by many factors. Two important factors are the

repetition time (TR), which is the time between the application of one RF pulse to the next, and the echo time (TE), which is the time from the application of the RF pulse till the maximum induced signal is received at the coil (Weishaupt et al., 2003). By changing the TR and TE used in different pulse sequences, different types of images are generated. For example, scans used to study volumetric images will contain pulse sequences with short TR and TE values to maximize tissue contrast. In contrast, scans used to study functional activation will use pulse sequences with longer TR and TE values (Westerbrook et al., 2005; Weishaput et al., 2003).

MRI can be used to measure functional activation in different areas of interest. Functional MRI (fMRI) exploits the differences in magnetism between deoxygenated and oxygenated hemoglobin to provide a measure of blood flow in the body. This type of imaging uses the Blood-oxygen-level-dependent (BOLD) signal to generate contrast in the tissue of interest.

Deoxygenated hemoglobin is paramagnetic, while oxygenated hemoglobin diamagnetic. Under conditions of basal activity in a region, there is a mixture of deoxy and oxygenated hemoglobin in arteries. Hydrogen atoms present in tissues surrounding areas with high levels of deoxygenated hemoglobin face an inhomogeneous magnetic field because of the paramagnetic properties of deoxygenated hemoglobin (Westerbrook, et al., 2005). Therefore, when an MR signal and RF pulse are applied to such a, fewer

protons precess in synchrony because of the effects exerted by local paramagnetic deoxygenated hemoglobin. The end result is a dampened signal is received at the coil.

When a tissue is being used, metabolism is increased resulting in an increased demand for oxygen levels. Particularly in the brain, where there are few local energy stores, energy must be continually supplied to active regions in order to maintain proper functioning. This energy is supplied by increased blood flow containing oxygenated hemoglobin (Buxton, 2002). With neural firing, the local vascular response is an overshoot of oxygenated hemoglobin flooding the capillary bed and reducing deoxygenated hemoglobin levels (Tofts, 2003). Due to the decreased in deoxygenated hemoglobin, and therefore decreased paramagnetic influence, the net result of is a local increased in MR signal (See Figure 3).

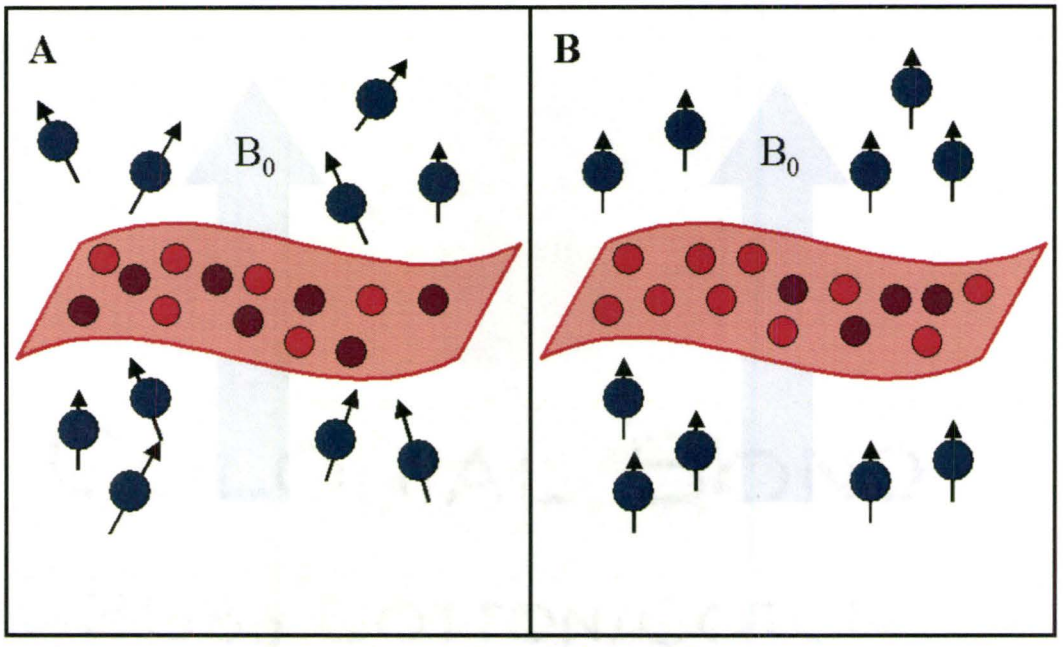

Figure 3: The generation of the BOLD signal in MRI. Under conditions of basal activity (A), there is a mixture of oxygenated (red) and deoxygenated blood (purple) in a region of interest. The influence of the paramagnetic properties of deoxygenated blood causes an inhomogeneous magnetic field. Therefore, the magnetic moments of the hydrogen atoms (blue) do not completely align in the direction of B_0, resulting in an overall diminished signal. In contrast, when an area is activated (B), increases in oxygenated blood and decreases in deoxygenated blood allow the magnetic moments of hydrogen to properly align with B_0, leading to an increase in signal.

Increased blood flow to supply an area of neural activity with oxygen is not an instantaneous process. Instead, it takes about 4-5 seconds before a peak response is observed. The hemodynamic response (See Figure 4) describes the time delay in the transition of deoxygenated and oxygenated hemoglobin. The delay in peak signal corresponds to the time necessary for oxygen to be delivered and released from the hemoglobin in an area of neural activity. Once neural activity in a region subsides, the blood flow returns to its basal activity state. Deoxygenated hemoglobin levels return to their pre-activity state, which corresponds to a decrease in signal intensity observed at the end of the hemodynamic response (Huettel et al., 2004).

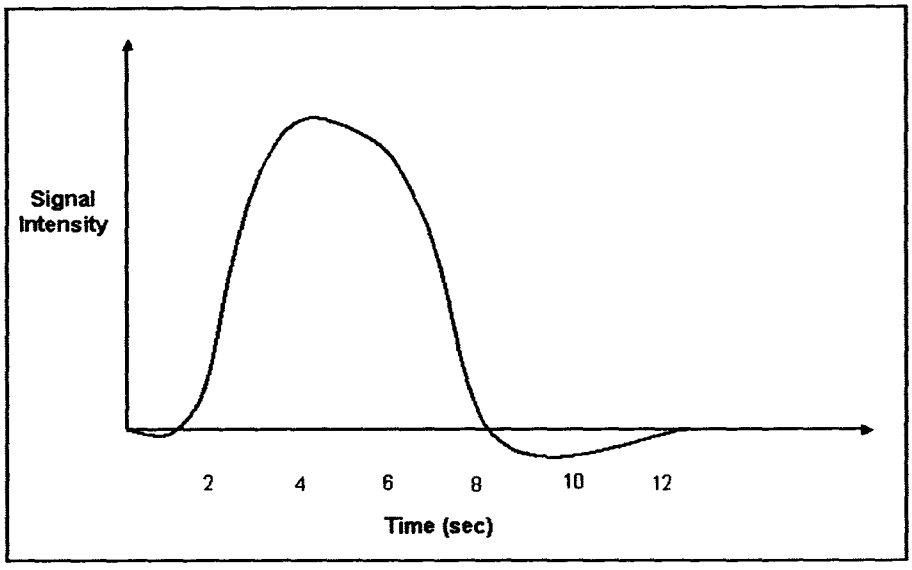

Figure 4: The time course of the hemodynamic response.

MRI can also be used to assess the levels of in vivo metabolites in localized brain regions with the use of Proton Magnetic Resonance Spectroscopy (1-H MRS). The chemicals measured as spectra peaks can provide useful insights about the metabolic status of brain tissue under investigation, which otherwise may appear normal on MRI. These biochemically active molecules provide information on the tissue viability and metabolism in a specified region of brain tissue. MRS imaging can therefore provide valuable information in determining the neuronal changes that take place in MDD.

There are two main nuclei of interest used in MRS clinical research today. Phosphorus spectroscopy is used to measure levels of phosphate containing metabolites, which are associated with energy metabolism in the brain (Stanley, 2002). However, hydrogen spectroscopy (1-H MRS) is used more commonly in spectroscopic studies of psychiatric disorders because it provides finer spatial resolution and it can provide

information on a wide variety of metabolites reflective of neuronal functioning (Burtscher & Holtas, 2001). There are a number of metabolites that are detected with MRS imaging. Each metabolite is visible as a different peak (or in some cases multiple peaks) along the MRS spectra (See Figure 4) and provides a different information regarding neuronal and glial cell density and viability as well as energy metabolism.

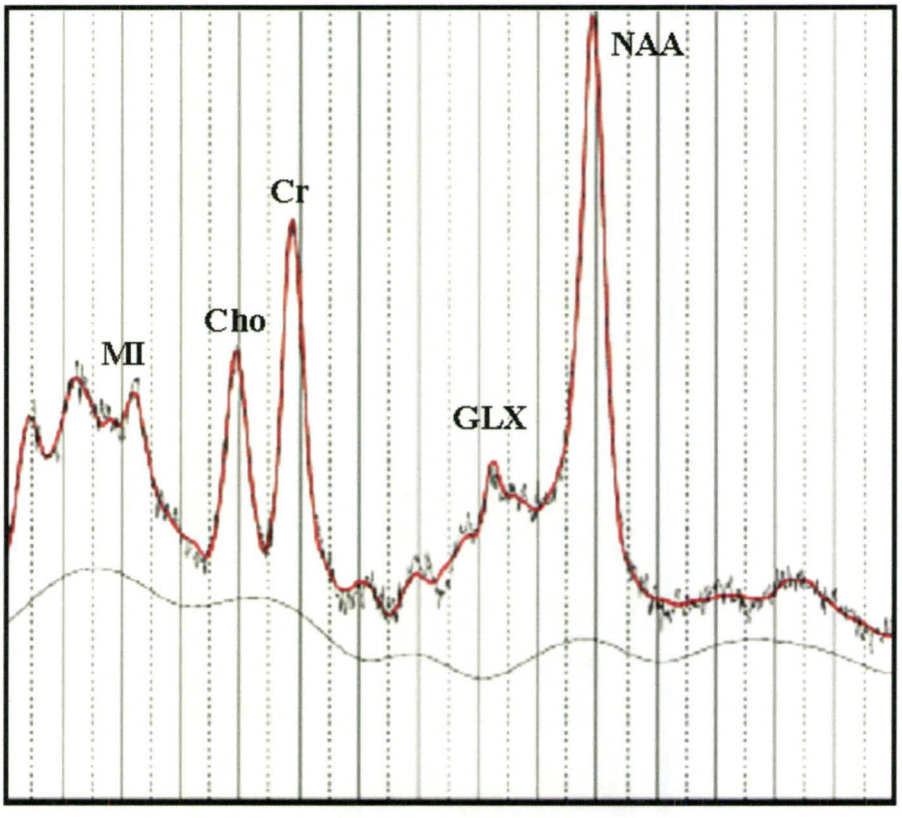

Figure 5: Sample MRS spectra. The presence of different chemical functional groups in each metabolite determines where its peak will occur on the MRS spectra.

MRS spectra are generated using the same principles that determine regular MRI signals. Metabolites that are visible with 1-H MRS contain primarily hydrogen atoms in different concentrations and orientations for each metabolite. These differences are due

to the presence of different chemical functional groups present in individual metabolites. The unique chemical composition of each molecule, therefore, creates different precessional frequencies for each metabolite, which ultimately determines the amount of chemical shift and the position of each metabolite peak on the MRS spectra (Dager et al., 2008).

Choline (Cho) is contained in the compounds phosphatidylcholine and glycerolphosphocholine, acetylcholine, and second messenger compounds, which are usually integrated into cellular membranes. Cho is often released under pathological conditions from its stores in cell membrane, myelin and cerebral lipid stores in the brain to form its visible resonance at 3.2ppm (Burtscher & Holtas. 2001). Cho is therefore considered a marker of cellular membrane metabolism and turnover. N-acetyl-aspartate (NAA) is another resonance detected by 1-H MRS. NAA, which is visible at 2.02ppm, is found only in the nervous system and is a neuronal marker of neuronal viability, density and pathology (Burtscher & Holtas, 2001; Dager et al., 2008; Vythilingam et al, 2003). Another compound, Myo-inositol (MI) is seen as a glial cell marker as it is actively transported into astrocytes and it is visible at 3.6ppm (Coupland et. at, 2005; Burtscher & Holtas, 2001). The MI peak on the MRS spectra also includes a contribution from syllo-inositol, a precursor of phosphoinositides, which are involved in cellular membrane second messenger systems (Moore et al., 1999). The excitatory neurotransmitter Glutamate (Glu), in addition to the astrocyte marker Glutamine (Gln) can also be seen as singlets or in combination (Glx) in the 1-H MRS spectrum. The Glx peak observed in 1-

H MRS spectra largely represents the intracellular stores of Glu and Gln in neurons and glial cells respectively (Burtscher & Holtas, 2001).

Findings From Magnetic Resonance Spectroscopy in Depression

Information regarding changes in neuronal density, viability, energy metabolism and glial cell viability can be obtained with the use of 1-H MRS. Moreover, this imaging technique details information regarding a pre-selected localized region of interest (ROI). This type of imaging has been used extensively to study MDD; there have been several metabolites that have shown altered 1-H MRS signals in studies with depressed individuals.

Reports of significant decreases in the NAA concentration have been shown in the PFC, basal ganglia and in the hippocampus in MDD (Brambilla et al 2005; Gonul et al 2006; Vythilingam et al 2003; Blasi et al 2004). As with other MRS studies in MDD, decreases in NAA have not been consistently reported. Studies have also failed to find any significant decreases in NAA in the DLPFC (Farchione et al 2002; Coupland et al 2005; Kumar et al 2002). A recent meta-analysis of over 14 MRS depression studies, found that overall, there were no differences in NAA between depressed subjects and controls across a number of brain regions (Yildiz-Yesiloqlu & Ankerst 2006). Studies examining NAA have often been limited by confounds of medication status, illness duration and age. Brambilla et al (2005) studied the difference in NAA levels in the

DLPFC associated with illness duration. It was found that more chronically ill patients had significant NAA decreases compared to controls, while less chronically ill patients did not differ significantly from controls. Gonul et al. (2006) examined the effects of antidepressant treatment on NAA levels in the medial PFC. A significant increase was seen in the post antidepressant treatment NAA levels such that there was no significant difference between MDD patients and controls. There is evidence to suggest there are significant changes in NAA concentrations in various regions in the brain reflecting possible changes in neuronal integrity in depression. However, these studies highlight the importance of controlling population heterogeneity when studying MDD.

In addition, several studies have investigated the levels of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and its precursor, the excitatory neurotransmitter glutamate in MDD. MRS research in MDD has identified altered Glx and GABA concentrations in various locations. Reduced concentrations of GABA have been shown in MDD in the occipital cortex compared to controls (Sanacora et al., 1999 & 2004). These levels appear to change with the subtype of depression. It was shown that individuals with melancholic MDD having the greatest decrease in GABA compared to controls (Sanacora et al., 2004). However, these decreases have not been reported consistently. Hasler et al. (2005) found no change in GABA levels in a group of remitted MDD patients compared to controls. In addition to the alterations in the occipital cortex, a recent study found decreased GABA levels in the dorsomedial/anterolateral prefrontal cortex in a group of unmedicated MDD patients (Hasler et al., 2007). These findings

suggests that GABA levels may fluctuate with the type and severity of illness. Changes in GABA concentrations have also been noted in response to different treatments. Significant increases following SSRI treatment (Sanacora 2002), and significant decreases following cognitive behavioural therapy (Sanacora 2005) in GABA concentrations in MDD have been reported.

Similarily, alterations in Glx levels have been shown in MDD. Several studies have examined the Glx levels in the ACC in MDD. The ACC is thought to be involved in the pathophysiology of MDD. Volumetric studies have found decreases in ACC volume in adult MDD populations, while functional studies have shown abnormal ACC resting metabolism (Rogers et. al, 2004). Reduced Glx levels have been reported in the ACC across different MDD populations. Decreases in adult (Auer et al., 2000) and childhood (Rosenberg et al., 2004 & 2005; Mirza et al., 2004) MDD Glx levels have been documented. In addition, Mirza et al., 2004 noted that a decrease in ACC Glx concentrations in their children with MDD was associated with greater functional impairment. Finally, a recent study found that decreased levels of Gln showed a negative correlation to anhedonic symptoms in an adult MDD population (Walter et al., 2009).

The Glx levels in the dorsolateral prefrontal cortex (DLPFC) have also been investigated in 1-H MRS studies in MDD. Two studies examining late life MDD produced conflicting results with both decreases (Ajilore et al., 2007) and increases (Binesh et al., 2004) in DLPFC Glx levels. However, a recent meta-analysis found

collective evidence of decreased Glx levels in the DLPFC; this finding was most notable with greater symptom severity (Capizzano et al., 2007). Collectively, these studies provide further evidence of altered Glu and GABA neurotransmission in MDD.

Alterations in Cho levels in various locations have also been reported in a number of studies in MDD. Increases in Cho, a marker of cell membrane turnover, have been noted in the PFC, including the OFC and DLPFC, and also in the basal ganglia (Steingard et al 2000; Farchione et al 2002; Kumar et al 2002; Vythilingam et al 2003). However, decreases or no differences in Cho concentrations have also been reported in the DLPFC (Caetano et al 2005; Renshaw et al 1997; Coupland et al 2005). These conflicting reports seem to be related to the characteristics of the particular subject population included in each study. Drawing comparisons across these studies is made difficult as differences in age, illness severity and duration as well as medication status at the time of the scan are all present. By including a more narrowly defined study population, the exact changes in Cho, reflecting cell membrane turnover, can be determined. Additionally, studying subjects experiencing their first episode of MDD will help to characterize the abnormalities present at illness onset.

Finally, alterations in MI concentrations, a marker of glial cell loss, have been observed in studies of MDD. In a study examining childhood depression, MI levels have shown significant increases in the DLPFC (Caetano et al 2005). Similarly, a study of adult and late life MDD have found significantly increased MI also in the DLPFC

(Binesh et al., 2004; Ajilore et al., 2007; Kumar et al 2002). Another study examining the medial prefrontal cortex found decreased levels of MI in an adult MDD population (Gruber et al., 2003). These studies suggest that there may be regional differences in MI concentrations in MDD.

It is evident from the review above that the current literature includes numerous 1-H MRS studies of MDD that identify various regional metabolic differences in this disorder. It is also clear however, that these metabolic abnormalities have not been consistently reported. Study population characteristics such as age, illness duration, illness severity, past medication use and current medication status have not been controlled in all studies, and could all potentially affect study outcomes. However, while various regions in the PFC have been examined with MRS, studies examining limbic structures, and the Hc in particular, have been lacking. Furthermore, the MRS literature in MDD has failed to characterize the metabolic changes present at illness onset; to date there has only been two studies examining MRS in first episode MDD patients (Gonul et al., 2006; Kaymak et al., 2009). By comparing the metabolic differences present in first episode patients to those present in patients with multiple past episodes we can gain valuable information about the neuronal and glial cell integrity present at illness onset, and also, how these markers change across time in this disorder.

Hippocampal Changes in Depression

Dysfunctions in various memory systems have been identified in MDD. Working memory, the temporary storage and manipulation of information, which is dependent on the DLPFC is impaired in MDD (Harvey et al., 2004). Additionally, recollection (declarative) memory, the conscious recall of specific information, is also affected in MDD. The recollection memory abnormalities seen in MDD highlight the hippocampus as an area of dysfunction in this disorder as various studies have identified the hippocampus (Hc) as a key brain structure involved in recollection memory (Buckner et al., 1995; Bernard et al., 2001; Mayes et al., 2001).

Recollection memory is one of the most consistently reported impairments in MDD (Zakzanis et al., 1998). Research assessing both recollection memory (explicit) and habit memory (implicit) has found that only recollection memory is impaired in MDD patients. Furthermore, this research showed that the previous number of past depressive episodes predicted the level of memory impairment (MacQueen et al., 2002). Another study examined the effects of antidepressant treatment on recollection memory impairment in MDD. It was observed that patients with a complete remission after 35 days of antidepressant treatment had shown the least amount of memory impairment prior to antidepressant administration. However normal scores on the California verbal learning test, a measure of recollection memory, were not observed until a period of long term remission had transpired (minimum of 6 months) (Deuschle et al., 2004). These

results suggest that recollection memory impairments may not be stable across illness states.

Volumetric changes in the hippocampus in MDD have been widely reported (Campbell et al., 2004; MacKinnon et al., 2009; Videbech et al., 2004). It is thought that these alterations may develop as a result of dysfunction in the hypothalamic-pituitary-adrenal axis (HPA). The HPA axis refers to a set of feedback interactions between the hypothalamus, the pituitary gland and the adrenal glands. One of the end results of this pathway is the release of glucocorticoids (GC) from the adrenal glands (Tuvnes et al., 2003). This pathway is activated during times of stress to help an individual respond adaptively. In the short term this coping mechanism is beneficial to the individual, however long term chronic exposure to stress and the resultant continued activation of the HPA system can be detrimental to the individual. Cumulative changes take place in the body and brain when the system is not properly down regulated after a period of stress, or if stressors persist. These changes have been termed "allostatic overload" (McEwen, 2004).

In MDD the regular cyclic nature of cortisol is abnormal. Nighttime levels tend to be increased, and the axis does not respond to suppression by dexamethasone (McEwen, 2004). A recent study of over 1200 subjects with a history of depression found increased cortisol levels at awakening and in the evening in both currently depressed and remitted subjects (Vreeburg, et al., 2009). Moreover, another study found

that these increased cortisol levels observed in MDD had a negative impact on tasks assessing verbal memory, visuospatial memory, working memory and selective attention (Hinkelmann et al., 2009). These state changes reflect an allostatic overload, and may play a role in the structural remodeling of the brain that has been observed in MDD.

The Hc is a major negative feedback site for the HPA axis, and therefore it has been has been one area studied as a possible site for neurotoxic effects of GC (Jacobson & Saplosky, 1991). It is hypothesized that excessive amounts of GC impairs neurogenesis in the Hc and leads to atrophy of dendritic processes contributing to an overall neurotoxic effect on the Hc (Saplosky, 2000). Repeated periods of stress cause atrophy of the pyramidal neurons in the Hc and decreased proliferation of nerve cells (McEwen, 2000). Studies of Cushing's disease (a disorder marked by chronically elevated GC) have shown that after correction of the underlying hypercortisolism, an initially decreased Hc volume was significantly increased (Starkman et al., 1999). This improvement suggests that excessive GC does negatively impact the Hc. Hc volume does appear to be reduced in depression, which could be caused in part by the neurotoxic effects of GC (Campbell et al., 2004).

Given the impact that an abnormal HPA response can have on the Hc, and the observation that the HPA system is dysfunctional in MDD, considerable research efforts have focused on the structure of the Hc in MDD. Volume changes in the Hc have been reported in a number of MDD populations including adolescent and adult MDD patients

(Janssen et al., 2004; Saylam et al., 2005). Three meta-analyses of Hc volume in MDD have identified smaller Hc volumes in patients with a prolonged longitudinal course of illness (Campbell et al., 2004; McKinnon et al., 2009; Videbech et al., 2004). However, varying results have been reported with decreases, increases, no changes and differences in the hemisphere reported to be abnormal all noted. These inconsistencies may be partially explained by the clinical heterogeneity within the different study populations. Such factors as the number of previous episodes, age at onset, early childhood stressful life events and the duration of MDD before the illness was treated appear to contribute to changes in Hc volume in MDD (MacQueen et al., 2003; MacMaster & Kusumakar, 2004; Vythilingam et al., 2002; Sheline et al., 2003).

Still, it is not understood if the Hc volume reductions seen in MDD remain in the remitted stage (Campbell & Macqueen 2006), or whether volume reductions are present at illness onset. A study examining a group of first episode depressed patients identified decreased scores of recollection memory, without a corresponding change in hippocampal volume (MacQueen et al., 2002). These findings would suggest that functional alterations in the hippocampus could precede the volume loss in MDD. Moreover, a recent meta-analysis examining over thirty Hc volumetric studies in MDD, found that the most pronounced decrease in Hc volume was observed in patients with a protracted course of illness. Volumetric differences were not observed for subjects who had experienced less than one episode of MDD, or who had been ill for less than two years, did not show Hc volume decreases (McKinnon et al., 2009). However, a recent

study found significant volume decreases in a group of female first episode patients that correlated to poorer performance on a test of immediate and delayed recall (Kaymak et al., 2009). While the evidence seems to suggest volumetric decreases and memory deficits in subjects who have experienced a protracted course of illness with multiple depressive episodes, the findings are not clear for patients who have experienced a shorter burden of illness. Moreover, few studies have investigated the Hc with functional imaging in MDD. This work will help to clarify the functional activation in this region at illness onset and whether this activation is affected by past episodes of MDD.

Alterations in Reward Processing in Depression

Emotional processing, which is altered in MDD, involves a complicated set of interactions across an integrated network of limbic and cortical structures. Findings of decreased volume and blood flow in the dorsal regions of the frontal lobes have been consistently reported in MDD (Marvel et al., 2004). Additionally, decreased glial cell and neuronal density have also been reported in this region in mood disorders (Ongur et al., 1998; Rajwoski et al., 2001). These alterations occur in frontal regions thought to sub-serve the executive functioning, attention and working memory deficits in MDD. A model, derived largely from positron emission tomography (PET) findings, has focused on a set of key regions involved in the pathophysiology of MDD. It highlights dysfunction in several prefrontal areas including the medial, orbital and dorsolateral prefrontal cortex as well as areas of the anterior cingulate cortex (Mayberg, 2003).

Anhedonia, one of the main symptoms of MDD, is a loss of pleasure in previously enjoyed activities (Mitterschiffthaler et al., 2003). Although this symptom is clinically well characterized in MDD, the exact neural underpinnings responsible for this symptom remain unknown. However, it would seem likely that these symptoms are mediated by changes in the brain reward systems (BRS). The BRS is engaged in the processing of reward, pleasure and motivation and involves an extensive network of prefrontal regions, many of which that are dysfunctional in MDD.

The BRS has been studied extensively in drug addiction research. This research has identified areas of brain activation during pleasurable conditions, which are now thought to be key in reward processing. Studies of cocaine-dependent subjects has shown that drug induced states are associated with various areas of activation including the nucleus accumbens (NAc) (Breiter et al., 1999). The NAc, part of the mesolimbic system involved in reward processing, receives dopaminergic output from the ventral tegmental area and in turn, sends projections to various prefrontal regions including the medial prefrontal cortex, the orbitofrontal cortex and the medial amygdala (Wightman & Robinson, 2002). Moreover, the ACC has shown activation during reward processing paradigms that involve the assessment of risk (Knutson & Cooper, 2005). With the previously noted changes in the PFC, and the general symptomology of MDD, it would follow logic that individuals with MDD would also have deficits in reward processing.

Reward processing has been investigated in MDD. One study investigated the mesolimbic system response after dextroamphetamine sulfate administration, a drug which causes dopamine release in the mesolimbic system. MDD patients showed a hypersensitive response to the drug administration, which was positively correlated to symptom severity of anhedonia (Tremblay et al., 2006). These findings connect dysfunction in the mesolimbic system to symptoms of anhedonia in MDD. A study of adolescent MDD investigating decision-making during a reward-processing paradigm found that MDD was associated with decreased activation in superior OFC regions and increased activity in inferior OFC and caudate regions in comparison to controls (Forbes et al., 2006). Additionally, in an investigation of affective word presentation in MDD, it was found that patients with MDD had decreased bilateral ventral striatal activation to positive words (Epstine et al., 2006). Finally, a recent deep brain stimulation (DBS) study investigated the effects of DBS to the NAc in MDD. In this study bilateral electrodes were implanted in the NAc in three subjects with refractory MDD. Stimulation of the NAc resulted in a significant improvement in symptom severity and was accompanied by increases in metabolic activity in areas of the ventral striatum and areas of the PFC (Schlaepfer et al., 2007). These imaging studies suggest that altered reward processing may contribute to the symptomology of MDD.

A number of studies examining anhedonia in MDD have utilized methods that indirectly recruit parts of the BRS. For example, Mitterschiffthaler and colleagues (2003) used the presentation of affective words to show decreased prefrontal activation in a

group of treatment-resistant depressed patients. Another study used audio taped maternal criticism to display decreased prefrontal activation in currently remitted patients with a history of MDD (Hooley et al., 2005). A recent study examined MDD patient responses to the presentation of positive and negative sights and smells. It was observed that depressed patients exhibited decreased ventral striatal activation in response to pleasant sights (McCabe et al., 2009).

Recently, MDD studies examining anhedonia in MDD have begun to explore reward processing more directly. Recent fMRI studies using paradigms with monetary gains and losses have demonstrated increased anterior cingulate activation during anticipation of rewards (Knutson et al., 2008); decreased striatal activation during reward processing (Forbes, et al., 2009; Pizzagalli, et al., 2009) and decreased middle frontal and rostral cingulate activation during reward selection and anticipation (Smoski et al., 2009).

In addition to examining general dysfunction in reward processing in MDD, research has also identified abnormalities in specific areas of the BRS in MDD. The medial prefrontal cortex (MPFC) is an area that has shown activation in response to unexpected rewards during a gambling task in healthy controls (Ramnani et al., 2004). A recent meta-analysis of functional activation changes associated with MDD found decreased activation in MPFC during resting conditions (Fitzgerald et al., 2007), while a second meta-analysis found the same area to show increased activation in response to emotional stimuli (Steele et al., 2007). An investigation of morphological differences in

the MPFC in MDD found that this area was significantly decreased in a population of elderly males with depressive symptoms compared to age matched controls (Taki et al., 2005). These studies all implicate the MPFC as an area of potential dysregulation in MDD.

Another region that contributes to the neurological dysfunctional in MDD is the orbitofrontal cortex (OFC). The OFC is thought to play a neuromodulary role in depressive symptoms (Drevets, 2000), and reward processing (O'Dorhety et al., 2001). O'Dorhety et al. (2001) has shown that the magnitude of fMRI activation in the OFC is correlated with the magnitude of the reward presented. Further evidence of OFC involvement in MDD is found in decreased glial cell numbers, neuronal size and density and cortical thickness, which have reported in this disorder (Rajkowska et al., 1999). Furthermore, decreased levels of n-acetyl-aspartate (NAA) and myo-inositol have also been identified in the prefrontal cortex, through magnetic resonance spectroscopy, further implicating this area in the neuropathology of MDD (Gonul et al., 2006; Coupland et al., 2005).

The ACC is another region involved in the processing of rewards that has been associated with altered functioning in MDD. a recent meta-analysis identified the ACC as one of the most consistently identified regions showing dysfunction in MDD (Fitzgerald et al., 2007). Konarski et al. (2007) observed an association between the number of previous episodes and lack of treatment response to hypometabolism of the

ACC. Moreover, a structural MRI study observed bilateral volume reductions in this area (Caetano et al., 2006). Additionally, recent studies have identified abnormalities in this region in recently diagnosed individuals. One study found smaller ventral ACC volumes in treatment naïve females with MDD (Tang et al., 2007). Halari and colleagues (2009) observed decreased ACC activation in a group of first episode MDD patients during attention and error detection tasks.

It is evident from numerous studies of MDD that many morphological and functional abnormalities have been identified in key regions involved in the BRS. Although these changes have been identified, the impact of past depressive episodes on these regional abnormalities has not been well characterized. There are a few recent reports of prefrontal abnormalities in regions associated with reward processing. However, the impact of these abnormalities on reward processing in recently diagnosed individuals has not been examined. The use of an fMRI gambling paradigm allows for the examination of the neural correlates of reward processing. By employing this technique to study patients with MDD, we will be able to observe the changes in functional activation present while these subjects evaluate the possibility of rewards. In addition, comparing first episode patients to those with multiple past episodes of MDD will determine if impairment is present in the BRS at illness onset and whether it worsens over time with increasing number of depressive episodes.

Objectives:

With reference to the noted abnormalities in frontal and temporal regions previously observed in depression, the research presented in the ensuing chapters used MRI methods to examine functional and metabolic alterations the Hc and frontal cortex in two specific groups of depressed patients. One of the major goals of this research was to systematically compare alterations in brain metabolites and function in differing tasks, in individuals experiencing their first episode of MDD to those who had experienced multiple past episodes of depression. This methodological approach allowed for an investigation of the impact of illness duration in MDD. Additionally, we used novel tasks to assess Hc and frontal functional activation as well as Hc metabolic changes associated with MDD.

The first manuscript presented in this thesis (Chapter 2) investigated changes in metabolite levels in depression with the use of MRS. One of the objectives of this work was to characterize what, if any, metabolic changes are present in individuals who were experiencing their first treated episode (FTE) of MDD. We compared this study population to healthy controls, and also to patients who had experienced multiple treated episodes (MTE). While research has indicated that the Hc displays volumetric decreases in MDD, particularly in subjects with a protracted course of illness, this brain region that has been largely overlooked in MRS studies of depression. Therefore the second objective of this work was to characterize MRS metabolites in a localized region selecting the Hc. We hypothesized that depressed patients would have decreased levels

of NAA, GLX and MI and increased levels of Cho. Moreover, we hypothesized that these alterations would be greater in the MTE patients compared to the FTE patients.

The second manuscript presented in this thesis (Chapter 3) investigated recollection memory in depression. Despite several meta-analyses supporting the hypothesis that in the aggregate Hc volumes are smaller in MDD, to date no studies have examined Hc functioning with neuroimaging during a Hc dependent task in the depression literature. Therefore, the primary objective of this study was to assess Hc activation in MDD during an Hc-dependent, recollection memory task. We were also interested in examining the impact of illness duration on the alterations in Hc activation. Therefore, we again studied patients experiencing their first episode of MDD and compared them to subjects who had experienced multiple episodes of depression and healthy controls. We hypothesized that depressed patients would have attenuated Hc activation during recollection memory trials. Furthermore, we hypothesized that this attenuation would be less pronounced in the FTE group compared to the MTE patients.

The third manuscript presented in this thesis (Chapter 4) investigated reward processing in depression. Dysfunction in several areas that are key to reward processing have shown volumetric and functional abnormalities in MDD. Many of the prior fMRI studies examining reward processing in depression have used affective face stimuli or scripted scenarios to make inferences about anhedonia. While these studies provide an indirect means of recruiting the reward circuitry in MDD, much can also be learned from

utilizing tasks that explicitly activate these reward circuits. Therefore, the main objective of this work was to use an fMRI gambling task to directly recruit reward circuitry in our study populations. Similar to the other manuscripts presented in this thesis, another goal of the work was to assess changes in the neurocircuitry underlying reward processing across illness duration. By including two patient population groups, those experiencing their first episode of MDD and those who had experienced multiple past episodes of depression, we hope to clarify functional differences in reward related neurocircuitry across the illness duration in MDD. We hypothesized that depressed patients would have decreased activation in prefrontal and striatal areas shown to be involved in reward processing (OFC, ACC, NAc). We also hypothesized that changes in activation would be more apparent in MTE patients when compared to FTE patients.

CHAPTER 2:

Hippocampal metabolic abnormalities at first onset and with recurrent episodes of major depressive disorder: A proton magnetic resonance spectroscopy study

This manuscript outlines our examination of the changes in Hc metabolite levels in patients experiencing their first episode of MDD, those who had experienced multiple past episodes of MDD and matched controls.

From these experiments we observed: A) Increased choline containing compounds in the Hc of patients who have experienced multiple past episodes of MDD in comparison to matched controls. B) This increase was still statistically significant after controlling for the decreased Hc volume observed in this group when compared to controls. C) Increased MI in the Hc of individuals experiencing their first treated episode of MDD in comparison to controls. D) Depressed individuals in this group did not have significant volume alterations in comparison to healthy controls.

This paper was published in the journal *NeuroImage* in March 2009. I was responsible for the execution and interpretation of all of the experiments, as well as the writing of the manuscript. My co-authors provided technical advice and assistance as well as supervision and funding for the work (see Preface for more details). This manuscript is reprinted with permission from Elisiver (See Appendix I).

Hippocampal metabolic abnormalities at first onset and with recurrent episodes of a major depressive disorder: A proton magnetic resonance spectroscopy study

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ABSTRACT
The neural underpinnings of major depressive disorder (MDD) are unknown but there is evidence for structural alteration in the hippocampus that may become more pronounced over the course of illness. The aim of the present study was to examine metabolite levels of N-acetyl-aspartate (NAA), Myo-inositol (MI), Glutamate–glutamine (Glx) and choline-containing compounds (GPC and GPC+PCh) in patients presenting for first treatment of a depressive episode compared to those with multiple past episodes and age and sex matched controls. We used single voxel proton magnetic resonance spectroscopy (1H-MRS) centered on the hippocampus. Choline-containing compounds were significantly increased in patients with a high past illness burden relative to controls after controlling for hippocampal volume. The group presenting for first treatment had only increases in MI levels compared with matched controls. These results suggest that abnormal membrane turnover in the hippocampus is greater in patients with highly recurrent illness, and provide support for the hypothesis that there are neuronal changes in this region over the course of illness.

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Converging lines of clinical and preclinical research support the suggestion that there are functional and structural changes in the hippocampus (Hc) of patients with major depressive disorder (MDD). Dysregulation of the Hypothalamic–Pituitary Axis (HPA) in patients with MDD is one of the most reliably described findings in biological psychiatry (Plotsky et al., 1998; Young and Korszun, 1998) and it now appears that this dysregulation is likely to have specific and long-lasting effects on the Hc (McEwen, 2003). The Hc is a stress-sensitive structure and decreased Hc volume might be due to remodeling of key cellular elements, involving retraction of dendrites, decreased neurogenesis in the dentate gyrus and loss of glial cells (Czeh and Lucassen, 2007; McEwen, 2001; Rajkowska, 2002).

Both potentially reversible remodeling and irreversible cell death is likely to be caused by dysregulation of glucocorticoid secretion and elevated activity of excitatory amino acid neurotransmitters (Sapolsky, 2000). As the Hc is a major site in the glucocorticoid negative feedback circuit, remodeling or neuronal damage and death may lead to less efficient inhibitory control of the corticotrophin releasing hormone-producing cells of the hypothalamus, resulting in increased glucocorticoids and worsening of the process (Bremner, 2000). A decrease in factors such as brain derived neurotrophic factor resulting in decreased neurogenesis, increased remodeling of dendrites, loss of glial cells, or increased excitotoxicity, could lead to low Hc volume and vulnerability to subsequent episodes of depression, (McEwen, 1999; Pittenger and Duman, 2008).

Two meta-analyses of studies examining Hc volume in patients with major depressive disorder (MDD) supported the hypothesis that in the aggregate Hc volumes were lower in patients with MDD compared to age and sex matched controls. (Campbell et al., 2004; Videbech and Ravnkilde, 2004). A recent meta-analysis of over thirty studies confirmed this finding and found further evidence that the difference between patients and controls was most apparent in those with a recurrent or chronic form of illness (McKinnon et al., 2009). Corollary behavioural testing has identified that depressed patients are differentially impaired on recollection memory tasks that are dependent on the Hc formation, and that illness progression exacerbates this impairment.

Proton magnetic resonance spectroscopy (1H-MRS) is a noninvasive imaging technique used to assess the levels of in vivo metabolites in localized brain regions. 1H-MRS levels of specific bioactive molecules are considered indices of tissue viability, integrity and metabolic turnover in a specified location (Burtscher and Holtas, 2001). For instance, N-acetyl-aspartate (NAA) is an amino acid highly localized to neurons (Urenjak et al., 1993) and often considered a marker of neuronal density and integrity (Stanley, 2002). Also, Choline (Cho) is often released under pathological conditions from its stores in cell membranes, and is therefore considered to be a marker of cellular membrane turnover and active neurodegeneration (Burtscher and Holtas, 2001; Malhi et al., 2002). Myo-inositol (MI), is precursor molecule for several brain metabolites, participates in signaling in the phosphatidylcholine system and is predominantly located in glial cells (Wolfson et al., 2000). As such, it is commonly considered a marker for glial cell loss.

Finally, Glutamate–glutamine (Glx) indexes glutamatergic neurotransmission (Kato et al.,1998) and elevated glutamate levels have been associated with increased neuronal firing and a shift in energy utilization from oxidative phosphorylation to glycolysis (Dager et al., 2004). As a consequence, elevated glutamate levels may result in increases in lactate, which is neurotoxic (Dager et al., 2004; Glitz et al., 2002). The measurement of these four metabolites using H-MRS therefore, allows us to indirectly examine neuronal and glial alterations in the Hc of patients with depression.

Despite the many studies reporting volumetric differences in patients with recurrent MDD and healthy comparison subjects, few studies have focused on this region with MRS, and to our knowledge, none have examined past illness burden in a systematic manner. The purpose of the current study was therefore to use H-MRS to examine hippocampal levels of NAA, Cho, Glx, and MI in two distinct patient groups and age and sex matched controls. One group consisted of patients who presented for first treatment of a depressive episode (FTE). The second group consisted of patients who had experienced a minimum of three past treated episodes (MTE). Given the volumetric differences associated with recurrent MDD, we examined these metabolites in voxel centered on the Hc, while controlling for Hc volume in our analysis of the individual metabolites.

We hypothesized that MDD patients would have decreased levels of Glx, NAA and MI and increased levels of Cho in comparison to healthy controls. Moreover, we

further hypothesized that we would observe a greater decrease of Glx, NAA and MI and greater increase of Cho in the MTE group in comparison to the recently diagnosed MDD group. To our knowledge this is the first study to examine metabolites in a treatment naïve population and to systematically compare patients with low and high past illness burden.

Materials and methods

Subject selection

The two patient groups were recruited from the Mood Disorders Program at St. Joseph's Centre for Mountain Health Services in Hamilton. This study was approved by the Research Ethics Board of St Joseph's Healthcare Hamilton. All subjects gave informed consent after a full explanation of the study protocol. Subjects with a history of head injury, neurological illness, alcohol or substance abuse, previous history of electroconvulsive therapy or transcranial magnetic stimulation within the last two years were excluded from the study. Healthy controls were free from medication and had no current symptoms or medical history of a mental health disorder. A psychiatrist confirmed a primary diagnosis of unipolar MDD according to DSM-IV criteria (American Psychiatric Association, 1994). One group of depressed participants (FTE) was comprised of individuals who were presenting for assessment for the first time and had no past treatment history (mean age 32.1±9.3). The second group of patients (MTE) included individuals who had extensive illness histories, having experienced three or more previous episodes of MDD and/or an illness duration of 5 of more years (mean age

47.3±10.6). All subjects with depression met criteria for non-psychotic unipolar major depressive disorder as determined with the Structured Clinical Interview for DSM-IV (SCID-I; First et al., 1998). In addition, psychiatric symptoms were assessed prior to scanning using Beck Depression Inventory (BDI; Beck and Beamesderfer, 1974) and the Hamilton Rating Scale for Depression (HRSD: Hamilton, 1960). A group of age and sex matched controls were recruited from the community, and subsets of 14 and 13 controls were selected to match by age and sex with the MTE and FTE groups respectively. Clinical details and demographic information of subjects can be found in Table 1.

MRS procedure

MRS data were collected using a GE short-bore 3-T MRI system (General Electric Healthcare, Milwaukee, WI) and a standard quadrature head coil. Each subject participated in a single voxel spectroscopy scan located in the hippocampus in addition to two high-resolution anatomical scans. At the start of the 1-HMRS scan session, following head immobilization and a localizer scan, a T1 weighted 3D-FSPGR axial anatomical scan was performed (TR/TE 9/ 2.1 ms, flip angle=12°, FOV=240 mm, slice thickness=2.0 mm, 124 slices, matrix size=320×192). A second anatomical scan was acquired in the sagittal direction (3D-FSPGR scan; TR/TE 18/3.4 ms, flip angle=20°, FOV=240 mm, slice thickness=1.2 mm, 124 slices, matrix size=512×256). The hippocampal voxel (see Fig. 1), was first prescribed on the sagittal anatomic and was centered on the head of the left hippocampus. Then, using the axial series, the left hippocampal voxel was adjusted to be lateral to the medial aspect of the left lateral

fissure. Voxel size was 20×20×20 mm3. In addition to the head of the hippocampus, the voxel as prescribed above, had small contributions from adjacent structures including the uncus and fornix medially, the lateral ventricle, and stria terminalis, tail of caudate laterally/dorsolaterally, and the parahippocampus ventrally. Single-voxel 1H-MRS spectroscopy was conducted using a short echo PRESS (Point RESolved Spectroscopy) sequence (TE=35 ms, TR=2000 ms, 256 acquisitions, 2500 Hz spectral width, 2048 points, duration 9:00 min). Analysis was conducted with LC Model (Provencher,1993). LC Model is an operator independent software that fits in vivo metabolite spectra by using model spectra previously acquired from similar scanning conditions from various compounds in phantom solutions. LC model normalizes the metabolite spectra obtained using an unsuppressed water peak as a reference. Only metabolites with a Cramer-Rao standard deviation less than 20% were included for analysis. Absolute concentrations of NAA, Glx, GPC, GPC+PCh and MI were derived and compared between groups.

Hippocampal volume analysis

The sagittal anatomic images acquired using a 3D/FSPGR/20 sequence (flip angle=20; echo delay time in-phase (TE), minimum; repetition time (TR)=300 ms; inversion recovery=300 ms; matrix= 512×256;fieldof view(FOV)=24cm; scanthickness=1.2mm) during the MRS procedure were used to assess hippocampal volume for all subjects. The protocol used to measure hippocampal volume has previously been detailed (Yucel et al., 2008), and is also available at: http://physics.stjosham.on.ca/_kaan/HippoProtocol.pdf.

Statistical analysis

Differences between each patient group and their age and sex matched controls were tested separately for each metabolite. Statistical tests were performed using SPSS version 16.0. Group differences in metabolite concentration, hippocampal volume and full width at half maximum (FWHM) were assessed with analysis of variance (ANOVA). The FWHM measure provides a estimate of the linewidth of the in vivo spectrum. It therefore provides a rough estimate of the quality of the spectra (Provencher, 2009). When hippocampal volume or FWHM was significantly different between groups they were entered as covariates in the analysis of covariance (ANCOVA) for the group comparisons of each metabolite. Data is presented as mean ± standard deviation.

Results

There were no differences in age between FTE patients (32.1±9.3) and their matched controls (mean=30.0±8.9) [$F(1,24)=0.17$, $p<0.74$] or MTE patients (mean=47.3±10.6) and their matched controls (mean=41.8±12.9) [$F(1,26)=3.12$, $p<0.23$]. Demographic information for MDD patients and controls are listed in Table 1. The MTE group had a significantly longer illness duration (mean=24.5± 13.1) than the FTE subjects (mean=10.9±9.6) [$F(1,20)=1.5$, $p<0.02$]. The HRSD scores for both groups overlapped, with the scores from the MTE group ranging from 1 to 16 and the FTE group from 6 to 27. The group mean HRSD score for MTE patients (mean=8.2±5.6) was significantly lower than the HRSD score for FTE patients (mean=16.3±5.6) [$F(1,24)=8.8$, $p < 0.007$]. One subject in the FTE group had taken citalopram for 3 days prior to

scanning, the rest of the subjects were treatment naïve when scanned. All control subjects, for both groups, were medication free. Among MTE patients, 71% of the subjects were being treated with antidepressants, 14% with antipsychotics, 7% with lithium and 21% were medication free at the time of testing.

Metabolite concentrations of NAA, Glx, GPC, GPC+PCh and MI for the hippocampal voxel are listed in Table 2. The ANOVAs performed revealed significant differences in hippocampal volume [$F(1,26)=6.4$, $p<0.02$) and FWHM [$F(1,26)=9.8$, $p<0.004$] in the MTE group when compared to their matched controls. MTE patients had significantly decreased left hippocampal volume (mean=2159.5±260.8) in comparison to controls (mean=2438.8±307.9). MTE patients also had significantly increased FWHM (mean=0.09±0.01) in comparison to matched controls (mean=0.06±0.01). ANCOVAs, including left hippocampal volume and FWHM as covariates, examining absolute concentrations of metabolites revealed significant effects between MTE patients and matched controls. When comparing the two groups, we found a significant effect for GPC+PCh [$F(1, 26)=5.9$, $p<0.03$] and GPC [$F(1,25)=6.2$, $p<0.021$) (see Fig. 2). MTE patients had significantly elevated GPC+PCh (mean=2.0±0.35) and GPC (mean=2.0+0.35) relative to control subjects (GPC+PCh mean=1.8±0.4; GPC mean=111.8+0.4). There were no other significant differences for any other metabolites in the between MTE patients and controls (see Table 2).

Left hippocampal volume [$F(1,26)=0.048$, $p<0.83$] did not differ between the FTE group and their matched controls. A significant difference in FWHM was found between these two groups [$F(1,26)=5.7$, $p<0.025$]. FTE patients had significantly increased FWHM (mean=0.07±0.016) in comparison to matched controls (mean=0.06±0.01). ANCOVAs, including FWHM as a covariate, revealed a significant effect of MI [$F(1,25)=4.381$, $p<0.047$]. FTE patients had significantly increased MI (mean=6.14±1.31) in comparison to matched controls (mean=5.23±0.93). There were no significant differences in the levels of other metabolites between FTE patients and controls (Table 2). The p-values reported above represent significant univariate tests that would not survive adjustment for multiple testing of the various metabolites. The pattern of results above held when differences in illness severity (BDI scores) were entered as additional covariates in ANCOVAs examining the metabolites of interest for the MTE and FTE groups (MTE vs. Controls; GPCPCH with FWHM, hippocampal volume and BDI as covariates [$F(1, 26)=4.99$, $p<0.036$]: GPC with FWHM, hippocampal volume and BDI as covariates [$F(1,25)=4.525$, $p<0.046$]) (FTE vs. Controls; MI with FWHM and BDI as covariates [$F(1,25)=4.550$, $p<0.044$]).

Discussion

Consistent with our previous report, we found that Hc volumes were smaller in MTE patients compared to matched controls, a difference that was not apparent between FTE patients and their matched comparison subjects (MacQueen et al., 2003). When accounting for Hc volume differences in this study, we found increased levels of choline-

containing compounds in a group of patients with extensive illness histories. These results were in contrast to patients presenting for first lifetime treatment, where there were few differences, other than heightened MI spectra, between FTE patients and matched comparisons.

Our findings of increased choline-containing compounds in the MTE group support previous Hc spectroscopy findings in MDD. Cho is a precursor to the neurotransmitter acetylcholine and is incorporated into the two major phospholipids in neuronal membranes: phosphatidylcholine and sphingomyelin (Loffelholz, 1989). The measured Cho signal is predominately composed of byproducts of phosphatidylcholine hydrolysis (Klein et al., 1993). It is thought that Cho is released from membrane stores as neuronal membranes breakdown, thereby causing increases in local levels and allowing Cho to serve as a measure of membrane turnover (Gadian, 1995), and cell density (Miller et al., 1996). Several H-MRS studies have found mixed results of Cho in the prefrontal cortices of depressed patients (Farchione et al., 2002; Caetano et al., 2005), however few have examined the Hc. In a voxel including the Hc, Mervaala et al. (2000) found significantly elevated Cho/Cr in a group of 34 treatment-resistant MDD patients. The current study supports previous findings of increased Cho in patients with extensive past illness, and extends these findings to include the Hc.

Cholinergic activity in the Hc is modulated by changes in the levels of circulating corticosteroids. Both acute stress and the administration of corticosterone induce rapid

activation of the septo-hippocampal cholinergic system, as evidenced by increases in acetylcholine release and high-affinity choline transport (Finkelstein et al.,1985; Gilad et al., 1985; Imperato et al., 1989; Hortnagl et al., 1993). Such changes suggest a role for septo-hippocampal cholinergic neurons in the response to stress and regulation of the glucocorticoid system. Moreover, glucocorticoids appear to enhance the vulnerability of the septo-hippocampal cholinergic neurons to noxious insult (Hortnagl et al., 1993). As a consequence, it seems reasonable to speculate that the high Cho levels recorded MTE patients may reflect stress reactive remodeling processes associated with the small Hc volumes that were also apparent in these patients.

Conversely, the absence of Hc volume loss in FTE patients is consistent with our observation of no differences in NAA or Cho levels or Hc volume in the FTE patients relative to controls. There was an increase in MI levels in FTE patients in comparison to age-matched controls. MI is thought to reflect glial cell integrity, and/or intracellular second messenger signaling (Kumar et al., 2002). It is possible that this finding represents increased glial cell density in the Hc early in illness, although whether high MI levels represent an early marker of Hc cellular pathology in MDD remains to be examined.

To our knowledge, this is the first 1-HMRS study of depressed patients to include Hc volume in the analysis of 1-HMRS spectra and to examine patients at different points in the course of illness in a systematic manner. Results of previous 1HMRS studies of MDD have been inconsistent (Campbell et al., 2004; Videbech and Ravnkilde, 2004).

The variability in results may reflect in part a lack of consideration for volumetric differences of the region studied and lack of consideration for the illness and treatment status of the patients studied. These variables may be inter-related if volumes are affected by illness course and treatment.

Despite previous reports in MDD of decreased Glutamate and/or Glx levels in brain regions like the frontal and anterior cingulate cortices (Ajilore et al., 2007; Caetano et al., 2005; Rosenberg et al., 2005) we did not observe reduced hippocampal Glx levels in either patient group. In addition, we did not observe decreases in NAA in either patient group. In a previous study significant increases in NAA/Cr concentrations were reported after a clinical improvement in depressive scores was achieved with antidepressant treatment (Gonul et al., 2006). This raises a potential limitation of this study, as most MTE subjects were taking antidepressant medications and were not severely depressed at the time of scanning, suggesting that these factors may have contributed to NAA levels. Furthermore, psychotropic medications administered for MDD may produce a normalization of metabolite levels (Gonul et al., 2006; Block et al., 2008) suggesting that further study of MTE patients will be required to examine the relationship between medication status and levels of choline metabolites. On a similar note, one of the FTE patients had started on citalopram just prior to scanning. In a recent study examining the effects of short-term citalopram use, however, Taylor and colleagues (2008) reported no significant differences in MI levels following a 10-day administration of citalopram.

These results suggest that treatment with citalopram for three days was unlikely to affect the MI levels observed in this patient.

Another limitation to this study is that it is difficult to ascertain with confidence the precise number of past episodes or duration of illness that patients experience. We are confident that none of our FTE patients had ever received psychiatric treatment prior to this presentation, but it possible that some had experienced short, mild depressions that were not identified on structured interview. Similarly, although we were confident that all MTE patients had a significant past illness burden based on a known treatment history, even with life charting methodology it can be difficult to ascertain the onset and duration of relatively mild episodes, particularly if patients have low-grade dysthymia or partial resolution of symptoms in the inter-episode intervals.

Another limitation concerns the issue of multiple comparisons in the statistical analysis. This issue is not unique to our work, as the issue of multiplicity is one for which the field of brain imaging must continue to seek a standardized solution.

A final limitation concerns the differences in BDI scores, age and sex across the FTE and MTE groups included in this study. While we did not directly compare the Hc volumes or 1-HMRS metabolites in these groups, such differences demand that caution be exercised when thinking about the study outcomes as they pertain to illness trajectories.

In summary, we found high Cho levels and small volumes in the Hc of MDD patients with a high past illness burden. While a number of HMRS studies have controlled for perturbations in Hc volume (e.g. Posttraumatic Stress Disorder; Schuff et al., 2008; long-term corticosteroid treatment; Brown et al., 2004), to our knowledge, this is the first study to include Hc volume in the analysis of 1-HMRS metabolites in MDD. We suggest that controlling for volumetric differences across study populations can provide for a more accurate assessment of potential metabolic differences identified using 1-HMRS. The pattern of results seen in the MTE group was not apparent in patients assessed after presenting for first lifetime treatment of depression, suggesting that the course of depression may importantly influence Hc volumes and Cho levels. The FTE group did, however, have high MI levels compared to matched controls, raising the possibility that an increase in glial cells occurs early in the course of illness, reflecting an early marker of cellular changes in the Hc of patients with depression.

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Table I. Clinical and demographic information for FTE, MTE and healthy control subjects.

	First Episodes (N=14)	Controls for First Episodes (N=13)	Multiple Episodes (N=14)	Controls for Multiple Episodes (N=14)
Age (years)	32.1 (9.3)	30.0 (8.9)	47.3 (10.6)	41.8 (12.9)
Female	5	5	10	10
Male	9	8	4	4
Education (years)	14.7 (2.6)	18.1 (3.6)	15.7 (2.0)	16.9 (4.4)
Duration of Illness (years)	10.1 (8.7)	NA	24.4 (13.1)	NA
Number of Episodes	5.4 (5.6)	NA	8.0 (8.9)	NA
Medications:				
Antidepressants	1	NA	10	NA
Antipsychotics	0	NA	2	NA
Lithium	0	NA	1	NA
Medication Free	13	NA	3	NA
BDI	25.7 (12.9)	0.64 (1.50)	12.6 (5.0)	0.8 (1.7)
HRSD	16.2 (7.2)		8.2 (5.6)	

Table 2. Absolute metabolite concentrations for FTE and MTE patients compared to healthy control subjects.

	Recently Diagnosed (N=14)		Controls for Recently Diagnosed (N=13)		Multiple Episodes (N=14)		Controls for Multiple Episodes (N=14)	
Metabolite	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glx	12.77	2.01	11.37	2.34	13.19	3.86	11.82	2.25
GPC	1.97	0.44	1.65	0.36	2.01	0.35	1.76	0.42
GPC+ PCh	1.97	0.43	1.65	0.36	2.01	0.35	1.76	0.40
MI	6.14	1.31	5.24	0.93	5.29	0.92	5.22	0.84
NAA	6.21	1.45	5.61	1.06	5.72	1.35	6.34	1.34

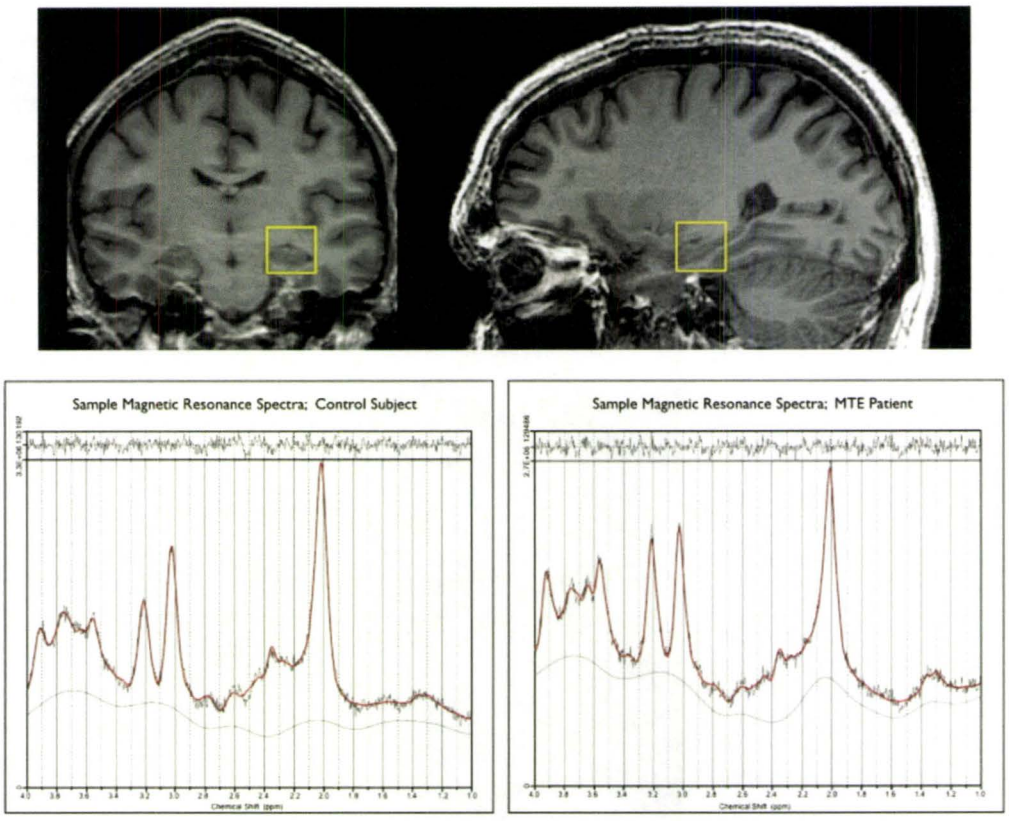

Figure 1: Graphic depicting the voxel placement for the 1H-MRS acquisition and two representative MRS spectra from a Control subject and an MTE patient. The image is presented according to radiological convention.

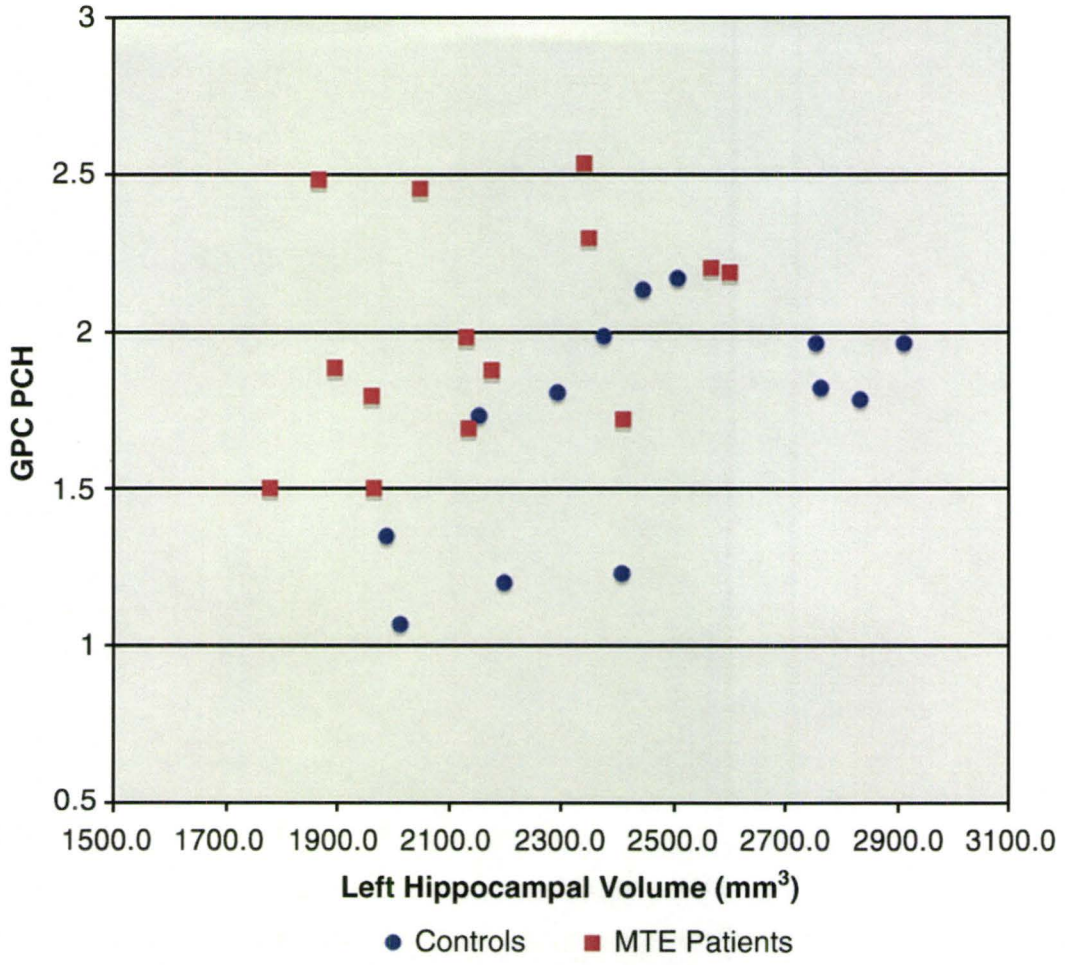

Figure 2: A scatterplot of individual datapoints for choline concentrations and left hippocampal volume in MTE patients and Controls.

CHAPTER 3:

Altered hippocampal activation in response to recollection memory trials in Major Depressive Disorder: An fMRI Study

This manuscript outlines our examination of the changes Hc functional activation during a Hc-dependent recollection memory task. We studied patients experiencing their first episode of MDD, those who had experienced multiple past episodes of MDD as well as matched controls for each depression group.

From these experiments we observed: A) Decreased Hc activation during Hc-dependent trials in patients who have experienced multiple past episodes of MDD in comparison to matched controls. B) We observed corresponding Hc-dependent memory errors in this same group of depressed patients. C) Increased Hc activation in patients experiencing their first episode of MDD on all trial types. D) We did not observe memory errors on any trial type in this group of depressed patients in comparison to controls.

This paper is in preparation to be submitted to the journal *Nature Neuroscience*. I was responsible for the execution and interpretation of all of the experiments. I wrote the manuscript. My co-authors provided technical advice and assistance as well as supervision and funding for the work (see Preface for more details).

Abnormal hippocampal activation in patients with minimal or extensive past history of major depression: An fMRI study

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ABSTRACT

Dysfunction in various memory systems has been identified in Major Depressive Disorder (MDD). Impairments on recollection memory tests are consistently reported in MDD (MacQueen et al., 2002). These abnormalities may reflect the underlying hippocampal (Hc) volume changes observed in MDD, particularly in patients with a protracted course of illness (McKinnon et al., 2009). The aim of the present study was to use functional magnetic resonance imaging (fMRI) to examine the effects of illness duration on hippocampal activation during recollection memory trials. Patients presenting for first treatment (FTE) of MDD (N = 14, mean age 26.35 $\pm$ 7.43), were compared to patients who had experienced three or more past treated episodes (MTE) of MDD (N = 22, mean age 44.91 $\pm$ 11.32). Age and sex matched controls were recruited for both groups. All subjects took part in an fMRI recollection memory task. Using region of interest analysis isolating the hippocampus we observed decreased recruitment of the Hc in the MTE patients in comparison to controls during recollection memory trials. In contrast, FTE patients showed similar Hc recruitment as matched controls on recollection memory trials and greater recruitment for habit and guessing trials. The findings of greater recruitment at first treatment, and decreased recruitment after multiple episodes suggest that the Hc is sensitive to the impact of disease burden and repeated episodes of MDD. Furthermore, greater recruitment of the Hc in first treatment patients raises the possibility that patients may engage compensatory processes during the early stages of illness that are attenuated with repeated episodes of illness.

Keywords: Major Depressive Disorder, Hippocampus, Recollection Memory, Functional Magnetic Resonance Imaging

1. Introduction

Although the exact neural underpinnings of Major Depressive Disorder (MDD) have not been fully elucidated, there is a multitude of evidence suggesting disruption of fronto-temporal networks (Mayberg, 2003; Seminowicz, et al., 2004). The hippocampus (Hc), a key region in these networks, has been implicated in the pathophysiology of MDD in both clinical and preclinical research. While the Hc has been studied extensively through volumetric Magnetic Resonance Imaging (MRI) (Koolschijn et al., 2009; McKinnon et al., 2009) and behavioural tasks targeting the Hc (Campbell et al. 2004), functional imaging studies investigating the Hc are lacking.

The Hc is a stress sensitive structure. Neurobiological responses to physical and psychological stress are mediated in part by the hypothalamic-pituitary-adrenal (HPA) axis. Engagement of the HPA axis prompts the release of glucocorticoids, which help with bodily responses to stress by modulating a number of metabolic functions including pro-inflammatory responses and the liberation of energy stores (Sapolsky et al., 2000). The Hc contains abundant concentrations of glucocorticoid receptors (De Kloet et al., 1998) and is a major site in the glucocorticoid (GC) negative feedback circuitry of the HPA axis (Jacobson and Sapolsky, 1991). However, chronic or repeated periods of stress can result in elevated GC levels, compromised hippocampal function (Diamond et al., 2006) and reductions in hippocampal volume (McEwan, 2000; McEwan & Sapolsky 1995). Such GC mediated changes in hippocampal volume are thought to result from reductions in neurogenesis, dendritic atrophy and impaired glucose metabolism (Reagan

& McEwen, 1997; Gould and Tanapat, 1999; Sapolsky, 2000). Hippocampal loss and remodeling and the corresponding loss in GC receptor density may lead to less efficient inhibitory control of the corticotrophin releasing hormone-producing cells of the hypothalamus and result in increased GC and the cyclic worsening of this process (Bremner, et al., 2000).

HPA dysregulation is a consistent finding in studies of MDD (Duschle et al., 1998; Young et al., 1994). A large number of studies have identified GC irregularities in MDD, with elevated afternoon basal levels and, in particular for older or more severely depressed patients, patterns of blunted GC reactivity and impaired post stress recovery (Burke et al., 2005). Stress related reductions in neuroplasticity, including decreased neurogenesis, increased remodeling of dendrites, loss of glial cells, or increased excitotoxicity, could lead to low Hc volume, disruptions in fronto-temporal circuitry and increased vulnerability to subsequent episodes of depression (McEwen, 1999; Pittenger and Duman, 2008).

Volumetric changes in the Hc have been extensively reported in MDD. Several meta-analyses have concluded that Hc volume is smaller in patients with MDD (Campbell, et al., 2004, Videbech, et al., 2004, McKinnon et al., 2009). Moreover, recent results from a meta-analysis of volumetric MRI studies, including over 1000 patients and controls, found that decreases in Hc volume are more pronounced in patients who have experienced a high past illness burden. (McKinnon et al., 2009).

Hc dependent memory is also affected in MDD. Recollection (declarative) memory, the conscious recall of specific information, is particularly affected in MDD (MacQueen et al., 2003). Various studies have identified the importance of the Hc in this type of memory (Buckner et al., 1995; Bernard et al., 2001; Mayes et al., 2001). Our prior research has shown that recollection memory deficits in MDD patients (MacQueen et al., 2003), particularly those who have experienced a protracted course of illness (MacQueen et al., 2002).

While alterations in Hc volume and Hc dependent memory have been extensively studied in depression, these findings are not unique to MDD. Decreased Hc volume has also been reported in posttraumatic stress disorder (Smith, 2005), schizophrenia (Heckers, 2001), Alzheimer's disease, Parkinson's disease and epilepsy (Geuze et al., 2005). Additionally, Hc dependent memory changes have also been noted across psychiatric disorders. Deficits in declarative memory are among the most consistently reported findings in schizophrenia (Weiss & Heckers, 2001). Similar alterations have been reported in bipolar disorder (Deckersbach et al., 2004; Glahn et al., 2005), Alzheimer's disease (Satler et al., 2007) and posttraumatic stress disorder (Golier et al., 2007). There has been extensive behavioural and volumetric MRI research examining the Hc across various psychiatric disorders; however, few studies have utilized functional MRI (fMRI) to examine Hc involvement in declarative memory.

While volumetric and behavioural abnormalities of the Hc have been frequently reported in MDD, to our knowledge no prior studies have examined Hc dependent memory with fMRI paradigm. Moreover, few studies on MDD have examined the impact of illness burden in a systematic fashion. Therefore, the purpose of the current research was to use fMRI technology to examine Hc activation during a declarative memory task. The task we selected has previously been shown to assess Hc dependent memory processes separately from Hc independent memory functions (Jacoby, 1998; Jacoby et al., 1996; Ruiz-Caballero & Gonzalez, 1997). We studied two distinct populations of MDD patients as well as age and sex matched controls. One MDD group was made up of patients who presented for first treatment of a depressive episode (FTE). The second MDD group enlisted patients who had experienced a minimum of three past treatment episodes (MTE). With previous evidence of Hc volumetric differences associated with MDD being more pronounced in patients with recurrent episodes of MDD, this study design allowed for the examination of the impact of low and high past illness burden on Hc function.

We hypothesized that consistent with prior reports of abnormalities in the Hc in MDD, both groups of depressed patients would show deficits on Hc dependent memory trials. Moreover, we further hypothesized that depressed patients would show decreased activation of the Hc on Hc-dependent recollection memory trials and that the MTE depressed group would show a greater attenuation of activation than the FTE group.

2. Methods

2.1. Subject Selection

The two patient groups were recruited from the Mood Disorders Program at St. Joseph's Centre for Mountain Health Services in Hamilton. This study was approved by the Research Ethics Board of St Joseph's Healthcare Hamilton. All subjects gave informed consent after a full explanation of the study protocol. Subjects with a history of head injury, neurological illness, alcohol or substance abuse, previous history of electroconvulsive therapy or transcranial magnetic stimulation within the last two years were excluded from the study. Healthy controls were free from medication and had no current symptoms or medical history of a mental health disorder. A psychiatrist confirmed a primary diagnosis of unipolar MDD according to DSM-IV criteria (American Psychiatric Association, 1994). One group of depressed participants (FTE) was comprised of individuals who were presenting for assessment for the first time and had no past treatment history (mean age 26.4 years $\pm$ 7.4). The second group of patients (MTE) included individuals who had extensive illness histories, having experienced three or more previous episodes of MDD and/or an illness duration of 5 of more years (mean age 44.9 years $\pm$ 11.3). All subjects with depression met criteria for non-psychotic unipolar major depressive disorder as determined by the Structured Clinical Interview for DSM-IV (SCID-I; First et al., 1998). In addition, psychiatric symptoms were assessed just prior to scanning using Beck Depression Inventory (BDI; Beck and Beamesderfer, 1974) and the Hamilton Rating Scale for Depression (HRSD: Hamilton, 1960). A group of age and sex

matched controls were recruited from the community, and subsets of 18 and 15 controls were selected to match by age and sex with the MTE and FTE groups respectively. Clinical details and demographic information of subjects can be found in Table 1.

2.2 Process Dissociation Task:

Subjects were asked to complete the recollection memory - process dissociation task, adapted for scanning from the previously described protocol (MacQueen et al., 2003; MacQueen et al., 2007). The process dissociation task was selected as it has been shown to distinguish recollection memory (Hc-dependent) separate from habit memory (Hc-independent) capacities (Ruiz-Caballero, & Gonzalez, 1997) and because it has been studied extensively in non-psychiatric populations (Jacoby, 1998; Jacoby et al. 1996).

In the first part of a scan session, subjects take part in a half hour training phase outside of the MRI. An IBM ThinkPad computer was used with MICROEXPERIMENTAL software (Schneider, 1988) to display the stimuli. During the training, subjects were repeatedly exposed to eighteen stimulus words pairs. Each word that appeared on the left side of the screen was paired with two associative responses that occurred with equal frequency in published norms (e.g. barn-yard, barn-farm). Word pairs were initially presented in word + word fragment combinations (eg. barn _ar_). Subjects are given two seconds to guess the correct answer to complete the word fragment (eg. barn yard or barn farm) before the correct completed word pair combination appears

on the screen. Unknown to the subject, word pairs were presented with an unequal distribution. For example, one word pair (eg. barn yard) appeared as the correct response in 67% of the trials, while the other word choice (eg. barn farm) appeared as the correct response 33% of the time. This was done to generate habit associations with the higher frequency word pair. The particular word combinations presented as either high or low frequency pairs were equally distributed across all subjects.

After the training phase subjects were relocated to the magnet. The task was projected onto a mirrored visor located on top of the head coil within the scanner bore. Stimulus presentation was controlled by Eprime Software. The scanning parameters were designed with a 3 second silent period following each TR to allow for the subjects to give verbal responses to complete the word pairs.

During the testing portion of the experiment, subjects were presented with a 17 memory tests that consisted of a study list, an arithmetic distractor and then a memory test. The study and test lists were made up of the word combinations previously learned during the training phase. The study list contained 6 habit word pairs (high frequency word match) and 3 recollection word pairs (low frequency word match). Subjects were instructed to read the word pairs and remember them for the following memory test. Complete word pairs (eg. barn yard) appeared on the screen for 1 second followed by

a fixation point shown on the screen for 0.5 seconds before the next word pair. Following the study list, a brief mathematical distractor task was presented.

In the memory test list subjects were shown 11 word + word fragment combinations (eg. barn _a r_) consisting of the 6 habit word pairs and three recollection word pairs from the study list. In addition, two word pairs that were not included in the study list were presented to assess a subject's tendency to guess (non-item trials). Subjects were instructed respond verbally and to complete the word fragments with the words on the immediately preceding study list or provide their best guess if they could not remember which word was presented in the study list.

The test list was presented with a jittered intertrial interval with periods of 3, 6 or 12 seconds of a fixation point between test list items. This portion of the experiment was broken into 5 scan runs.

Recollection scores were obtained by subtracting the recollection trial (when study-list pairs were the same as the low frequency pair during training) probability from the habit trials (where study-list pairs were the same as the high-frequency pair during training) probability. An estimate of habit memory is obtained by the formula habit = low frequency probability/1-recollection (from MacQueen 2002).

2.3 Image Acquisition

The imaging session lasted approximately 1 hour during which subjects complete the process dissociation task and a T1 weighted anatomical scan. Imaging was performed using a 3T GE MRI scanner. Thirteen axial slices (3 mm thickness) centered on the hippocampus were imaged with a temporal resolution of 3 seconds using a echo planar pulse sequence [echo time (TE) = 43 ms; repetition time (TR) = 3000 ms; matrix = 128 × 64; flip angle = 90°]. Stimuli were presented according to an event related design with a jittered stimulus presentation. An anatomical scan in the sagittal orientation was obtained following the fMRI portion. The scanning parameters for the anatomical image series were 3D SPGR pulse, sagittal plane, fast IRP sequence, TR=10.8ms, TE=2ms, TI = 400 ms, flip angle= 20°, matrix 256x256, FOV=24, slice thickness 1mm, no skip, field of view 124 contiguous slices.

2.4 Analysis

Acquired images were transferred to a workstation, preprocessed and analyzed using Brain Voyager QX version 1.10.4 (Brain Innovation B.V., Maastricht, The Netherlands). The functional data sets were slice-time corrected, linear detrended, 3D-motion corrected and realigned, and normalized to Talairach space (Talairach & Tournoux, 1988). High-resolution T1-weighted three-dimensional (3D) anatomical MR

data sets were transformed into Talairach space, used for co-registration and averaged to generate a composite image onto which functional activation results were projected. An event related model for each participant was used to examine the Blood Oxygen Level Dependent (BOLD) signal at every voxel. Using a random-effects multiple general linear model recollection memory, habit memory, non-item and study list presentations were set as the explanatory variables accounting for differences in BOLD signals within and between groups. Contrasts were corrected for multiple-comparisons using the false discovery rate methodology .05 (Genovese, Lazar, & Nichols, 2002), and the average statistical value for the resulting regions of interest are reported.

3. Results

3.1 Demographics

There were no significant differences in age between the FTE group (26.3 ± 7.4) and their matched controls (26.7 ± 7.7), or the MTE group (44.9 ± 11.3) and their matched controls (42.1 ± 11.5). Demographic information for MDD patients and controls are listed in Table 1. The MTE patients included in the study had a mean illness duration of 20.1 ± 12.8 years, a mean BDI score of 16.1 ± 9.2 and a mean HRSD score of 10.9 ± 5.1. While the FTE patients had a mean illness duration of 8.2 ± 4.9 years, a mean BDI score of 26.3 ± 11.2 and a mean HRSD score of 17.9 ± 7.0. There were significant differences in age, illness duration, BDI and HDRS scores between the MTE and FTE groups. MTE subjects were significantly older [$F(1,35) = 28.37$, $p < 0.001$] and had a significantly longer illness duration [$F(1,35) = 11.00$, $p < 0.002$] compared to FTE

subjects. FTE subjects were significantly more depressed compared to MTE subjects (BDI: [$F(1,28) = 7.26$, $p < 0.012$; HRSD: [$F(1,34) = 11.00$, $p < 0.002$]). 86% of the MTE subjects were being treated with antidepressants (13 SSRIs, 5 SNRIs, 2 tetracyclic antidepressant, 2 MAOIs, 2 atypical antidepressant, 1 tricyclic antidepressant), and 23% with antipsychotics (1 typical and 3 atypical). Three FTE subjects were receiving antidepressant treatment at the time of scanning. For these three subjects the administration of medication had commenced 6, 4 and 2 days respectively prior to scanning. All other FTE patients had no lifetime history of exposure to any psychotropic medications.

3.2 Memory Performance

Recollection memory was significantly impaired in the MTE group (mean = 0.521 ± 0.27) in comparison to their matched controls (mean = 0.708 ± 0.13) [$F(1,39) = 7.302$, $p < 0.010$]. There was one outlier in the MTE group; recollection memory was still significantly impaired after removal of this outlier [$F(1,38) = 6.949$, $p < 0.012$]). In contrast, significant differences in recollection memory were not observed for FTE patients (mean = 0.722 ± 0.12) and their matched controls (mean = 0.673 ± 0.18) [$F(1,25) = 0.716$, $p < 0.406$]. The FTE and MTE groups performed as well as their matched controls on both habit memory trials (FTE vs. controls [$F(1,25) = 0.598$, $p < 0.447$]; MTE vs. controls [$F(1,39) = 0.299$, $p < 0.587$] and in their tendency to guess on non-item trials (FTE vs. controls [$F(1,22) = 0.260$, $p < 0.615$]; MTE vs. controls [$F(1,36) = 0.317$, $p < 0.577$]. Results of memory performance are reported in Table 2.

3.3 Imaging Results

Between Groups Analysis of Memory Performance:

Region of interest analysis was conducted to assess hippocampal activation in response to task conditions. Regions of interest were traced on a summed anatomic image at the group level to include the hippocampus bilaterally and subsequently for each between groups contrast (MTE vs. controls, FTE vs. controls). Between groups activation patterns were determined for the contrast of each type of memory trial (recollection, habit and non-item trials) against the presentation of the study list. See Table 3 for a full listing of results.

Significant differences were observed between MTE patients and controls during recollection memory trials. Control subjects showed heightened activation in the right Hc and the left Parahippocampal gyrus for the contrast of recollection memory trials versus the study list (See Figure 1). There were no differences for the contrasts of habit trials versus the study list or non-item trials versus the study list between the MTE patients and their controls. In contrast FTE patients showed increased activation in comparison to their matched controls. For the comparison of recollection memory trials versus the study list, FTE patients showed greater bilateral Parahippocampal gyrus (BA 36 & 27) activation, although these results did not survive correction for multiple comparisons (See

Figure 2). The same trends were observed for habit memory and non-item trials versus the study list. FTE patients had significantly increased bilateral activation of the hippocampus during habit memory and in the Parahippocampal gyrus bilaterally during non-item trials in comparison to matched controls.

4. Discussion

Consistent with our previous studies examining the Hc in depression, we report findings of attenuated Hc activation during Hc-dependent recollection memory task with corresponding impairments on recollection memory trials in a group of multiple episode MDD patients in comparison to matched healthy controls. The current results support previous findings of altered Hc volume and function in MDD (MacQueen et al., 2002; Campbell et al., 2004; Videbech et al., 2004). In addition, we report findings of heightened activation during habit memory and non-item trials in a group of recently diagnosed MDD patients, without corresponding impairments in Hc-dependent memory in comparison to controls.

To our knowledge, this is the first fMRI study to use the process dissociation task in depressed individuals. The Hc has been studied extensively with volumetric MRI methods in depression. Recent meta-analyses of studies examining Hc volume in patients with MDD supported the hypothesis that in the aggregate Hc volumes are lower in patients with MDD compared to age and sex matched controls (Campbell et al., 2004, Videbech et al., 2004). Furthermore, evidence suggests that the difference between

patients and controls is most apparent in those with a recurrent or chronic form of illness These findings are compatible with our results in that we observed decreased activation in our MTE group, but not in our FTE group, suggesting that illness duration negatively impacts Hc volume (MacQueen et al., 2003; McKinnon et al., 2009; Milne et al., 2009).

Our findings of attenuated Hc activation in the MTE patient group were further examined by analyzing the individual within group patterns of activation for the same contrasts of interest (See Table 4). We found that while the matched controls for the MTE group showed robust Hc/Parahippocampal activation in response to recollection memory trials, the MTE group had deactivation of the Hc and Parahippocampal gyrus. These results add further evidence to suggest that the course of depression may importantly influence Hc activation in response to recollection memory tasks.

While we observed a pattern of decreased Hc activation with corresponding recollection memory deficits in the MTE patients, these same observations were not apparent in a group of recently diagnosed depressed patients. We did not observe significant differences between the FTE patient group and their matched controls during recollection memory trials. Moreover, there were no differences between these two groups in their ability to perform our task. Differences were observed, however, during habit and non-item trials. FTE patients showed increased Hc and Parahippocampal gyrus activation on habit memory trials and on non-item trials in comparison to controls, without corresponding differences in task performance. Although it did not survive

correction for multiple comparisons, we did find a trend towards greater recruitment of the Hc in FTE patients during recollection memory trials in comparisons to controls. These results may suggest that at the onset of MDD, there is universal (global) heightened engagement of the Hc across trial types. In contrast, healthy controls selectively recruit the Hc only during recollection memory trials, accounting for the lack of significant difference for this trial type.

Greater activation across trial type in FTE patients may suggest that in the early course of MDD, patients are able to compensate with increased Hc recruitment in order to successfully complete the task. However, with a protracted course of illness consisting of repeated depressive episodes and corresponding prolonged exposures to GC (McEwan, 2004; Sheline et al., 1996), the Hc is no longer able to fully engage the Hc for proper recruitment as we observed in the MTE patient group. In some related 1-HMRS work (Milne et al., 2009), we have associated reduced hippocampal volumes in MTE patients with elevated hippocampal choline levels. As choline is considered a marker of neuronal membrane breakdown (Gadian, 1995), elevated levels may reflect stress reactive remodeling processes at the hippocampus. This study further reported an absence of Hc volume loss in FTE patients that was accompanied by increased levels of myo-inositol. From these findings it was suggested that early in the course of MDD there may be increases in glial cell density at the hippocampus (Milne et al., 2009). In the current context it is interesting to speculate that if the heightened Hc engagement in FTE patients

reflects some kind of compensatory processes, then the associated increases in local metabolic needs may be satisfied by changes in the density of glia.

Imaging studies of healthy subjects have highlighted the importance of Hc recruitment for declarative memory. It has been suggested that the Hc plays a role in assessing novel items (Tulving et al., 1994b), information retrieval success (Nyberg et al., 1996), visual and spatial memory (Bellgowan et al. 2003), and recollection memory (Brown & Aggleton 2001, Rugg & Yonelinas 2003, Yonelinas 1997). Recently, a review of imaging studies examining memory in the medial temporal lobes found that Hc activation was most consistent with proposed memory models that link the Hc to recollection memory (Diana et al., 2007).

Relatively few functional imaging studies have examined declarative memory in psychiatric populations. Schizophrenia research has demonstrated a number of temporal lobe abnormalities across different tasks. Decreased activation of hippocampal and Parahippocampal regions have been noted in response to verbal recognition (Wood & Flowers, 1990) word-stem cued retrieval (Heckers et al., 1998) and non-verbal recognition (Ragland et al., 2000) tasks. These functional activation deficits in schizophrenia appear to be consistent with the abundant evidence suggesting smaller Hc volumes in this disorder (Heckers 2001; McCarley et al., 1999; Nelson et al., 1998)

In the extant literature there is one recent study that has attempted to study Hc related memory in MDD (Werner et al., 2009). Applying a subthreashold paired

associate task using faces and occupations, the authors failed to identify differences in Hc activation between MDD patients and controls. Group differences in other regions associated with memory, including frontal and parietal regions, were observed (Werner, et al., 2009). The lack of Hc differences might suggest that the task employed was insufficiently sensitive to Hc activation changes to isolate group differences in this region. Moreover, Werner and colleagues (2009) postulated that the small sample size (N=11) and relatively young age of depressed patients (mean = 37.18) may have contributed to the lack of Hc findings.

The present study has several limitations. Notably, some patients were taking antidepressant medication and it is possible that the observed differences in activation were influenced by treatment. Another limitation to this study is that it is difficult to ascertain with confidence the precise number of past episodes or duration of illness that patients experience. We are confident that none of our FTE patients had ever received psychiatric treatment prior to this presentation, but it possible that some had experienced short, mild depressions that were not identified on structured interview. Similarly, although we were confident that all MTE patients had a significant past illness burden based on a known treatment history, even with life charting methodology it can be difficult to ascertain the onset and duration of relatively mild episodes, particularly if patients have low-grade dysthymia or partial resolution of symptoms in the inter-episode intervals.

Finally, because of the cross-sectional nature of the study design, it is impossible to confirm whether the past burden of illness in the MTE group led to or resulted in part from, the altered Hc activation that we observed in this group compared to those with minimal illness burden. Longitudinal functional imaging studies that repeat the same task in patients across various points in illness history are lacking, likely because of the complexity involved in this approach.

In summary, we found attenuated activation in the Hc of MDD patients with a high past illness burden during a Hc dependent recollection memory task. In addition, MTE patients showed recollection memory deficits during the task. Importantly, these findings were not present in a group of patients presenting for first treatment of depression. Instead, FTE patients showed heightened Hc activation without corresponding memory deficits on all memory trial types. These findings add fMRI evidence that the Hc is a region that is sensitive to the impact of disease burden and is negatively impacted with repeated episodes of depression.

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Table I. Clinical and demographic information for MDD patients and matched controls.

	First Episodes (N=14)	Controls for First Episodes (N=14)	Multiple Episodes (N=22)	Controls for Multiple Episodes (N=18)
Age (years)	26.3 (7.4)	26.7 (7.7)	44.9 (11.3)	42.1 (11.5)
Female	4	7	15	13
Male	9	7	7	5
Education (years)	13.4 (1.6)	17.7 (2.9)	15.5 (2.1)	16.8 (3.7)
Duration of Illness (years)	8.2 (4.9)	NA	20.1 (12.8)	NA
Number of Episodes	3.2 (3.3)	NA	5.6 (2.4)	NA
Medications:				
Antidepressants	3	NA	19	NA
Antipsychotics	1	NA	5	NA
Lithium	0	NA	2	NA
Medication Free	11	NA	3	NA
BDI	26.3 (11.2)	1.5 (2.3)	16.1 (9.2)	0.9 (1.4)
HDRS	17.9 (7.0)	NA	10.9 (5.1)	NA

Table 2. Memory performance across trial type during the process dissociation task

Trial Type	FTE (N=14)	Controls FTE (N=14)	*P* value	MTE (N=22)	Controls for MTE (N=18)	*P* value
Recollection	0.72 (0.12)	0.67 (0.18)	0.406	0.52 (0.27)	0.71 (0.12)	0.012
Habit	0.42 (0.20)	0.36 (0.18)	0.447	0.43 (0.13)	0.46 (0.19)	0.587
Guessing	0.60 (0.09)	0.60 (0.11)	0.615	0.62 (0.10)	0.64 (0.09)	0.577

Table 3. Localization of memory-related fMRI activation identified through between groups comparisons.

	X	Y	Z	Region	Brodmann Area	t-value	# Voxels
Recollection Trials – Study Trials							
NC > MTE	26	-20	-14	Hippocampus		3.679	1041
	-27	-25	-16	Parahippocampal gyrus	35	3.334	26
FTE > NC	24	-43	-6	Parahippocampal gyrus	36	3.165	177*
	-22	-34	-3	Parahippocampal gyrus	27	3.224	918*
Habit Trials – Study Trials							
FTE > NC	27	-31	-4	Hippocampus		3.268	2431
	-19	-34	-2	Hippocampus/Parahippocampal gyrus	27	3.477	2394
Non-Item Trials – Study Trials							
FTE > NC	24	30	-3	Parahippocampal gyrus	27	3.161	713
	-22	-33	-3	Hippocampus/Parahippocampal gyrus	27	3.660	2547

* Results not corrected for multiple comparisons

Table 4. Within group patterns of activation during memory-related fMRI process dissociation task.

	X	Y	Z	Region	Brodmann Area	t-value	# Voxels
Recollection Trials – Study Trials							
MTE	28	-21	-16	Hippocampus		-3.393	5120
	-26	-19	-2	Parahippocampal gyrus		-3.211	3718
	-23	-21	-22	Parahippocampal gyrus	35	-3.272	1083
NC for MTE	23	-15	-15	Hippocampus/Parahippocampal gyrus	28	4.480	8829
	-21	-12	-15	Hippocampus/Parahippocampal gyrus	28	4.291	5700
FTE	29	-26	-10	Hippocampus (Head)		3.549	1419
	-34	-25	-12	Hippocampus (Head)		3.333	685
	-21	-41	-7	Parahippocampal gyrus	36	3.837	1766
NC for FTE	23	-2	-18	Amygdala		3.674	1526
	30	-30	-2	Hippocampus (Tail)		-3.611	2600
	-22	-31	-2	Hippocampus (Tail) /Parahippocampal gyrus	27	-3.928	2759

Table 4. Continued

	X	Y	Z	Region	Brodmann Area	t-value	# Voxels
Habit Trials – Study Trials							
MTE	31	-42	-6	Parahippocampal gyrus	19	3.594	1901
	27	-20	-2	Parahippocampal gyrus		-3.345	229
	-29	-36	-6	Hippocampus		3.118	486
NC for MTE	21	-15	-15	Hippocampus/Parahippocampal gyrus	28	3.776	3838
	-26	-14	-15	Hippocampus		3.278	3038
FTE	26	-18	-14	Hippocampus (Head)		4.442	5085
	-21	-43	-6	Hippocampus (Head) / Parahippocampal gyrus	36	4.872	5782
	-23	-36	-3	Hippocampus (Tail)		5.869	3509
NC for FTE	23	-3	-17	Amygdala		3.990	1761
	30	-30	-4	Hippocampus (Tail)		-4.179	2958
	-21	-7	-21	Amygdala/Uncus		2.654	420
	-28	-30	-3	Hippocampus (Tail)		-4.370	3391
Non-Item Trials – Study Trials							
NC for MTE	24	-15	-14	Hippocampus		4.517	10,461
	-26	-18	-15	Hippocampus		4.372	7599
FTE	24	-17	-14	Parahippocampal gyrus	28	4.066	2243
	-35	-11	-15	Hippocampus (Head)		4.218	1844
	-22	-36	-6	Parahippocampal gyrus	36	4.339	2635
NC for FTE	24	-4	-18	Amygdala		3.302	994
	31	-28	-5	Hippocampus (Tail)		-3.808	2308
	32	-32	-6	Hippocampus (Tail)		-4.429	3202

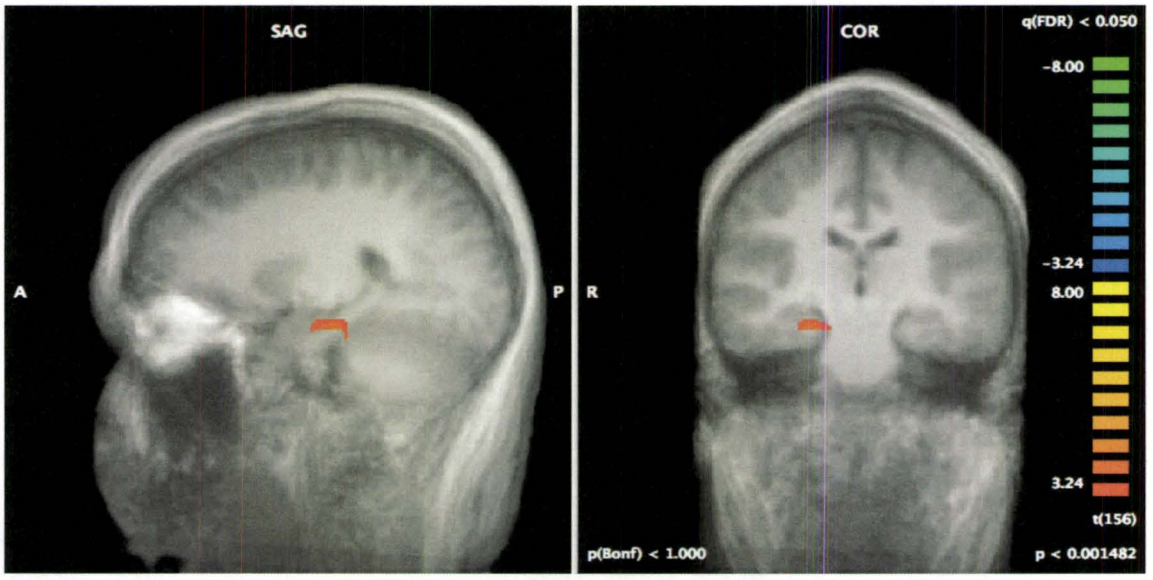

Figure 1: Group differences in hippocampal activation during recollection memory trails. Healthy control subjects have increased activation in the hippocampus compared to MTE patients. FDR corrected statistical maps are superimposed on averaged anatomical group images. Images are presented according to radiological convention.

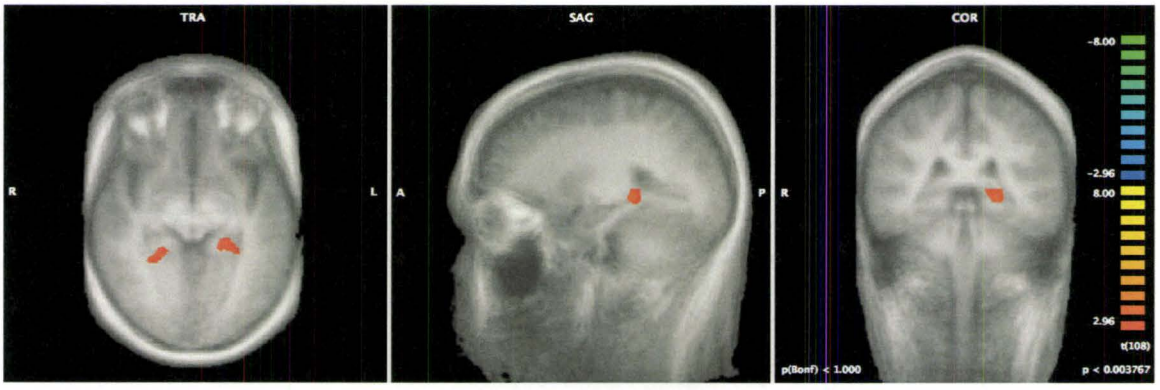

Figure 2: Group differences in hippocampal activation during recollection memory trails. FTE patients show a trend towards heightened bilateral hippocampal activation compared to healthy control subjects. Statistical maps are superimposed on averaged anatomical group images. Images are presented according to radiological convention. Results are not FDR corrected.

CHAPTER 4:

An fMRI study of reward circuitry in patients with minimal or extensive past history of major depression.

This manuscript outlines our examination of the changes in reward processing neurocircuitry in depression. We studied patients experiencing their first episode of MDD, those who had experienced multiple past episodes of MDD as well as matched controls.

From these experiments we observed: A) Health to illness gradients of activation in the nucleus accumbens, anterior cingulate (HC > FTE > MTE) in response to rewarding outcomes. B) Alterations in orbitofrontal and medial prefrontal activation in response to punishing outcomes in the depressed populations. C) There were no differences in task performance between either of the depressed groups and their matched controls.

This paper was submitted to the journal *Neuropsychopharmacology* in October 2009. I was responsible for the execution and interpretation of all of the experiments. I assisted in writing the manuscript. My co-authors provided technical advice and assistance as well as supervision and funding for the work (see Preface for more details).

An fMRI study of reward circuitry in patients with minimal or extensive history of major depression

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Abstract

Functional abnormalities in regions associated with reward processing are apparent in people with depression, but the extent to which disease burden impacts on the processing of reward is unknown. This research examined the neural correlates of reward processing in patients with major depressive disorder and varying degrees of past illness burden. Twenty-nine depressed patients and twenty-five healthy subjects with no lifetime history of psychiatric illness completed the study. Subsets of fourteen patients were presenting for first lifetime treatment of a depressive episode and fifteen patients had at least 3 treated episodes of depression. We used functional magnetic resonance imaging to study blood oxygen level-dependent signals during the performance of a contingency reversal reward paradigm. The results identified group differences in the response to punishers bilaterally in the orbitofrontal and medial prefrontal regions. In addition, areas such as the nucleus accumbens, anterior cingulate and ventral prefrontal cortices were activated greatest by controls during reward processing, less by patients early in the course of illness, and least by patients with highly recurrent illness - suggesting that these areas are sensitive to the impact of disease burden and repeated episodes of depression. Reward processing in people with depression may be associated with diminished signaling of incentive salience, a reduction in the formation of reward-related associations and heightened sensitivities for negatively valenced stimuli, all of which could contribute to symptoms of depression.

Introduction

Anhedonia is a central feature of major depressive disorder (MDD) and is defined as a decrease in the ability to seek out and experience pleasurable activities (American Psychiatric Association, 2000). As one of the earliest and most frequent precursors to adult-onset MDD (Wilcox et al., 2004), anhedonia is thought to potentiate existing genetic vulnerabilities to depression. (Loas et al., 1996). A dysregulation of the neural system involved in the processing of reward may contribute to the experience of anhedonia. Indeed in patients with MDD, suboptimal decision-making strategies, heightened sensitivities to negative feedback and punishment (Elliott et al., 1997), and failures to modulate responses to rewards (Pizzagalli et al., 2008; Must et al., 2006) are closely aligned with the symptoms of anhedonia.

Functional imaging studies have identified a number of brain regions important in the processing of reward. At the core to this network is the nucleus accumbens (NAc). Frontal regions, such as the orbitofrontal cortex OFC appear to be important in assigning value to rewarding stimuli and in aspects of approach behavior and response inhibition (Elliott et al., 2004; Dolan et al., 2007). The ventral medial prefrontal cortex (VMPFC) plays a role in the contextual processing of reward (Elliott et al., 2004; Knutson et al., 2001), the response to unexpected rewards (Ramnani et al., 2004), forming abstract representations of the reward value (Elliott et al., 2000) and the assessment of reward outcomes (O'Doherty et al., 2001). Finally, the more dorsolateral frontal regions and the

anterior cingulate cortex (ACC) engage in the integration of reward information to support response selection (Knutson et al., 2003; Ullsperger et al., 2004).

Functional abnormalities in the regions associated with reward processing are apparent in people with depression (Taylor Tavares et al., 2008), but whether disease burden influences the processing of reward is unknown. Accordingly, the objectives of the present study were 1) to examine the neural correlates of reward processing in patients with MDD and 2) to determine whether patients with a high past illness burden differ from patients with minimal past illness burden when processing reward. We therefore studied a group of patients with major depressive disorder and healthy controls, and for further analysis subdivided the MDD group into those presenting for first treatment of an episode (FTE) of depression and those with multiple treated episodes (MTE) of depression.

Materials and Methods

Participants and Design

Patients were recruited from the Mood Disorders Clinic at St. Joseph's Centre for Mountain Health Services in Hamilton. Subjects received a full explanation of the study protocol that was approved by the Research Ethics Board of St Joseph's Healthcare Hamilton. Written informed consent was obtained. A psychiatrist confirmed a primary diagnosis of unipolar, nonpsychotic MDD according to DSM-IV criteria. A total of 29 patients were included in the study. Patients were further classified based on their illness

duration. The FTE group (N=14) consisted of patients presenting for treatment of a depressive episode with no prior treatment history. The second group (N=15) consisted of patients who had at least three previous episodes of MDD and/or an illness duration of at least five years. Twenty-five control subjects were recruited from the community and were matched on age, sex and handedness for each patient group. Healthy control subjects were free from medication and had no current symptoms or medical history of a mental health disorder. Exclusion criteria for all participants included history of head injury, unmanaged medical or neurological illness, alcohol or substance abuse, or history of electroconvulsive therapy or transcranial magnetic stimulation within the last two years. Depressive symptoms were assessed at the time of scanning using the Beck Depression Inventory (BDI; Beck et al., 1961).

fMRI Paradigm and Procedure

Participants completed a reward processing task that was previously used in healthy individuals to identify activation of the OFC during periods of monetary gains and losses (O'Doherty et al., 2001). Prior to the scan, subjects were given a brief orientation of the task. During the first part of the training subjects were shown two unfamiliar fractal images (approximately 5x5 cm2), which appeared in random order on either side of an IBM ThinkPad screen. Subjects were asked to identify one of the two symbols consistently for 5 trials. Participants then received instructions for a "Money Game" (See Figure 1). Subjects were told to select the image they felt would be the most rewarding choice over a number of trials, based on previous monetary outcomes.

The two fractals were predetermined to be either rewarding or punishing over the course of a number of trials (4-6 trials). The most rewarding fractal was associated with rewards or gains in the range of $80 to $250 (large gain), and punishment or loss values of $–60 to $10 (small loss). The most punishing fractal was associated with large losses that ranged from $-250 to $-600, and small gains of $-30 to $60. After making a selection, the dollar amount associated with the choice was presented on the screen along with a running total of the amount gained or lost from the start of the game. Unbeknownst to the subject, the gain: loss values for the reward and punishment fractals appeared in specific ratios (reward fractal – 70:30, punishment fractal – 40:60).

Subjects were told that after selecting the most profitable fractal a number of times the conditions would switch (the original rewarding fractal now becoming the punishing fractal, and the original punishing fractal now becoming the rewarding fractal), and that they should adapt to this switch. The training phase continued until subjects were able to successfully complete two condition reversals. Prior to entering the scanner the subjects were informed that the dollar amounts were fictional and that they would not receive any of money 'earned' during the experiment. Instead, at the completion of the study, subjects received a $25 gift card to a local movie rental store.

Following training, each participant completed the task while undergoing an fMRI scan. The task was projected onto a mirrored visor located on top of the head coil within the scanner bore. Subjects made their responses by pressing one of two buttons of

a response box attached to their right hand. The presentation of stimuli and recording of responses was controlled by Eprime presentation software (http://www.pstnet.com).

During the fMRI portion of the experiment the protocol was altered slightly. Initially, during the baseline portion of the experiment, subjects were asked to consistently identify the occurrence, left or right, of one of two fractals. The fractals were the same ones used in the training phase, although subjects were now asked to identify the opposite fractal. Once the subject had correctly identified the specified fractal for 20 trials, the experiment progressed to the "Money Game". Subjects received the following instructions: 'You will now play the Money Game. Decide which fractal is more profitable, reverse your choice when needed'. After a selection, the dollar amounts associated with their choices and the running totals were shown on the screen, in the same position as the training phase, for 3 seconds. The screen was then cleared and a fixation point was shown for 4, 7, 10 or 13 seconds (jittered inter-trial interval) prior to the next trial presentation. The 'Money Game' continued for approximately 30 minutes in the scanner.

Image Acquisition

The scanning session lasted approximately 1 hour during which subjects completed the reward processing task and a T1 weighted anatomical scan. Images were acquired using a GE 3T whole body short bore scanner with 8 parallel receiver channels (General Electric, Milwaukee, WI). A three-dimensional volume SPGR pulse sequence

with 124 contiguous slices (1.6 mm thick) was used to acquire anatomical images in the axial plane. Functional images were acquired with an optimized gradient-echo EPI sequence, and covering 32 axial slices (4 mm thick, no gap), beginning just below the most ventral part of the inferior temporal cortices (bilaterally) and encompassing the entire cerebrum (TR/TE= 3000/35 ms, FOV=24 cm, matrix = 64 x 64, flip angle 90°). Acquired images were transferred to a workstation, preprocessed and analyzed using BrainVoyager QX version 1.10.4 (Brain Innovation B.V., Maastricht, The Netherlands). The functional data sets were slice-time corrected, linear detrended, 3D-motion corrected and realigned, and normalized to Talairach space (Talairach & Tournoux, 1988). High-resolution T1-weighted three-dimensional (3D) anatomical MR data sets were transformed into Talairach space, used for co-registration and averaged to generate a composite image onto which functional activation results were projected.

Data Analysis

An event related deconvolution model for each participant was used to examine BOLD signal at every voxel. Using a random-effects multiple general linear model, the reward acquisition, punishment reversal, large gain, small gain, large loss, and small loss conditions were set as the explanatory variables accounting for differences in blood oxygen level dependent signals within and between groups. Reward acquisition included the contiguous series of trials in which both small punishers and larger rewards were delivered. The Punishment Reversal period was defined as the series of trials during which larger punishers and small rewards were delivered. To examine the response to

changes in the magnitude of rewards, select trials in which large positive rewards were awarded were contrasted with trials in which small rewards were allocated. Finally, to examine responses to changes in the magnitude of punishers, trials in which large punishers were delivered were contrasted with trials associated with small punishers.

Imaging data was analyzed first at the level of the entire group of subjects with MDD (N=29) compared against healthy controls (N=25). From that analysis for each condition of interest, areas of significant activation were used to generate an activation map. This contrast specific activation map was then used to localize regions of interest in the subsequent analysis of the FTE vs. controls, MTE vs. controls and FTE vs. MTE comparisons. Contrasts were corrected for multiple-comparisons using the false discovery rate methodology (threshold set to.05) (Genovese et al., 2002) and the average statistical value for the resulting regions of interest are reported.

Results:

Demographic, Clinical and Behavioural Measures

There were no significant differences in age between the FTE group (26.4 $\pm$ 7.4) and their matched controls (28.4 $\pm$ 7.9), or the MTE group (47.7 $\pm$ 9.4) and their matched controls (46.3 $\pm$ 11.4). The MTE subjects included in the study had mean illness duration of 23.9 $\pm$ 12.7 years and had extensive documented past treatment histories prior to inclusion in this study. The FTE subjects included in the study identified the onset of

depressive symptoms an average of 8.3 ± 4.9 years prior to presentation, although none had ever sought treatment prior to participating in this study. FTE subjects had a significantly lower past burden of illness compared to MTE subjects [$F(1,28) = 18.67$, $p < 0.001$]. Despite a more extensive illness history, MTE subjects had less severe symptoms scores at the time of assessment with mean BDI score of 13.4 ± 5.7 and a mean HRSD score of 9.4 ± 4.8, compared to FTE subjects who had mean BDI score of 26.3 ± 12.7 [$F(1,28) = 15.86$, $p < 0.001$] and a mean HRSD score of 17.9 ± 7.0 [$F(1,28) = 14.84$, $p < 0.001$]. Eighty percent of MTE patients were treated with antidepressants (7 SSRIs, 4 SNRIs, 2 atypical antidepressants, 1 tetracyclic antidepressant, 1 MAOI). Three FTE patients had received minimal exposure to antidepressant treatment prior to scanning; all had less than one week's lifetime exposure to psychotropic medication. See Table 1 for demographic information.

During task performance the FTE patients had significantly faster response times than controls in reaction to both the reward acquisition phase (FTE mean = 915.9 ms, control mean = 1053.5 ms; $F=(1,26)=4.63$, $p < 0.04$]) and the punishment reversal phase (FTE mean = 940.4 ms, control mean = 1050.8 ms; $F(1,25) = 6.40$, $p < 0.02$). There were no significant differences between these two groups on the number of successful switches in reward contingencies (FTE mean = 12.4, controls mean = 12.6, [$F(,27) = 0.247$, $p < 0.62$]). When examining MTE patients and their matched controls, there was a significant difference in the latencies averaged across all trial types [$F(1,27) = 5.41$, $p < 0.028$]. MTE patients had a significantly delayed reaction time (mean = 1219.7 ms) in

comparison to matched controls (mean = 1059.9 ms). However, there was no significant difference in reaction time for the reward acquisition phase between these two groups (MTE mean = 1202.5 ms, controls mean = 1050.0 ms) or the punishment reversal (MTE mean = 1230.0 ms , controls mean = 1078.1 ms) periods specifically. Additionally, there was a trend towards a fewer number of successful switches in reward contingencies condition in the MTE group in comparison to controls (MTE mean = 10.7, control mean = 11.9; $F(1,28) = 3.87$, $p < 0.06$).

Imaging data

Reward versus punishment

Between group activation patterns were determined for the contrast of reward acquisition versus punishment reversal. Reward acquisition was defined as the consistent selection of the correct rewarding fractal for 4-6 trials. The punishment reversal phase was defined as the trials when the subject selected the old rewarding fractal and continued to do so until the new rewarding fractal was acquired. This contrast allowed for the examination of all rewarding conditions in comparison to all punishing conditions.

In the first level of analysis, significant differences were observed between the larger MDD group and controls in a number of areas. Control subjects had increased activation in comparison to MDD patients bilaterally in the NAc, anterior cingulate (BA 32) and also in the right hippocampus (Figure 2.). Using an activation map containing these same regions we then examined this contrast between controls and FTE and MTE

patients respectively. Control subjects had increased activation in comparison to MTE subjects bilaterally in the NAc, anterior cingulate (BA 25 & 32) hippocampus. Additional significant findings for this contrast included differences in the medial prefrontal cortex (BA 10) activation between MTE patients and control subjects, FTE patients compared to matched controls, and MTE patients compared to FTE patients. Activation for this region showed a graded response between the three groups, such that MTE > FTE > healthy controls.

Magnitude of Reward or Punishment

Between groups analyses evaluated the neural responses associated with variable magnitudes of rewarding or punishing stimuli. The effects of the magnitude of rewards were assessed by contrasting large rewards and small rewards. Analysis of the larger patient group revealed significantly increased activation bilaterally of the NAc and inferior frontal cortices and ACC (BA 47 on the right, and BA 32 on the left, respectively) in controls versus MDD patients. Examination of the response to the magnitude of rewards in the FTE and MTE patient groups revealed similar activation in these same areas. Areas of significant activation revealed in pair-wise contrasts across the three groups in the large gain vs. small gain comparison revealed changes the ACC and bilateral NAc. Here, Controls had greater activation than both FTE and MTE patients and FTE patients in turn, had greater activation of these regions than MTE patients. This pattern was considered an activation gradient across the study groups; healthy Controls > FTE patients > MTE patients. Other areas of significant differences between groups are reported in Table 2.

Losses of large or small magnitude were contrasted to determine the effect of the magnitude of punishment. Patients had significantly less activation than controls to large penalties (large losses > small losses) in a number of regions. In particular, patients had less activation of the bilateral lateral orbital frontal regions (BA 13) and less activation than controls in the medial prefrontal cortex (BA 10). The same contrasts were then employed to examine the effect of illness burden in depressed patients. Controls had increased activation in the medial prefrontal cortex and lateral inferior frontal cortex in comparison to both FTE and MTE patient groups, although the comparison to MTE patients did not survive correction for multiple comparisons. Finally, FTE patients had increased neural responses in the medial prefrontal cortex (BA 10) in comparison to MTE patients, although this comparison did not remain after correction for multiple comparisons. These results created a trend towards a graded response between the three groups such that healthy controls > FTE subjects > MTE subjects in BA 10 when responses to large and small losses were compared. Other areas of significant differences between groups are reported in Table 2.

Discussion

Our results suggest that patients with depression have functional abnormalities in brain regions involved with the processing of reward. In particular, patients had less activation than controls in the NAc, VMPFC and ACC under conditions of reward acquisition and with changes in the magnitude of gain (Figure 2.). Moreover, patients

responded to losses by engaging the lateral OFC and VMPFC regions to a lesser extent than controls.

These findings were refined by further examination of the patients based on whether they had a minimal or extensive history of illness. Several regions showed a gradient of responses that was dependent on treatment history, including the ACC, OFC, and NAc. Control subjects had the greatest degree of activation in these regions, with less activation in FTE patients, and least by MTE patients.

Understanding the dysregulation in the reward network may provide insight into the neural underpinnings of anhedonia, a common but poorly studied feature of MDD. These results suggest that altered reward circuitry in MDD may initially involve diminished signaling of incentive salience by the NAc. Alterations in the NAc and OFC might then lead to the formation of fewer anticipatory reward-related associations. This reduction could promote symptoms of anhedonia, as fewer experiences would be tagged as rewarding. Changes in OFC function may also result in diminished capacity to gauge punishers and a generalized heightened sensitivity for negative affective-laden stimuli. Further, the outcome of reduced affective signaling and the generation of fewer reward-related associations may directly impact on VMPFC capacities to associate changes in reward contingencies with self-relevant information. Whether changes in VMPFC activity reflect a lessening of higher order integrative processing or a self- protective psychological response, such changes could contribute further to symptoms of anhedonia.

Below we examine the group differences in the response to rewards and punishers, and explore further the functional roles of key regions important in the neuropathology of MDD.

Responses to Changes in Reinforcement Contingencies and Punishers and the VMPFC

We observed group differences in the recruitment of the VMPFC when trials during which the subjects chose rewarding stimuli (Reward Acquisition) were contrasted with trials in which the response contingencies had reversed (Punishment Reversal). Here, recruitment of the VMPFC was greatest in MTE patients, less in FTE patients and the least in healthy controls. It is possible that these group differences are driven by either heightened engagement of the VMPFC under rewarding conditions, or reduced engagement (or deactivation) of this region during contingency reversal periods and punishment. Consequently, to clarify the directionality of these VMPFC findings we examined the activation of this region (within group) during both the reward acquisition and punishment reversal periods as they related to baseline rest period of the paradigm (Table 3; Figure 3.). Consistent with the literature, all three groups demonstrated activation within the VMPFC during reward acquisition (Elliott et al., 2004; Knutson et al., 2001).

In contrast, during the punishment reversal phase, the VMPFC was activated by controls, undistinguished from baseline in FTE patients, and deactivated by MTE patients. These results suggest that group effects in the reward acquisition / punishment

reversal comparison discussed above were not so much a consequence of differences in the responses to the receipt of rewards but more so reflective of 1) the processes involved in signaling changes in reinforcement contingencies and 2) the response to loss. While not mutually exclusive, it may be that both factors contribute to the altered reward processing and anhedonia in MDD, and as such will be explored further below.

During the contingency reversal periods the subject finds that the stimulus that has come to be associated with gain is now suddenly paired with loss, and that the stimulus that was previously associated with loss is now more rewarding. Of note, therefore, are studies of reward processing that have suggested a role for the VMPFC in the response to unexpected rewards (Ramnani et al., 2004) and in the contextual processing of reward (Elliott et al., 2004; Knutson et al., 2001). Current theories propose that this region is active in the assembly and integration of abstract representations of reward value (Elliott et al., 2000). Thus, the attenuated activation of this region in MDD during the contingency reversal period may signify diminished responses to novel rewards and to changes in reward contingencies.

Response to negative feedback is particularly accentuated in depressed patients when the feedback carries emotional significance (Murphy et al., 2003). Patients with depression can be distinguished from controls by a "double dissociation" of VMPFC responses to positive and negative affective stimuli (Keedwell et al., 2005). Reduced VMPFC activation to sad stimuli in MDD may reflect a negative bias in visual emotion

processing, corresponding to a heightened sensitivity for sad visual stimuli. Drevets (2007) has recently observed that patients with depression have enhanced sensitivity to negative feedback during the performance of a reversal learning task. Compared to controls, patients' demands for reversal shifting were associated with attenuated activity in the dorsomedial and ventrolateral prefrontal cortices. Reduced engagement of prefrontal regions in depression may have signaled failures to suppress emotional responses and to modulate activation in limbic structures following negative feedback. Accordingly, in the current study, an attenuated VMPFC response to punishing stimuli in depressed patients may be the result of heightened sensitivity to the negative affective content of such stimuli and indicative of a failure to regulate neuronal responses to this feedback.

The VMPFC is also associated with the processing of experiential and emotionally self-relevant information. For example, a recent fMRI study by Schmitz & Johnson (Schmitz & Johnson, 2006) presented participants with both positive and negative trait adjectives and found increased activation within a network that included the VMPFC when subjects matched descriptive words with self-referent statements. Areas of the medial PFC are implicated in processes that involve the maintenance of a representation of self in order to direct future behavioural responses to both reward and emotional contingencies (Stuss & Levine, 2002). Collectively, these studies suggest that this region is engaged when individuals judge outcomes according to experiential and emotionally laden self-referent information. Depression may therefore involve reduced

capacity to associate changes in reward contingencies with self-relevant information. In particular, diminished responses to novel rewards, and reduced self-referential processing may be core to the symptoms of anhedonia.

Punishment and the Orbitofrontal Cortices

Response to differences in the magnitude of loss was reduced in depressed patients compared to controls. Attenuated responses were apparent in the ventrolateral regions of the orbitofrontal cortices. Contrasts between the MDD subgroups and their controls, however, revealed only trends toward greater recruitment of this region by controls subjects. Collectively, these findings are consistent with evidence that points to alterations of the OFC in MDD, including post mortem studies (Rajkowska et al., 2007), structural imaging studies (Steffens et al., 2003), and a functional imaging study that used a go/no-go task with emotional words as stimuli (Elliott, et al., 2002).

Heightened ventrolateral OFC activation in our control subjects is consistent with studies identifying this region as important in the processing of punishment (O'Doherty et al., 2001: O'Doherty et al., 2003). Similarly localized lateral OFC findings led O'Doherty and colleagues to suggest a medial-lateral OFC dissociation for rewards and punishers, although subsequent work (Elliott et al., 2003; O'Doherty et al., 2003) noted activation in this region during both punishing and rewarding outcomes. In a study of reversal learning by O'Doherty et al., (2003) activity in the lateral OFC predicted subsequent shifts in contingencies. These findings suggest that the more lateral regions

may be important for the generation of stimulus reinforcement associations that represent anticipatory value (O'Doherty et al., 2003; Fellows, 2004). Accordingly, the ventrolateral findings in the present study may indicate that there are diminished capacities to gauge the magnitude of loss and develop expectations as to the probable outcomes of subsequent choices in patients with MDD.

The Processing of the Magnitude of Rewards

Another cross-group graded pattern of activation was evident when we looked specifically at reward trials and examined the effects that the size of the reward had on brain activation. Contrasting trials with large gains to trials with small gains revealed activation of the NAc that was greatest for control subjects, attenuated for FTE patients, and least by patients with extensive past illness (Figure 4.). Group differences in NAc activation were also observed in the comparison of reward acquisition with punishment reversal trials. In this instance, controls had greater NAc activation than MTE patients.

The NAc is an integral part of the mesolimbic reward system and reward processing (Knutson et al., 2001; Ramnani et al., 2004; Ernst et al., 2004; Kuhnen & Knutson, 2005; Spicer et al., 2007). The NAc may be particularly involved in anticipating reward and responding to unexpected outcomes, but this hypothesis is controversial. Others have suggested that this area may be active in signaling incentive salience, and important in processes that assign motivational value and amplify cue triggered "wanting" of rewards (Berridge, 2007).

Few studies have explored NAc activity in MDD. One study using PET to examine striatal dopamine uptake found a decrease in NAc dopamine metabolism in a group with marked affective flattening (Bragulat et al., 2007). The dysfunction in dopamine metabolism in this area could be reflective of the decreased volume seen in the NAc in MDD (Baumann et al., 1999). Recently, Knutson (Knutson et al., 2008) used a monetary incentive delay task that required unmedicated MDD patients and matched healthy controls to respond to rapidly presented cues in anticipation of gains or losses. Unexpectedly, they found no group differences in NAc activation when subjects were anticipating gain, however a trend was observed with reduced MPFC and NAc responses when depressed subjects were monitoring gain outcomes, consistent with findings of altered NAc activation in response to rewards in subjects with MDD. Healthy subjects may recruit this area to anticipate rewards more effectively than depressed subjects. Notably, the pattern of results suggest that early in the course of MDD there may be abnormalities in the NAc function that are exacerbated with repeated episodes or chronic illness.

Strengths and Limitations

A strength of the current study is the relatively large number of subjects who completed the functional task (fifty four in total). This allowed the patient group to be broken into subsets of patients while maintaining a reasonable number of patients in each sub-group. While intentionally varying on burden of illness, the sample was an otherwise

well-characterized and homogenous group of non-psychotic, unipolar outpatients who were free of major co-morbidity.

The present study has several limitations. Notably, some patients were taking antidepressant medication and it is possible that the observed differences in activation were influenced by treatment. Because of the cross-sectional nature of the design, it is impossible to confirm whether the accrued burden of illness in the MTE group led to or resulted in part from, the altered patterns of reward processing that were observed in this group compared to those with minimal illness burden. Longitudinal functional imaging studies that repeat the same task in patients across various points in illness history are lacking, likely because of the complexity involved in this approach. The contingency reversal paradigm also has limitations. Although prior studies have suggested that the NAc plays a role in the anticipation of rewards (Knutson & Cooper, 2005), the protocol employed in the current study was not designed to differentiate between reward anticipation and reward outcome processing. It will be important for future work to address the function of the NAc in reward anticipation in MDD.

Conclusions

Regional differences in reward processing were apparent between depressed patients and controls during the performance of a contingency reversal reward paradigm. The pattern of results suggest that the recruitment of the NAc in MDD is attenuated during the processing of reward, and as a consequence both the signaling of incentive

salience and the capacity to form reward-related associations of anticipatory value may be reduced.

Across groups, the recruitment of areas such as the NAc, ACC and ventral prefrontal cortices during reward processing was greatest in controls, less by first treatment patients, and least by multiple treatment patients - suggesting that these areas are sensitive to the impact of disease burden and repeated episodes of depression.

The responses to punishment and changes in reward contingencies in MDD are marked by reduced recruitment of the VMPFC and lateral OFC. Based on the extant literature, we have suggested that the VMPFC findings point to reduced capacities to regulate responses to negative affective stimuli and reduced abilities to associate self-relevant information with changes in reward contingencies. In the aggregate, these data highlight fundamental shifts in the processing emphasis of depressed patients during the experience of reinforcing or punishing events. The results also emphasize that the capacity of relevant neural systems to be activated in either a primary or compensatory manner may be importantly determined by the burden of illness that patients experience.

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Table I. Demographic & Clinical information separated by MDD treatment group and corresponding matched controls.

	FTE (N=14)	Controls for FTE (N=14)	MTE (N=15)	Controls for MTE (N=15)
Age (years)	26.4 (7.4)	28.4 (8.2)	47.7 (9.5)	46.3 (11.4)
Female	5	6	11	10
Male	9	8	4	5
Education (years)	13.4 (1.6)	17.4 (3.0)	16.3 (2.1)	16.5 (3.4)
Duration of Illness (years)	8.2 (4.9)	NA	23.9 (12.7)	NA
Number of Episodes	3.2 (3.3)	NA	7.7 (8.6)	NA
Medications:				
Antidepressants	3	NA	12	NA
Antipsychotics	1	NA	4	NA
Lithium	0	NA	1	NA
Medication Free	11	NA	3	NA
BDI	26.3 (11.2)	1.2 (1.8)	13.4 (5.7)	1.0 (1.6)
HRSD	17.9 (7.0)	NA	9.4 (4.7)	NA

Table 2. Localization of reward-related fMRI activation identified through between groups comparisons.

	X	Y	Z	Region	Brodmann Area	t-value	# Voxels
Reward Acquisition – Punishment Reversal							
MDD > NC	11	49	10	Ventral Medial Prefrontal Cortex	10	3.556	3744
	-10	49	6	Ventral Medial Prefrontal Cortex	10	3.493	4637
MTE > NC	9	51	10	Ventral Medial Prefrontal Cortex	10	3.270	4710
	-9	51	5	Ventral Medial Prefrontal Cortex	10	3.266	5281
MTE > FTE	9	51	11	Ventral Medial Prefrontal Cortex	10	3.399	3747
	-6	51	7	Ventral Medial Prefrontal Cortex	10	3.043	2574
FTE > NC	2	54	0	Ventral Medial Prefrontal Cortex	10	3.627	164
	-10	49	1	Ventral Medial Prefrontal Cortex	10	3.730	388
NC > MDD	10	17	-8	Anterior Cingulate Cortex	32	3.172	440
	14	6	-6	Lentiform Nucleus/Nucleus Accumbens		3.357	220
	-4	2	-5	Caudate Head/Nucleus Accumbens		3.333	253
	34	-26	-9	Hippocampus		3.174	244
NC > MTE	12	23	-6	Anterior Cingulate Cortex	32	3.031	2083
	-6	11	-10	Anterior Cingulate Cortex	25	2.750	411
	14	3	-5	Lentiform Nucleus/Nucleus Accumbens		2.980	426
	-9	-1	-8	Lentiform Nucleus/Nucleus Accumbens		2.983	325
	32	-24	-11	Hippocampus		3.255	5041
	-34	-24	-14	Hippocampus		2.913	995

Table 2. Continued

	X	Y	Z	Region	Brodmann Area	t-value	# Voxels
Large Gain – Small Gain							
NC > MDD	15	6	-5	Lentiform Nucleus/Nucleus Accumbens		3.997	254
	-7	2	-4	Lentiform Nucleus/Nucleus Accumbens		3.889	478
	11	20	-8	Medial Orbitofrontal Cortex/Anterior Cingulate Cortex	32	3.906	1260
	-4	20	-9	Anterior Cingulate Cortex	32	3.538	271
NC > MTE	16	4	-3	Lentiform Nucleus/Nucleus Accumbens		3.024	1433
	-8	4	-4	Lentiform Nucleus/Nucleus Accumbens		2.927	1800
	11	21	-9	Anterior Cingulate Cortex	25	3.269	3870
	-7	21	-11	Anterior Cingulate Cortex	25	3.045	1863
NC > FTE	20	4	-2	Lentiform Nucleus/Nucleus Accumbens		3.065	44
	-7	8	-1	Caudate Head/Nucleus Accumbens		3.153	262
	5	29	3	Anterior Cingulate Cortex	24	2.862	184
	-4	30	-6	Anterior Cingulate Cortex	24	3.232	164
FTE > MTE	14	5	-5	Lentiform Nucleus/Nucleus Accumbens		3.002	403
	12	21	-6	Anterior Cingulate Cortex	32	2.722	486
	-5	21	-9	Anterior Cingulate Cortex		3.003	1112

Table 2. Continued

	X	Y	Z	Region	Brodmann Area	t-value	# Voxels
Large Loss – Small Loss							
NC > MDD	10	50	12	Ventral Medial Prefrontal Cortex	10	2.893	696
	-9	50	11	Ventral Medial Prefrontal Cortex	10	2.907	2152
	-19	43	5	Anterior Cingulate Cortex	32	2.923	1849
	34	14	-8	Lateral Inferior Frontal Cortex	13	3.129	3370
	-29	-15	-8	Lateral Inferior Frontal Cortex	13	3.360	2605
	-38	33	7	Lateral Inferior Frontal Cortex	13	3.270	192
NC > MTE	7	50	12	Ventral Medial Prefrontal Cortex Bilateral	10	2.920	444*
	-5	52	-3	Anterior Cingulate Cortex/Medial Orbitofrontal Cortex		3.140	157*
	39	9	-6	Lateral Inferior Frontal Cortex	13	3.198	1046*
	-31	11	-14	Lateral Inferior Frontal Cortex	13	3.122	408*
NC > FTE	-2	55	0	Ventral Medial Prefrontal Cortex	10	3.559	340
	13	39	2	Anterior Cingulate Cortex	32	3.311	1121
	-1	45	-5	Anterior Cingulate Cortex	32	3.420	1589
	33	16	-8	Lateral Inferior Frontal Cortex	13	3.259	505
	-44	24	-1	Lateral Inferior Frontal Cortex	47	3.148	245
FTE > MTE	11	52	13	Ventral Medial Prefrontal Cortex	10	2.089	400*
	43	22	9	Lateral Inferior Frontal Cortex	13	2.218	1358*
	-31	12	-14	Lateral Inferior Frontal Cortex	13	2.117	334*

* Results not corrected for multiple comparisons (MDD = larger group of depressed patients, MTE = Multiple treated episode depressed patients, FTE = First treated episode depressed patients).

Table 3. Within groups activation patterns associated with the contrast between Reward Acquisition (Big rewards + Small punishers) and baseline rest, and Punishment Reversal periods (Big Punishers + Small rewards).

	X	Y	Z	Region	Brodmann Area	t-value	# Voxels
Big Rewards + Small Punishers							
MTE	-5	53	-2	Ventral Medial Prefrontal Cortex (Bilateral)	10	3.342	1738
FTE	-2	55	-1	Ventral Medial Prefrontal Cortex (Bilateral)	10	3.337	200
NC for MTE	-6	60	11	Ventral Medial Prefrontal Cortex (Bilateral)	10	2.981	336
NC for FTE	11	56	7	Ventral Medial Prefrontal Cortex	10	3.177	181
	-4	56	7	Ventral Medial Prefrontal Cortex	10	3.039	307
Big Punishers + Small Rewards							
MTE	14	52	13	Ventral Medial Prefrontal Cortex	10	-3.945	6617
	-11	50	10	Ventral Medial Prefrontal Cortex	10	-3.664	5885
FTE	-	-	-	No activation	-	-	-
NC for FTE	27	42	7	Ventral Medial Prefrontal Cortex	10	3.767	522
	-15	46	4	Ventral Medial Prefrontal Cortex	10	3.235	820
NC for MTE	11	56	7	Ventral Medial Prefrontal Cortex	10	3.113	39
	-4	56	7	Ventral Medial Prefrontal Cortex	10	3.077	115

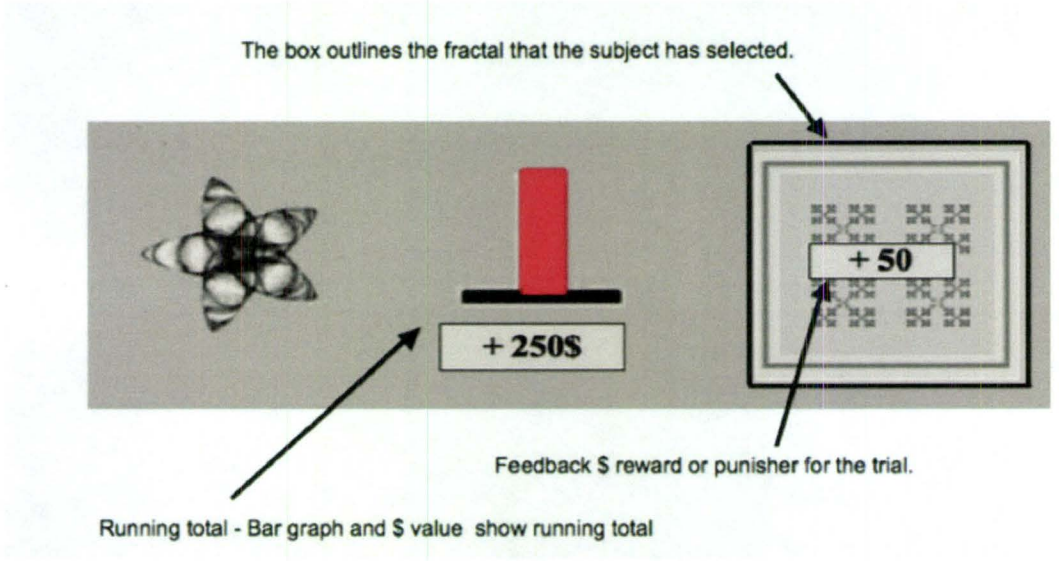

Figure 1: The Reward-Punishment paradigm was based on O'Doherty et al. (2001). On each trial the subject selects the fractal they feel is most rewarding. Once the most profitable fractal has been selected a number of times, the contingencies are reversed. The subject must detect the reversal and change their responses accordingly.

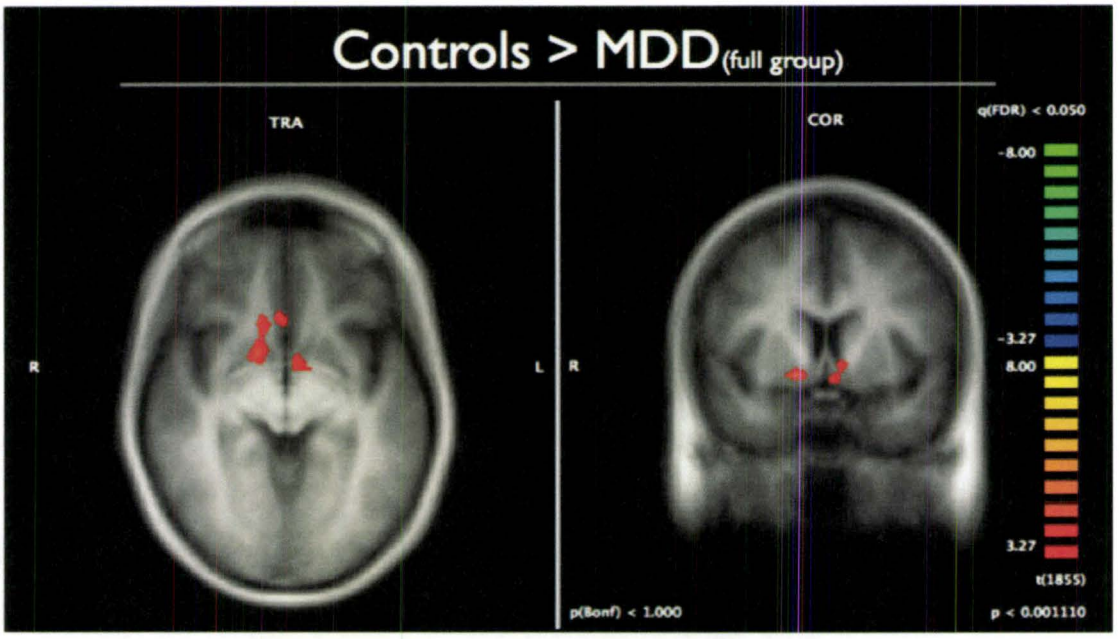

Figure 2: Full group differences in the activation of the nucleus accumbens and Anterior Cingulate cortices during reward acquisition relative to punishment / contingency reversal. FDR-corrected statistical maps are superimposed on averaged anatomical group images. Images are presented according to radiological convention.

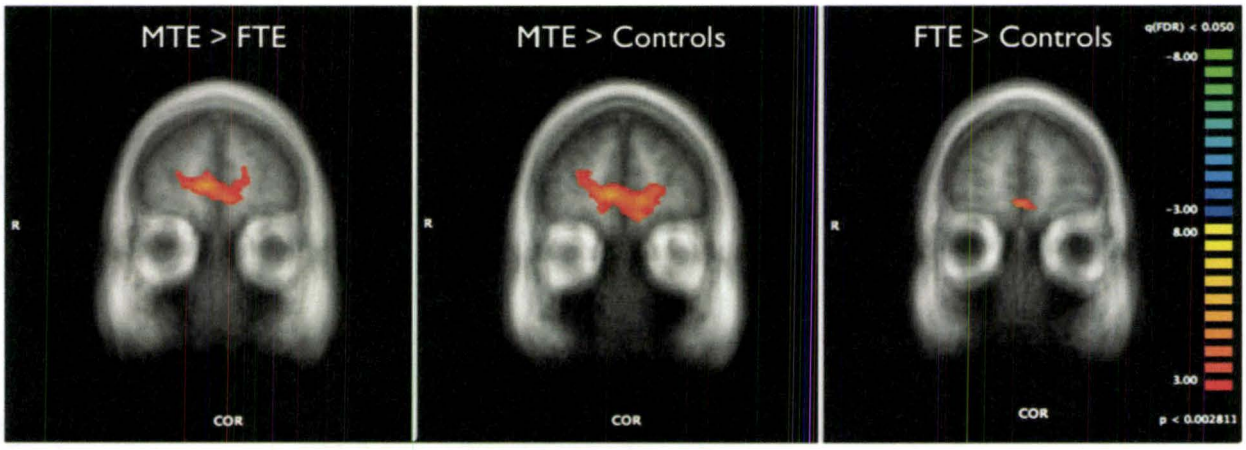

Figure 3: Subgroup differences in neural responses in the ventral medial prefrontal cortices during reward acquisition relative to punishment / contingency reversal. FDR corrected statistical maps are superimposed on averaged anatomical group images. Images are presented according to radiological convention.

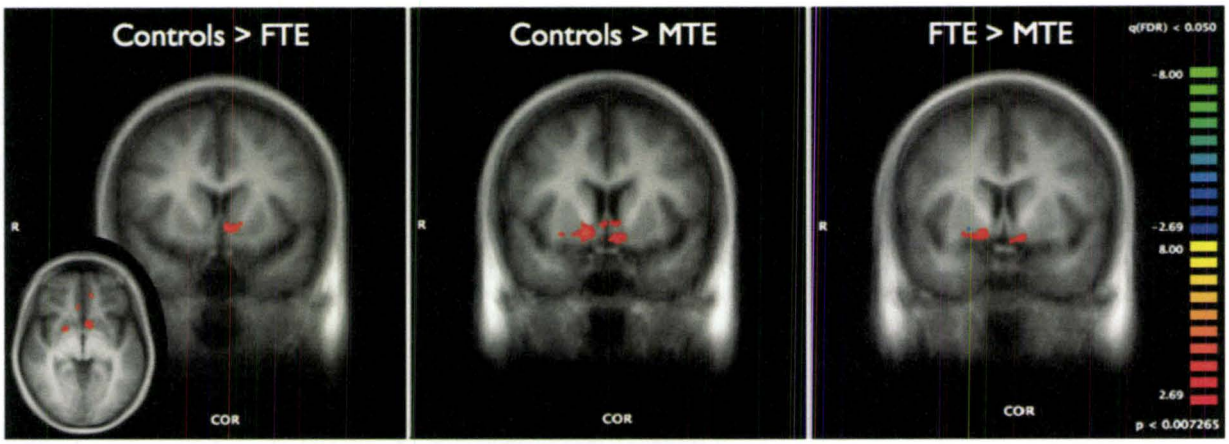

Figure 4: Subgroup differences in the activation of the Nucleus Accumbens during Large gains relative to small gains. FDR-corrected statistical maps are superimposed on averaged anatomical group images. Images are presented according to radiological convention.

CHAPTER 5:

DISCUSSION

Prior research has shown that individuals with MDD have alterations in frontal and temporal regions associated with emotional regulation. While these changes have been documented through a number of neuroimaging techniques, the chronology of these alterations develop has not been fully characterized. The MDD literature has begun to examine changes in prefrontal regions in recently diagnosed MDD individuals. Temporal regions, particularly the Hc, have received more attention in this subset of depressed subjects. The major goal of the work described in this thesis was to characterize functional and metabolic brain changes in individuals who were experiencing their first episode of MDD in comparison to those who had experienced multiple past depressive episodes. We used two tasks that had previously not been used to examine depressed patients using fMRI methodology. In addition we used MRS to isolate the hippocampus, an area that has been largely overlooked in MRS depression research. The findings from this work suggest that at illness onset, patients with MDD have changes in frontal and temporal functional activation, however, with prolonged illness duration associated with multiple episodes of MDD, these alterations become more pronounced.

Hippocampal Findings

Research has shown reductions Hc volumes in the range of 5-10% in patients with a history of depression (Campbell, et al., 2004 Videbech, et al., 2004). These findings are particularly evident in individuals with extensive histories of MDD (Bremner, 2002; Drevets, 2000; McKinnon et al., 2009; Sheline, 2000) suggesting that illness duration

greatly impacts Hc volume. There is less agreement surrounding Hc volumetric and functional changes in the early course of this disorder. Prior research has failed to find Hc volume changes in first episode MDD patients, although Hc dependent memory was impacted (Campbell, et al., 2004; Campbell & MacQueen, 2006). Moreover, a recent meta-analysis of over 30 Hc volumetric studies in MDD, found a lack of significant Hc reductions in patients who had experienced an illness duration of less than two years and only 1 disease episode (McKinnon et al., 2009).

Research exploring the mechanisms of Hc volume reductions in MDD have focused on cellular changes caused by prolonged periods of stress resulting in heightened GCs (Mayer et al., 2006; Mirescu, 2006). Evidence has shown decreased Hc neurogenesis in a number of animal stress models (Gould et al., 1997; Heine et al., 2004; Pham et al., 2003). Moreover, other factors such as dendritic retraction (Sousa et al., 2000; Woolley et al., 1990), cellular shrinkage or atrophy (McEwen, 2000; Stockmeier et al., 2004) and alterations in the number and generation of new glial cells (Stockmeier et al., 2004) have all be suggested as possible models for Hc volume reduction in MDD.

Magnetic Resonance Spectroscopy Findings

In Chapter 2, we used MRS imaging to characterize metabolic changes across illness duration in MDD in a voxel isolating the Hc. This type of imaging provides in-vivo information regarding neuronal and glial cell viability in a specified region. We studied the Hc in individuals experiencing their first treated episode of MDD and

compared them to both healthy controls and patients who had experienced a minimum of three past episodes. We observed increased levels of choline-containing compounds in MTE patients with corresponding Hc volume decreases compared to controls. In addition, we observed increased MI levels in FTE patients without volumetric differences compared to their matched controls.

In line with prior evidence that has shown decreased Hc volume in MDD patients, we demonstrated further evidence of volumetric decreases in patients with a protracted course of illness. We also observed corresponding increases in choline-containing compounds in our Hc voxel in MTE patients. The measured Cho peak in MRS contains by products of phosphotidylcholine hydrolysis (Klein et al., 1993). During membrane breakdown, Cho stores are released generating a local increase in the detectable Cho signal (Miller et al., 1996). Therefore, we suggest that the decreased volume seen in our MTE patients may be attributable to increased neuronal membrane breakdown and turnover.

In contrast, we did not observe Hc volumetric differences in our FTE depressed population. However, we did observe increased MI levels. MI is thought to reflect glial cell integrity, and/or intracellular second messenger signaling (Kumar et al., 2002). Decreases in astroglial have been reported in animal models of chronic stress (Alonso, 2000; Czeh et al., 2006). Post mortem studies of depressed patients have failed to generate a consensus about glial cell reductions in the Hc (Harrison, 2002; Lucassen et

al., 2001; Muller et al., 2001; Stockmeier et al., 2004). However, increases in glial cell packing in the Hc in depressed patients in comparison to controls have been reported (Stockmeier et al., 2004). Our findings of increased MI in our FTE patients support this work, and suggest that increases in glial cell density may be apparent in the early course of the disorder.

Our spectroscopy work provides an important advancement in the MRS research on MDD as we controlled for hippocampal volume in our analysis of metabolite levels. The inclusion of volumetric analysis in the examination of metabolite levels has been conducted in studies examining other disorders (Schuff et al., 2008; Brown et al., 2004). However, to date, this methodological approach has not been applied to MDD research. With prior reports of volumetric differences in a number of prefrontal and temporal brain regions in depression, this approach to MRS studies provides a more accurate comparison between study populations.

Functional Magnetic Resonance Imaging Findings

In Chapter 3, our purpose was to use fMRI technology to characterize changes in Hc functional activation during an Hc-dependent recollection memory task. In addition to volumetric decreases identified in MDD, prior work has highlighted memory deficits in Hc-dependent tasks (MacQueen, 2003; Zakzanis et al., 1998). However, particular Hc-dependent tasks, including the process-dissociation task, have not been studied in MDD with fMRI methodology. We studied those experiencing their first treated episode

of MDD and compared them to individuals with an extensive past history of depression and healthy controls using an Hc-dependent recollection memory task.

Our results from Chapter 3 support previous findings of Hc volumetric abnormalities and memory deficits in MDD. Patients who had experienced multiple past episodes displayed diminished functional Hc activation and corresponding memory deficits during recollection trials in comparison to controls and FTE patients. Additionally, while those experiencing their first episode of MDD perform at the same level as controls across memory trial type, they show heightened patterns of Hc activation. We suggest that this heightened activation is compensatory in nature, and reflective of increased neuronal recruitment necessary for successfully task completion. These results identify that illness duration negatively impacts Hc function and capacity in MDD. Functional changes in the early course of illness may reflect compensatory mechanisms necessary for successful task completion, however with multiple episodes of MDD, possibly associated with longer GC exposure, the Hc is no longer able to compensate and decreased Hc activation and memory errors become evident.

While numerous studies have examined Hc volumetric changes in MDD and behavioural changes during tasks that recruit the Hc, only 1 prior study has used fMRI methods to examine Hc functioning in MDD (Werner et al., 2009). In their face-occupation associative learning paradigm, the authors observed decreased parahippocampal activation in their depressed group during memory retrieval (Werner et

al., 2009). Our findings extend prior findings of Hc abnormalities in MDD to include abnormalities in functional Hc recruitment. Moreover, this is the first imaging study of Hc-dependent memory to systematically compare the effects of illness duration in MDD.

The results presented in Chapter 2 and 3 provide complementary findings of changes in Hc structure and function in MDD. In the first study, MTE patients displayed decreased Hc volume and increased Cho levels reflecting increased membrane turnover. In the second study, a group of MTE patients with similar illness duration and clinical characteristics had decreased Hc engagement during Hc-dependent memory trials. Taken together, the results may suggest Hc membrane breakdown leading to decreased Hc volume. These structural alterations in the Hc may correspond to decreased capacity for functional recruitment of the Hc during Hc dependent tasks.

Similarly, our FTE observations from Chapter 2 & 3 suggest complementary results for recently diagnosed patients. In our FTE patients we observed increased MI levels in the Hc. In a second similar group of FTE patients, we observed increased Hc recruitment across all memory trials. Given the heightened engagement of the Hc across all memory conditions in the FTE population during our task in Chapter 2, it is interesting to speculate that the increased activation might be a result of the increased glial cell density in the Hc observed in Chapter 2.

Glial cells act as supporting cells in the central nervous system. They provide nutrition, maintain pH homeostasis, form myelin and participate in signal transduction. Recent research has indicated that glial cells slightly out number neurons in the Hc (Joelving et al., 2006). Therefore, glial cells make a considerable contribution to the overall Hc volume. Moreover it has been suggested that, because of their extensive contacts with both neuronal synapses and cerebral blood vessels, glial cells contribute to the regulation of the microvasculature in the brain (Iadecola & Nedergaard, 2007). It is interesting to speculate that the increased MI we observed in our group of FTE patients might provide a biomarker that is consistent with a generalized heightened metabolic load associated with compensatory cross-condition recruitment of the Hc observed in our second group of FTE patients.

Our results from Chapter 2 and 3 suggest that illness duration in depression has a profound impact on the Hc. Our findings suggest that at illness onset, the Hc compensates with increased glial cell density. Heightened Hc activity seen across memory types in our process dissociation task may be supported by this increase in glial cell density. However, with multiple episodes of MDD reflecting a longer illness duration and potentially greater exposure to the deleterious effects of stress and GCs, the Hc is reduced in size, displays signs of neuronal membrane breakdown, and is no longer able to function at full capacities. This is evidenced by our findings of decreased Hc volume, reduced Hc activation in recollection memory trials, as well as recollection memory errors.

Reward Processing Findings

Anhedonia, the loss of interest or pleasure in previously enjoyed activities, is a common symptom associated with MDD. Moreover, this symptom is also a central component in neurobiological theories of depression (Tomarken & Keener, 1998). However, the neural regions associated with anhedonia have not been fully characterized. The BRS encompasses a number of striatal regions that project to prefrontal areas active in reward processing in healthy individuals. As outlined in Chapter 1 and 4, a number of these regions display functional and volumetric alterations in MDD. Few studies have used explicit reward processing tasks to examine reward processing in MDD, and instead, there are studies that have used indirect methods involving subject rating positive or negative stimuli to demonstrate reward processing (Mitterschiffthaler, et al., 2003; McCabe, et al., 2009). Moreover, of the handful of studies that have used explicit reward processing tasks in depression (Knutson, et al., 2008; Forbes, et al., 2009; Pizzagalli, et al., 2009; Smoski et al., 2009), none have examined those who are experiencing their first episode of MDD.

In Chapter 3, we used an fMRI gambling task to examine reward processing in depression. Our gambling task recruited several components of the BRS, and provided the means to examine the neural processing associated with the evaluation of rewards, which may reflect symptoms of anhedonia in MDD. We observed 'gradients' of activation (a hierarchy of group differences) across our three study groups (FTE, MTE

and healthy controls) in regions that have been previously associated with the processing of rewards and punishers in healthy controls.

During the processing of rewards MDD patients recruited the NAc and ACC to a lesser degree than matched healthy controls. The NAc is an integral part of the mesolimbic reward system (Knutson et, al., 2001; Ramnani et al., 2004). The ACC is engaged when individuals choose between rewarding values (Blair et al., 2006). Decreased volume (Baumann et al., 1999; Caetano et al., 2006) and hypometabolism (Bragulat et al., 2007; Konarski et al., 2007) have been noted in both regions in individuals with depression. In the current study, we observed gradients of activation in both of these regions across our study groups, such that controls showed the greatest activation, then FTE patients followed by MTE patients recruiting these areas the least (HC>FTE>MTE). These findings identify that depressed patients show diminished activation in areas associated with the processing of rewards, and that this dysfunction worsens overtime.

While many studies have examined symptoms of anhedonia and related brain structures with fMRI, few have used explicit reward processing tasks to examine the BRS in depression. Similar to our NAc findings, Pizzagalli and colleagues (2009) reported altered reward processing during a monetary incentive delay task. During their task, unmedicated, currently depressed subjects had decreased NAc activation in response to rewarding trails. Another recent study using a 'Wheel of Fortune' task found similar

results. In this study, MDD patients displayed decreased NAc activation during reward selection, anticipation and feedback in comparison to controls (Smoski et al., 2009). We build upon these results to extend observations of altered NAc activation to FTE patients.

We also observed a gradient of activation in the ventral medial prefrontal cortex (VMPC). When we examined the reward acquisition phase in contrast with the punishment reversal phase, we observed that MTE patients showed the greatest activation in the VMPFC, then FTE patients followed by controls showing the least amount of activation (MTE>FTE>HC). This finding was driven by the lack of engagement of the VMPFC during punishing outcomes in FTE and MTE patients. Within group analysis revealed that all subject groups recruited the VMPFC during the reward acquisition phase, however, only the controls recruited this region during punishment reversal phase. The depressed patients did not engage the VPMFC during this period; FTE patients failed to recruit the area at all and MTE patients showed decreased activation during the punishment reversal phase. Therefore, it is the lack of engagement / downregulation of the VMPFC of our depressed patients during the processing of punishing outcomes that generated the differences between depressed and healthy control groups. The VMPFC, which receives dopaminergic projections from the NAc, is proposed to be important in the response to unexpected rewards (Ramnani et al., 2007) and in the contextual processing of rewards (Knutson et al., 2009; Elliott et al., 2003). Moreover, studies suggest that this region is also engaged when individuals judge outcomes according to experiential and emotionally laden self-referent information (Drevets et al., 2007;

Schmitz & Johnson, 2006; Struss et al., 2003). Our findings suggest that depressed patients show reduced capacity to associate changes in reward contingencies with self-relevant information. Our findings of a gradient of activation between our three study groups suggest that these alterations become more pronounced overtime with repeated episodes of depression.

To date, reward processing had not been examined in individuals experiencing their first episode of MDD. However, recent studies have shown alterations in regions associated with the processing of rewards in this group of MDD subjects. During tasks of selective attention, attention switching and response inhibition recently diagnosed, medication-free adolescents showed diminished ACC activation in comparison to healthy controls (Halari et al., 2009). Moreover, another study examining first episode females with MDD found decreased right ACC volumes (Tang et al., 2007). These findings are in accordance with our observations of decreased ACC activation during reward processing in FTE patients.

Our reward processing findings add greatly to the research on anhedonia and MDD because we have systematically examined the functional changes in reward processing in MDD in patients who are experiencing their first episode of MDD and those who have experienced multiple past episodes of depression. Our findings of health-to-illness gradients of activation in several key reward processing regions suggest that these regions are altered at illness onset. Decreased activation in FTE patients in several

key regions during the processing of rewards and punishers suggest that abnormalities in the BRS may signal functional neuronal changes that occur early in the illness trajectory of MDD. Moreover, the finding that these alterations become worse over time with repeated episodes of MDD, suggests that illness duration greatly impacts the neurocircuitry underlying reward processing.

Future Directions

The studies presented in this thesis are among the few in the extant depression literature that have examined illness duration in a systematic fashion. We identified a number of temporal and frontal alterations across the course of illness with MDD. Future work could expand on the results presented in this thesis. For example, FTE patients could be studied in a longitudinal fashion following an initial course of treatment to determine if there are differences between treatment responders and non-responders on both recollection memory and reward processing tasks. MRI research has begun to be used in this approach to identify changes in regional activation between those who achieve remission and those who do not. In a longitudinal study examining an 8-week treatment with fluoxetine and neural processing during the n-back task, it was observed that patients with the lowest linear-load response in the dorsal anterior cingulate at baseline had the greatest clinical response following treatment (Walsh et al., 2008). Moreover, Fu and colleagues (2008) used an fMRI paradigm that presented sad faces of differing intensity in order to examine neural activation and treatment response in

depressed patients. The authors found that neural processing of sad faces distinguished depressed patients from healthy controls. Additionally, their analyses was somewhat successful in classifying the treatment response of patients, with the identification of 75% of patients who achieved a partial clinical response, and 62% of those who achieved a full response following fluoxetine treatment (Fu et al., 2008). Pre- and post-treatment differences have also been noted following cognitive behaviour therapy. Prior research has shown that patients with low subgenual cingulate cortex sustained reactivity to emotionally valenced words displayed the strongest clinical improvement following cognitive behaviour therapy (Siegle et al., 2008)

While pre- and post-treatment fMRI studies will add greatly to the understanding of the underlying neurobiology associated with treatment responses, to date, fMRI has not been used in this manner to assess recollection memory or reward processing in MDD. Additionally, if the neural differences separating treatment responders from non-responders can be elucidated through fMRI, it could lead to non-invasive methods that would help to determine which patients would respond in an optimal way to specific types of treatments. This would be particularly advantageous in developing treatment protocols for individuals in the early course of their illness. Moreover, because we identified functional and metabolic alterations in individuals experiencing their first treated episode of MDD, future studies could be conducted in individuals at risk for developing MDD to determine if there are measurable neural alterations prior to the development of clinical episodes of depression. Recently, research has begun to focus on

individuals at risk for psychiatric disorders. In a study examining young people at risk for MDD (categorized as at risk because they had a biological parent with a MDD diagnosis) it was observed that healthy controls showed increased pregenual ACC activation during an emotional Stroop task. Those at who were at risk for MDD failed to show activation of this area during the task (Mannie et al., 2008). These findings might suggest that the ACC is affected particularly early in the development of MDD. As this area is also involved in reward processing, these results may signal the involvement of a more extensive network dysfunction as opposed to alterations solely in the ACC. Studying this group of individuals with other fMRI paradigms, such as our reward processing task, could help to provide a more complete understanding of factors that might put people at risk to develop MDD.

Conclusions

The goal of the present work was to shed light on the functional activity and metabolite levels in individuals with varying degrees of illness duration in depression. The combined work of the three studies included in this thesis suggests that there are definite brain alterations when individuals experience their first episode of MDD. Our results also suggest that overtime, the consequence of multiple episodes of MDD results in more pronounce alterations of these systems. The Hc was identified as showing functional and metabolic alterations both in patients experiencing their first episode of MDD, and those who had experienced multiple past episodes. However, the nature of

these alterations distinguished between these two groups. We suggest that neural processes in the Hc, which may be compensatory in nature at illness onset, are not sustained following multiple episodes of MDD. Moreover, we identified a number of changes in the functional activation patterns of depressed patients during a reward-processing task. Our findings of gradients of activation across several prefrontal and striatal regions, suggest that the neurocircuitry involved in reward processing is also sensitive to illness duration in depression. We suggest that similar to our Hc findings, alterations in reward processing are evident in the early course of depression. However, with multiple episodes of MDD these alterations in functional activation become more pronounced.

It is our hope that the findings from these papers will help to spur future research examining the impact of illness duration across MDD. We show that functional and metabolic changes identified in depression research may not be static across the time course of the disorder, but rather may evolve and change as people experience a more protracted course of illness. Fully characterizing the alterations present in the brain at the first episode of MDD will give a more complete understanding of the neural underpinnings of depression and may also help to guide more targeted therapeutic interventions for more successful treatment outcomes early in this disorder.

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