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# THE FUNCTIONAL NEUROANATOMY
# OF EMOTION REGULATION
# IN MAJOR DEPRESSIVE DISORDER

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For my little girls,
Ella and Lily.

There are times
when I think
I can't possibly
love you more.

And then I do. ♥

"Like the entomologist hunting
for brightly colored butterflies,
my attention was drawn
to the flower garden of the grey matter,
which contained cells
with delicate and elegant forms,
the mysterious *butterflies of the soul*,
the bearing of whose wings may some day
clarify the secret of mental life."

- Santiago Ramón y Cajal, Recollections of My Life

# ABSTRACT

A mood-congruent processing bias toward negative emotional information is a hallmark characteristic of the pathophysiology of major depressive disorder (MDD). Previous neuropsychological studies have provided evidence of this phenomenon in memory and attention paradigms. In addition, functional neuroimaging studies consistently report increased neural responses to negative emotional stimuli, including words and faces.

The amygdala plays an essential role in the determining the salience of a stimulus and is particularly tuned to the evaluation of facial expressions. Two pathways to the amygdala have been proposed: one involving higher-order cortical regions that permits explicit stimulus perception and one involving subcortical structures that allows rapid detection of non-conscious or implicit stimuli of emotional significance.

Several studies have explored the nature of explicit emotional face processing in MDD. However, few have focused on how the brain responds to non-conscious or implicit emotional information. Altered processing of emotional information, below the level of explicit conscious awareness, may contribute to the establishment and maintenance of illness-associated symptoms involving dysfunctional conscious perceptions and social interactions. Providing further evidence, recent research suggests that the mechanisms underlying antidepressant treatment may involve a reversal of the negative emotional processing bias associated with MDD.

The purpose of this thesis was to investigate the functional anatomical neural network of structures involved in mood-congruent processing biases toward non-conscious emotional information in MDD and to evaluate differences in this network associated with antidepressant treatment.

In Study I, a novel backward masking task was developed to examine differences in the hemodynamic response of the amygdala to sad (SN), happy (HN) and neutral (NN) faces presented below the level of explicit conscious awareness using functional magnetic resonance imaging (fMRI). Participants included individuals with current major depressive disorder (dMDD), MDD in full remission (rMDD) and healthy controls (HC). A subset of dMDD participants completed the fMRI task before and after eight weeks of antidepressant treatment with sertraline hydrochloride. An amygdala region-of-interest analysis revealed a greater hemodynamic response in the amygdala to masked-sad vs. masked-happy faces (SN-HN) in dMDD vs. HC. In contrast, HC participants showed a greater response to masked-happy faces. rMDD participants also showed a negative processing bias toward masked-sad faces similar to the dMDD group. In dMDD, the negative processing bias reversed and a positive processing bias emerged following antidepressant treatment.

In Studies II and III, a regional analysis of the cortical and subcortical networks involved with the amygdala in processing emotional information was used to evaluate differences between the dMDD and HC participants and dMDD participants before and after antidepressant treatment from Study I. In Study II, dMDD participants showed a

greater hemodynamic response to SN-HN in the hippocampus and anterior inferotemporal cortex. As well, participants showed a greater response to SN-NN or HN-NN in areas of the medial and orbital prefrontal cortex and superior temporal gyrus. In Study III, dMDD participants showed a greater response to SN-HN in the pre- vs. post-treatment condition in the pregenual anterior cingulate cortex, superior temporal gyrus and anterior inferotemporal gyrus. Additional regions of the sensory and visceromotor networks also showed an increased hemodynamic response to SN-NN before versus after treatment. The regions associated with these differential responses are known to participate with the amygdala in evaluating and responding to the salience of emotional stimuli.

Taken together, these studies provide insight into the underlying neurocircuitry associated with the processing of non-conscious emotional information. Furthermore, they reveal networks that are influenced by antidepressant treatment to alter brain function and establish a reversal of the negative processing bias and development of a normative positive processing bias in major depressive disorder.

# LIST OF PUBLICATIONS (INCLUDED IN THE THESIS)

This thesis is based on the following papers, referred to by their Roman numerals:

I.    **Victor TA**, Furey ML, Fromm SJ, Öhman A, Drevets WC (2010). Relationship between amygdala responses to masked faces and mood state and treatment in major depressive disorder. *Archives of General Psychiatry*, 67(11): 1128-1138.

II.    **Victor TA**, Furey ML, Fromm SJ, Bellgowan PSF, Öhman A, Drevets WC (2012). The extended functional neuroanatomy of emotional processing biases for masked faces in major depressive disorder. *PLoS ONE*, 7(10):e46439.

III.    **Victor TA**, Furey ML, Fromm SJ, Öhman A, Drevets WC. Changes in the neural correlates of implicit emotional face processing during antidepressant treatment in major depressive disorder. *The International Journal of Neuropsychopharmacology.* Submitted October, 2012.

# LIST OF PUBLICATIONS (NOT INCLUDED IN THE THESIS)

IV. Savitz J, Frank MB, **Victor TA**, Bebak M, Marino JH, Bellgowan PS, McKinney BA, Bodurka J, Teague, TK, Drevets WC. (2012). Inflammation and neurological disease-related genes are differentially expressed in depressed patients with mood disorders and correlate with morphometric and functional imaging abnormalities. *Brain, Behavior, and Immunity*, Oct 12. [Epub ahead of print]

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACC | Anterior Cingulate Cortex |
| ADT | Antidepressant Treatment |
| Amyg | Amygdala |
| ANOVA | Analysis of Variance |
| APA | American Psychiatric Association |
| AR(1) | Autoregressive Model of the Order 1 |
| ATQ | Automatic Thoughts Questionnaire |
| BA | Brodmann Area |
| BD | Bipolar Disorder |
| BOLD | Blood Oxygen Level-Dependent |
| dMDD | Current Major Depressive Disorder |
| dMDD-post | Current Major Depressive Disorder, Post-treatment |
| dMDD-pre | Current Major Depressive Disorder, Pre-treatment |
| DSM | Diagnostic and Statistical Manual of Mental Disorders |
| EKG | Electrocardiogram |
| EPI | Echo Planar Imaging |
| fMRI | Functional Magnetic Resonance Imaging |
| FOV | Field of View |
| FSPGR | Fast Spoiled Gradient Echo |
| HAM-D | Hamilton Depression Rating Scale |
| HC | Healthy Control |
| HC-post | Healthy Control, Post-treatment |
| HC-pre | Healthy Control, Pre-treatment |
| Hipp | Hippocampus |
| HN | Masked-happy face followed by an unmasked-neutral face |
| IDS-SR | Inventory of Depressive Symptomatology: Self-Rating |
| Ins | Insula |
| ISI | Interstimulus Interval |
| ITG | Inferotemporal Gyrus |
| latOFC | Lateral Orbitofrontal Cortex |
| MDD | Major Depressive Disorder |
| mg/d | Milligrams per day |
| MNI | Montreal Neurological Institute |
| NH | Masked-neutral face followed by an unmasked-happy face |
| NN | Masked-neutral face followed by an unmasked-neutral face |
| NS | Masked-neutral face followed by an unmasked-sad face |
| OFC | Orbitofrontal Cortex |
| PET | Positron Emission Tomography |
| PCC | Posterior Cingulate Cortex |
| PFC | Prefrontal Cortex |
| pgACC | Pregenual Anterior Cingulate Cortex |
| PTSD | Post-Traumatic Stress Disorder |
| rMDD | Major Depressive Disorder in Full Remission |
| rSTG | Rostral Superior Temporal Gyrus |

| | |
|---|---|
| SAD | Social Anxiety Disorder |
| SCID | Structured Clinical Interview for DSM Disorders |
| SD | Standard Deviation |
| SN | Masked-sad face followed by an unmasked-neutral face |
| SPM5 | Statistical Parametric Mapping |
| SSRI | Selective Serotonin Reuptake Inhibitor |
| STAI-S | State-Trait Anxiety Inventory- State |
| STAI-T | State-Trait Anxiety Inventory- Trait |
| STG | Superior Temporal Gyrus |
| TCQ | Thought Control Questionnaire |
| TE | Echo Time |
| Thal | Thalamus |
| TR | Repetition Time |
| vlPFC | Ventrolateral Prefrontal Cortex |
| WASI | Wechsler Abbreviated Scale of Intelligence |
| WHO | World Health Organization |

# 1  INTRODUCTION

According to the World Health Organization, major depressive disorder (MDD) is the leading cause of years lived with a disability across all age groups. By the year 2020, depression is expected to become the second leading cause of disability-adjusted life years, or years of life lost due to premature mortality, second only to ischemic heart attack. Over 120 million people are estimated to suffer from depression worldwide. MDD is also associated with an increased risk of suicide and approximately 15-20% of depressed individuals end their lives by committing suicide, a leading cause of death in young adults [1].

Major depressive disorder is characterized by persistent depressed mood and/or loss of interest and pleasure, in addition to feelings of hopelessness, worthlessness, or guilt, diminished concentration or indecisiveness, fatigue, recurrent thoughts of death or suicide, and difficulties with psychomotor activity, weight loss or gain, sleep and appetite [2]. The clinical symptomatology of MDD involves brain systems responsible for the regulation of mood, attention, anxiety, reward processing, motivation, social function, and autonomic function (e.g. sleep, energy, weight, libido) [2].

Despite the enormous burden on an individual's life and society, the pathogenesis of major depressive disorder has been poorly characterized until recent years, due to a lack of gross brain abnormalities or clear animal models associated with the recurrent MDD [3]. Advances in neuroimaging technologies, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have provided methods of non-invasive assessment of the functional, structural and neurochemical abnormalities underlying the pathophysiology of mood disorders [3]. In particular, the elucidation of the functional neuroanatomical correlates of emotion regulation has been of importance in advancing psychiatric research. Knowledge concerning the neurophysiological correlates of emotion regulation will aid in the understanding of how these processes are altered and may be reversed with proper treatment. *The significance of the proposed study lies in its contribution to the understanding of the specific neuroanatomical correlates, neurophysiological processes, and neural pathways involved in emotional processing and the potential to establish effective treatment mechanisms in major depressive disorder.*

## 1.1  FUNCTIONAL MAGNETIC RESONANCE IMAGING

Methods for functional brain imaging have been developed that use magnetic resonance imaging (MRI) to observe activity-related, hemodynamic response changes with spatial and temporal resolution. Functional MRI (fMRI) is predicated on the magnetic properties of the hydrogen atom. When a subject is placed in the scanner, a radio frequency pulse is applied which affects proton alignment in the hydrogen atom. Upon completion of the pulse sequence, the protons attempt to realign, thereby emitting energy that can be interpreted as signal strength. The most commonly used method of functional imaging examines changes in blood oxygenation, such that neural activity is measured indirectly by the blood oxygen level-dependent contrast (BOLD) [4,5]. Because of the different magnetic properties of oxy- and deoxyhemoglobin, increased levels of blood oxygenation are associated with increased signal for T2*-weighted gradient echo imaging and echo planar imaging (EPI). Increased neural activity is associated with this increased signal [4]. Because the change in signal is due to properties inherent in the blood, MRI is non-invasive and eliminates the need for injection of contrast agents or radiation exposure. Therefore, this method provides an advantage in the evaluation of neural activity over other brain imaging techniques, such as positron emission tomography (PET).

A limitation of BOLD susceptibility-contrast based fMRI is that the magnetic resonance signal obtained using T2*-weighted images is also sensitive to other sources of magnetic susceptibility. A problematic artifact in these images is the artifact associated with air in the bony sinuses of the skull (e.g. sphenoid sinus), which attenuates the MR signal in orbitofrontal and anterior temporal cortex [6]. The extent of MR signal dropout from such susceptibility artifacts worsens as the magnetic field strength of the MRI scanner increases. By shortening the echo time (TE) and employing smaller voxel sizes in the present study, signal susceptibility artifacts were sufficiently reduced in the orbitofrontal cortex to allow for sensitive measures of the BOLD signal in this region.

## 1.2  NEURAL CIRCUITRY INVOLVED IN DEPRESSION

The convergence of neuroimaging, neuropathological and lesion studies has resulted in the identification of networks within the brain involved in the regulation of

emotional behavior in mood disorders [7]. These regions include the limbic-cortical-striatal-pallidal-thalamic circuit which is comprised of multiple connections between areas of the orbital and medial prefrontal cortex (PFC), hippocampus, amygdala, and related structures in the striatum and thalamus [8]. These circuits were initially thought to be involved in depression based on the increased risk for developing MDE in persons with basal ganglia disease or lesions of the striatum and orbital cortex [9]. Dysfunction in these circuits has been proposed to result in the pathological emotional symptoms of a major depressive episode (MDE) [10].

Two additional extended cortical circuits within the brain have been identified based largely on neuroanatomical studies of monkeys [11]. The first, referred to as the "orbital prefrontal network", involves sensory association areas of the inferior temporal cortex, insula and frontal operculum. This circuit is responsible for sensory integration, critical assessment of objects and the anticipation of reward [11]. The second extended cortical circuit is the "medial prefrontal network" or "visceromotor" network. This network involves the amygdala and interconnections with the medial prefrontal cortex, caudolateral orbital cortex, anterior and medial temporal cortex and posterior cingulate cortex, as well as subcortical structures such as the striatum, thalamus, hypothalamus and brainstem [11,12]. The visceromotor network, in contrast to the medial prefrontal system with its sensory-related functions, is involved in the modulation of visceral responses to emotional stimuli and is implicated the pathophysiology of mood disorders [12]. Both networks have shown abnormalities in depressed individuals during fMRI studies of reward and emotion, however the visceromotor network has been more specifically implicated in mood disorders [13,14].

The subgenual and pregenual regions of the anterior cingulate cortex (ACC) have been implicated in the neurobiology of MDD and bipolar disorder (BD) [15,16]. Both areas are known to be associated with autonomic, neuroendocrine, and monoamine neurotransmitter modulation in response to stressful and emotionally provocative stimuli ([16] for a review). In PET imaging, after correcting for the effects of a left-lateralized reduction in gray matter volume, cerebral blood flow and metabolism are abnormally increased in the subgenual ACC in depressed patients versus controls [15,17,18]. Providing further support for this finding, cerebral blood flow (CBF) increases in the subgenual ACC in response to induced sadness in healthy individuals [19-21] and following effective antidepressant treatment, glucose metabolism

decreases to normative levels in MDD [17,21-23]. Pregenual ACC cerebral blood flow and metabolism findings are more variable than the subgenual ACC, however most imaging studies report increases during major depressive episodes, consistent with elevated CBF during emotional conditions found in healthy and anxiety-disordered individuals [16], and normalized metabolism following successful treatment [15,24]. Electrical stimulation of this region elicits fear, panic, and a sense of foreboding in humans and vocalization in experimental animals [[25] for a review].

In addition to the ACC, several other prefrontal cortical regions exhibit neurophysiological abnormalities in major depressive disorder, including the dorsomedial and dorsolateral PFC, ventrolateral PFC, and posterolateral orbital cortex. In the dorsomedial and dorsolateral PFC, resting CBF and glucose metabolism are reduced in depressed individuals compared to controls [26-28], while CBF increases in these areas during performance of tasks that elicit emotional responses or evaluations in healthy controls [29-31]. The dorsomedial PFC may also play a role in attenuation of emotional responses, as increases in CBF are inversely correlated with decreases in heart rate and anxiety ratings during anticipation of a shock [32]. In depressed individuals, CBF and glucose metabolism in the dorsomedial and dorsolateral PFC have been shown to normalize with effective antidepressant treatment in some studies [23,26,27,33], but not all [34,35]. Lesions of this area in rats result in an increased heart rate response to fear-conditioned stimuli, and stimulation of this area inhibits heart responses and defensive behaviors evoked by stimulation of the amygdala [36].

In the ventrolateral and posterolateral orbital cortex, CBF and metabolism are reportedly increased in unmedicated, unipolar depressed individuals [30,37,38], and decrease with successful antidepressant treatment [21,39,40]. In healthy individuals, CBF in the posterolateral orbital cortex increases during induced sadness or anxiety. Electrophysiological and lesion studies suggest a role for the posterior orbital cortex in the modulation of defensive, autonomic, and behavioral response patterns to reward and perseveration on inappropriate strategies [41,42]. Furthermore, the role of the posterior orbital cortex may be partially influenced by interactions with the amygdala and other limbic structures due to direct projections to each other and overlapping projections to the striatum, hypothalamus, and periaqueductal gray (PAG) [43,44]. Compatible with this finding, glucose metabolism in the orbital cortex and amygdala

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are inversely correlated in depressed individuals [22]. As well, similar to the subgenual ACC, gray matter and glial cells are reduced in MDD in the posterolateral orbital cortex [28,45]. If this abnormality is associated with altered synaptic connections between these areas above, then orbital dysfunction may contribute to exaggerated emotional responses to stressors and ruminative ideation [24]. During a major depressive episode (MDE), activation of the posterolateral orbital cortex may be part of an effort to attenuate emotional expression, break negative thought patterns, and inhibit defensive behaviors and visceral responses to stressors [15,16]. If the posterolateral orbital cortex is activated in MDD in an attempt to correct abnormal responses and modulate emotional expression, abnormalities may result in the inability to interrupt perseverative negative thoughts and anxious responses to otherwise non-threatening stimuli [46].

While the prefrontal cortex has multiple regions implicated in the abnormal neurophysiology associated with MDD, several other subcortical structures also play a very important role, such as the amygdala, striatum, and thalamus. In humans, electrical stimulation of the amygdala can produce anxiety, fear, dysphoria, recollection of emotionally provocative events, and an increase in cortisol secretion [[47] for a review]. In MDD, resting cerebral blood flow and glucose metabolism are abnormally elevated in the amygdala and medial thalamus [30,37,48-50]. The magnitude of this abnormality when corrected for spatial resolution effects is an actual increase in CBF and metabolism of about 50%-70% [37,51]. Antidepressant treatment induces and maintains remission of MDD and is associated with the normalization of amygdala metabolism [39].

## 1.3   RECIPROCAL INTERACTIONS BETWEEN AMYGDALA AND PREFRONTAL CORTEX

The amygdala is an almond-shaped structure located within the medial temporal lobe of the brain. It is composed of a group of nuclei with extensive interconnections throughout cortical and subcortical regions. The amygdala contains cells that are selectively tuned toward stimulus characteristics that permit the rapid detection of salient information [52,53]. The amygdala has also been shown to influence the sensory cortices such that they are differentially sensitive to salient signals [54,55]. The amygdala has been extensively studied as a region involved in the

pathophysiology of fear and anxiety processes in the brain, often using the approach of classical fear conditioning. This paradigm has shown that fear is processed through a series of circuits that detect and respond to danger [56]. The pathway underlying fear conditioning involves the communication of information to the amygdala through the thalamus and cortex to networks in the brain that influence behavioral, autonomic, and endocrine responses [57,58]. Functional neuroimaging studies have implicated the role of the amygdala in the processing of fear and evaluation of emotional significance to stimuli [59,60]. The amygdala has been shown to play a role in fear conditioning [61,62], recognizing facial expressions [63-65], and abnormal amygdala response in the context of depression and anxiety [66,67].

Two pathways have been proposed to explain how sensory information reaches the amygdala. More specifically, these two pathways involve thalamo-cortico-amygdala (cortical) and thalamo-amygdala (subcortical) projections. These projections pass through the thalamus and terminate in the lateral amygdala which generates responses to visual and auditory inputs. Information then continues from the lateral amygdala to the central nucleus of the amygdala, by way of the basal and accessory basal nuclei, which control the expression of the emotional response [58]. The thalamo-amygdala pathway is thought to be rapid response system for visual and auditory stimuli and involved in mediating fear responses to stimuli presented below the level of conscious awareness [60]. In contrast, the thalamo-cortico-amygdala pathway is involved in processing more complex sensory information requiring cortical inputs necessary for information processing [58,68,69]. Functional neuroimaging studies have shown increases in amygdala activity during the presentation of subconscious stimuli, in which the most robust relationships are found between the amygdala and subcortical structures, providing further evidence for the role of a subcortical pathway in the human brain [70,71].

The amygdala receives multiple inputs from sensory processing cortical areas, including reciprocal connections with the PFC. When the amygdala is activated by a sensory stimulus by receiving input from the thalamus or cortex, it can also regulate cortical areas with projections to the amygdala, thereby controlling the type of input it receives [56]. The medial PFC provides important feedback to the amygdala and hippocampus, which may represent a 'top down' processing of their response, enabling the reduction of a fear response once an imminent danger is no longer

present or the meaning of a threatening stimulus changes context [72] and extinction of conditioned responses to stimuli [73,74]. In both animal data and human brain imaging, the amygdala has been implicated in fear conditioning and the PFC with a role in the process of extinction of fear responses [72]. In rats, it has been demonstrated that damage to the medial PFC results in fear that is difficult to extinguish [75]. This suggests that alterations in medial prefrontal cortical regions may predispose certain people in some circumstances, such as stressful situations, to learn fear responses that persist under normal circumstances. Garcia et al. [76] showed that prefrontal neurons reduce their spontaneous firing activity in the presence of a conditioned stimulus as a function of the degree of fear. As well, the reduction was related to amygdala activity. They provide evidence that in the presence of threatening stimuli, the amygdala modulates both fear expression and prefrontal neuronal activity. This suggests that the abnormal amygdala-induced modulation of activity in the PFC may be involved in the pathophysiology of disorders with anxiety components [76]. In preliminary PET data in humans with anxiety disorders, dysfunction of medial prefrontal cortical structures (i.e. the ACC and infralimbic cortex) is associated with the abnormal expression of anxiety and fear [67]. Given the reciprocal connections the amygdala and prefrontal cortical structures, the amygdala is in a position to activate the PFC and modulate interactions between the PFC and medial thalamus and influence both emotional and stress responses [22]. Pathological changes in the top-down control of this network of regions may be responsible for the manifestation of the symptoms of major depressive disorder. Impairment could result in an exaggerated response to threatening or negative stimuli and a reduction in the response to rewarding or positive stimuli [12,77,78].

## 1.4 MOOD-CONGRUENT EMOTIONAL PROCESSING BIASES

Studies of emotional processing biases often center on the wealth of information contained in human faces. In his pioneering work on emotion, Charles Darwin hypothesized that emotions were genetically determined and that basic emotions were universal in all mammals [79]. More recently, Paul Ekman's research on facial expressions as representatives of emotion has resulted in six universal expressions: anger, disgust, fear, happiness, sadness and surprise [80]. This work continues to influence our understanding of emotion and provides the foundation for the vast amount of research performed to evaluate emotion-specific neurophysiological

responses [81] and gain further insight into neuropsychiatric disorders associated with abnormalities in emotional processing.

A mood-congruent processing bias toward negatively-valenced emotional stimuli is a consistent feature in the pathophysiology of major depressive disorder (MDD). This bias is described as a stimulus processing bias toward negative emotional information as compared to neutral or positive information. This bias is evident in behavioral measures that evaluate memory and attention [82-85]. In studies of memory, depressed patients show increased memory recall for negatively toned information in comparison with positively toned information [82,85]. In studies of attention, both medicated and unmedicated depressed subjects show faster reaction times during the presentation of sad words as compared to happy words during the affective go/no-go task that evaluates shifts in attention biases. [84,86]. As well, depressed patients have been shown to attend to sad facial expressions compared to neutral expressions using the face dot-probe task [83]. Taken together, these behavioral studies show a consistent preference toward negatively-valenced information in depression.

Consistent with behavioral findings, mood-congruent processing biases are also evident in neurophysiological indices [3,87-92]. In normal controls, several functional imaging studies have shown greater amygdala activity in response to overt negatively valenced fearful or sad faces in comparison with neutral or happy faces [64,65,93-95]. In addition, healthy controls have been shown to exhibit a linear increase in activity in the fusiform cortex and ventral striatum with increases in the intensity of happy expressions [96]. In the same study, depressed patients showed a similar pattern of response as the intensity of sad expressions increased and this finding was further extended to include the hippocampus and amygdala. In major depressive disorder, normal increases of the hemodynamic response are attenuated in the left amygdala during exposure to fearful facial expressions, whereas the response to sad faces is exaggerated in depressed individuals relative to controls [22]. Attenuated responses of the left amygdala during presentation of fearful faces relative to neutral and sad faces have been found in both depressed children [97] and adults [98]. This response (attenuation of tissue that is already abnormally activated) is consistent with findings of abnormally elevated CBF and glucose metabolism in the amygdala of depressed individuals at rest [24,37]. Finally, the hemodynamic response to *explicitly* presented sad faces is exaggerated in the amygdala in depressed individuals compared to healthy

controls [96], and this abnormality normalizes following antidepressant drug treatment (ADT) [99]. Given the differences in hemodynamic response of the amygdala at rest and during the presentation of emotional facial expressions between depressed individuals and controls, it is plausible that amygdala dysfunction may alter the interpretation of social or emotional stimuli in mood disorders [24].

Drevets et al. [98] investigated amygdala and PFC functioning toward sad faces in individuals with MDD using PET. Increases in CBF in the left amygdala and prefrontal cortical regions were found in the depressed relative to control individuals. Increased amygdala activation in the depressed individuals was explained by a lack of habituation to the sad faces. In the control individuals, amygdala activation decreased with multiple presentations of the sad faces, suggesting a habituation response to emotional stimuli that are familiar [64,97]. The table below reviews a number of regions exhibiting abnormal hemodynamic responses in the orbitomedial PFC and areas with connections to this region associated with differential amygdala responses to emotionally valenced stimuli between depressed and healthy control individuals.

Differential Hemodynamic Responses To Facial Emotion: Depressed vs. Healthy Controls.

| ⬆ In Healthy | ⬇ In Depression to | Sad, Fear<br>Sad, Fear<br>Fear | Ventromedial PFC (24/32/10m)<br>Pregenual ACC (24)<br>Left Ventrolateral Amygdala,<br>Hypothalamus,<br>Periaqueductal Gray Nucleus |
|---|---|---|---|
| ⬇ In Healthy | ⬆ In Depression to | Sad, Fear<br>Sad, Fear<br>Sad<br><br><br>Fear | Dorsal Temporal Pole<br>Parahippocampal Gyrus<br>Posterior Cingulate,<br>Anteroventral Striatum,<br>Dorsomedial PFC (9/32)<br>Infralimbic C (25),<br>Dorsal Area 32 |

## 1.5   BACKWARD MASKING OF EMOTIONALLY VALENCED STIMULI

Conscious feelings do not appear to be required to produce emotional responses that, like cognitive processes, involve subconscious processing mechanisms [60,100]. In addition to the study of the conscious processing of emotional stimuli using facial emotion described above, there has been an increasing amount of literature concerning the non-conscious processing of emotional stimuli using a technique known as

backward masking. Arne Öhman and his colleagues were the first researchers to demonstrate differences in automatic learning via a classical conditioning paradigm combined with backward masking in healthy controls and individuals with anxiety disorders [101]. In the technique of backward masking, two stimuli are presented with the second stimulus following the first so quickly that the neural processing of the original stimulus is interrupted by the presentation of the second and is therefore not detected by the individual. Behavioral studies suggest that stimulus-stimulus associations can be formed implicitly (i.e. without the subject's awareness) when the stimuli involved are biologically salient (e.g., snakes, spiders, or angry faces) [102,103].

Early strategies for the backward masking procedure involved conditioning the presentation of a facial expression to predict an aversive shock. This has been done in two ways described in Whalen et al. [101]: The first method involves presenting the facial stimuli overtly (unmasked) during conditioning and then recording participant responses (i.e. skin conductance responses (SCRs)) during masked presentations. This method seeks to determine if responses to previously learned associations can occur when later presented below conscious awareness. In a second method, backward masking during conditioning is used to assess whether subsequent presentations of unmasked faces produce greater responses to the face that predicted the aversive event in the masked presentations. This method seeks to determine if individuals can learn the conditioned association when they are initially presented below conscious awareness, as indicated by a subject's inability to describe having seen the masked stimulus. Using these methods, studies have demonstrated that greater SCRs can be produced to backwardly masked stimuli following previously learned overtly presented associations between the stimulus and aversive event and also greater SCRs to overtly presented stimuli that were previously conditioned using masked stimuli presented below the level of conscious awareness [100,104-110].

Presentations of masked emotional stimuli have been fairly reliable in obtaining differential behavioral (i.e. SCRs) and neurophysiological responses in healthy and anxiety-disordered individuals. Backward masking is of important relevance to anxiety disorders and disorders with anxiety components given that this technique is designed to evaluate the automaticity of emotional processing in the brain. These disorders are associated with an automatic information processing bias for negative information, such as involuntary and spontaneous obsessions, panic attacks, or ruminative negative

thoughts.  Given the high comorbidity of anxiety-related symptoms with mood disorders and the common neural structures and pathways implicated in the pathophysiology of these disorders with regard to emotional processing, backward masking is a technique that may prove to be useful in determining the underlying dysfunction of processing emotional stimuli.  This technique allows for the assessment of automatic processing of emotional stimuli as opposed to a controlled level of information processing, which may play a key role in understanding the manifestation of dysfunctional emotional processing in mood and anxiety disorders.

While the majority of what is known about backward masking has been established by measuring changes in SCRs to aversively conditioned stimuli, many studies have used this technique in fMRI studies as well, focusing on the role of the amygdala in the processing of emotional faces.  Based on animal studies indicating the thalamo-amygdala projections are involved in rapid conditioning [111], Le Doux [60] proposed that processing emotionally valenced stimuli presented below the level of conscious awareness would involve the amygdala.   In fMRI studies where emotional faces were presented below the level of awareness using a "masked" face paradigm, the amygdala was activated in healthy individuals [93,94], indicating that conscious perception is not necessary for detection at the subcortical level.   Whalen et al. [94] studied amygdala responses to backwardly masked-fearful and happy facial expressions in the absence of explicit knowledge and reported increased bilateral amygdala activation to masked-fearful vs. masked-happy faces in healthy individuals.  Morris and Öhman [71] demonstrated a significant neural response in the right amygdala to masked presentations of a conditioned angry face, and a significant neural response in the left amygdala to unmasked presentations of the same face.  Specifically in individuals with MDD, Sheline et al. [112] reported that hemodynamic responses in the left amygdala were increased in MDD subjects exposed to masked-fearful vs. masked-happy faces. Sheline et al. [112] further showed that this exaggerated left amygdala activity to masked-fearful faces was attenuated in MDD individuals following sertraline treatment.  The magnitude of the BOLD response to masked-fearful faces did not change over the same time interval in healthy controls.

As discussed above, the amygdala plays a pivotal role in evaluating the emotional *salience* of sensory stimuli through participation in two distinct types of distributed networks, one involving cortical regions that allow conscious or explicit stimulus

perception, and the other involving subcortical structures that allow rapid, non-conscious assessment of stimulus features [53,60]. Notably, *healthy subjects* show greater amygdala responses to happy versus sad faces when stimuli are presented below conscious awareness. This finding suggests the existence of a normal *positive* processing bias that is supported by subcortical networks which mediate rapid, automatic emotional evaluations [113,114]. Thus, it is plausible that the *negative* processing bias that characterizes MDD may be mediated by this rapid, non-conscious processing network involving the amygdala and may also implicate the dysfunctional limbic-cortical network involved in mood and anxiety disorders. Neurophysiological responses to non-conscious emotional stimuli in specific regions of the limbic-cortical network remain largely unknown.

## 1.6 NEUROIMAGING ABNORMALITIES AND ANTIDEPRESSANT TREATMENT

The neuroimaging measures with abnormalities in MDD that show changes in response to effective antidepressant drugs or mood stabilizers include: resting glucose metabolism in the amygdala, anterior cingulate cortex, and ventral striatum, hemodynamic responses to emotional faces in the amygdala and ACC, gray matter volume of the subgenual ACC, and serotonin type 1A receptor binding in the raphe and mesiotemporal cortex [23,33,112,115-120]. These findings implicate neurobiological systems shown by electrophysiological, lesion analysis, or PET/fMRI studies to play major roles in emotional behavior. These neuroimaging abnormalities have been replicated across laboratories, and are associated with histopathological correlates in post mortem studies of primary MDD ([121] for a review).

Studies designed to evaluate the effects of antidepressant treatment on the neurocircuitry involved in MDD may elucidate the mechanisms underlying the outward expression of the benefits of pharmacotherapy. Longitudinal studies in which depressed subjects are imaged before and during treatment show that CBF and metabolism decrease in the left amygdala, orbital cortex, ventromedial PFC, pregenual and subgenual ACC and anterior insula following effective antidepressant drug therapy, ECT, phototherapy, repeated transcranial magnetic stimulation (rTMS), and sleep deprivation (see [46] for a review). The reduction in amygdala metabolism during treatment correlates positively with clinical improvement (decrease in depression

ratings) and with the reduction in plasma cortisol levels [116]. Amygdala hemodynamic responses to emotionally valenced stimuli are also suppressed by chronic antidepressant drug treatment [99,112,122]. These data are consistent with preclinical evidence suggesting that chronic antidepressant drug treatments directly suppress amygdala function [123-127]. In addition, similar effects on the amygdala have been shown following acute antidepressant drug administration. For example, citalopram administered three hours prior to scanning decreases the amygdala response to fearful expressions [128] and similar results have been shown after seven days of administration with reboxetine and citalopram [129,130].

A recent hypothesis has proposed a cognitive neuropsychological model of antidepressant action, such that the primary therapeutic mechanism of antidepressant treatment is to normalize negative processing biases, thereby influencing the interpretation of salient personal and social information [131,132]. As well, it has been hypothesized that the clinical effects of antidepressant treatment lag behind the normalization of emotional processing biases because environmental and social interaction is required before a change in the emotional bias can overtly affect mood and behavior [132]. Evidence in support of this hypothesis has been shown in healthy individuals during the short-term administration of citalopram, which increased amygdala response to happy faces [133] and in depressed patients who after a single dose of reboxetine showed enhanced behavioral responses to positive stimuli [134]. These findings suggest that changes in emotional processing biases commonly occur under antidepressant treatments that are diverse with respect to their primary pharmacological mechanisms of action. Furthermore, these changes in the processing of emotional information at an early stage may contribute to and influence the change in mood and improvement in symptoms associated with MDD later in the course of treatment [134].

Among antidepressants, sertraline is a first line antidepressant drug for treating MDD given its established efficacy and more benign adverse risk and side effect profiles. For these reasons, sertraline was selected as the antidepressant treatment for the pharmacotherapy component of the thesis work. Sertraline has been shown to be as effective and well-tolerated as other selective serotonin reuptake inhibitors (SSRI)s, which function to inhibit the uptake of serotonin from the synapse. Sertraline has a relatively low tendency for altering the metabolism of other drugs relative to similar SSRIs because it has low potency for inhibiting the hepatic cytochrome P450 enzymes.

Risks or side effects associated with sertraline and other SSRIs include induction of hypomania, allergic reactions, gastrointestinal side effects, insomnia, agitation, anxiety, sedation, sexual dysfunction, and rarely, the syndrome of inappropriate secretion of antidiuretic hormone. Sertraline has rarely been fatal in cases in which suicidal patients took an intentional medication overdose. However, despite the improved tolerability of SSRIs over other antidepressants, such as monoamine oxidase inhibitors and tricyclic antidepressants, they have a slow onset of response and about one third of individuals treated with SSRIs are considered non-responders [135,136]. Therefore, the development of new antidepressant pharmacotherapies continues to be of importance in improving the lives of those who suffer from MDD.

## 1.7   HABITUATION AND EXTINCTION MECHANISMS

The hemodynamic response to presentations of emotionally valenced stimuli follows a biphasic pattern of activation in healthy individuals. For example, fMRI studies have shown that the amygdala is activated during the initial period of exposure to fear-conditioned stimuli, but then becomes deactivated (or habituated) during repeated exposures to the same stimulus. Eventually, through the process of extinction, the activity decreases to below the baseline BOLD signal level [61,62,137]. While the amygdala is postulated to play a key role fear conditioning, the PFC has been implicated in the extinction process of responses to emotionally valenced stimuli. For example, a study of animal phobics showed orbital CBF was unchanged during the initial exposures to phobic stimuli when fear was the greatest, but as habituation occurred, posterolateral orbital CBF increased, inversely correlating with measures of heart rate and changes in anxiety ratings [138]. In addition to paradigms with phobia-related components, evidence of amygdala habituation of the BOLD signal response has been found with multiple presentations of affective facial expressions in healthy individuals [64,97,139].

In mood-disordered individuals, habituation and extinction of hemodynamic response patterns involving both the amygdala and prefrontal cortical areas are of unique interest given the finding that these individuals do not normally habituate to the presentation of certain emotional stimuli. In a study by Drevets et al. [98], the hemodynamic response to initial exposures of sad faces presented to individuals with MDD and bipolar disorder (BD) did not differ from control subjects, however

repeated presentations of these sad faces resulted in habituation of the response in the controls, but not depressed individuals. The increased amygdala CBF was sustained across continued presentations of the sad faces in the depressed individuals. This effect has also been found during the presentation of negatively valenced words presented to depressed and control individuals. During exposure to negatively valenced words, increases in amygdala hemodynamic activity were found in both groups, however the response persisted abnormally in the depressed individuals and normalized in controls [140]. This phenomenon of sustained amygdala activity in MDD in response to emotionally valenced stimuli is of particular importance given evidence that the amygdala is involved in acquisition and expression of emotional information (e.g. aversive conditioning) [60,141,142]. If proper PFC functioning is necessary to extinguish learned emotional responses, it is plausible that the increased amygdala response would excessively stimulate cortical areas. This may lead to the tendency of depressed individuals to ruminate on memories of emotionally aversive or guilt-provoking life-events, possibly due to a dysfunction in the reciprocal interactions of the PFC and amygdala in the modulation of emotional responses [66]. Furthermore, these emotional responses may be generated and maintained on a subconscious level, outside explicit cognitive awareness.

# 2  AIMS

The objective of the present thesis was to elucidate and understand the role of neurophysiological abnormalities associated with non-conscious emotional processing in major depressive disorder (MDD) using functional magnetic resonance imaging (fMRI).

The primary aims of the project were as follows:

1.  To pilot a novel fMRI backward masking task in healthy controls designed to present emotional face stimuli below the level of explicit conscious awareness.

2.  To compare differences in neural responses to non-consciously presented sad, happy and neutral face stimuli between healthy controls and patients with current MDD.

3.  To compare differences in neural responses to non-consciously presented emotional face stimuli in an unpaired, cross-sectional study design between patients with MDD in full remission and patients with current MDD.

4.  To assess the effects of antidepressant treatment with the selective serotonin reuptake inhibitor, sertraline, on neural responses to emotional face stimuli in patients with current MDD.

# 3   MATERIALS AND METHODS

## 3.1   PARTICIPANTS

### 3.1.1   Recruitment and Selection Procedure

In Paper I, 25 healthy controls (HC; 15 female, aged 28.8 (± 6.7 years), 22 unmedicated participants with major depressive disorder in a current depressive episode (dMDD; 12 female, aged 31.1 ± 7.8 years) and 16 unmedicated participants with major depressive disorder in full remission (rMDD; 11 female, aged 30.8 ± 9.8 years) were recruited from the National Institute of Mental Health (NIMH) Mood and Anxiety Disorders Program and the National Institutes of Health inpatient and outpatient clinical services, Bethesda, Maryland, USA.  In some cases these individuals were recruited using advertisements posted in the Washington D.C. metropolitan area, as approved by the NIMH Human Studies Committee.  All participants were right-handed and between the ages of 18-50 years old. The race distribution of the samples in the study was similar to that of the greater Washington D.C. metropolitan area.

In Paper II, additional analyses were performed on the data acquired from the 25 HC and 22 dMDD participants described in Paper I.

In Papers I and III, 10 of the 22 dMDD participants (6 female, aged 33.2 ± 5.0 years) underwent antidepressant treatment with the selective serotonin reuptake inhibitor, sertraline, and completed the fMRI task before and after eight weeks of pharmacotherapy. Ten of the 25 HC participants (7 female, aged 28.4 ± 5.7 years) also completed the fMRI task before and after an equivalent eight-week time period.

### 3.1.2   Inclusion and Exclusion Criteria

Prior to enrollment in the study, the individuals participated in a phone screen and in-person screening evaluation at the National Institutes of Health to determine eligibility. The screening health evaluation included a medical and psychiatric history, current medication and pharmacotherapy history, laboratory testing (including

hematology, blood chemistry, thyroid function, urinalysis, urine drug screen and HIV testing), pregnancy test for women, electrocardiogram, physical examination and neuromorphological MRI. The psychiatric diagnosis was established by the Structured Clinical Interview for DSM-IV (SCID). [143] with a clinician and a semi-structured interview with a psychiatrist. A family history of psychiatric disorders was established by the Family Interview for Genetic Studies (FIGS) [144].

Healthy Volunteers: Individuals were selected who did not meet criteria for any major psychiatric disorder, had no known first-degree relatives with mood disorders, and whose current score on the Hamilton Depression Rating Scale (HAM-D; 24-item) was in the non-depressed range ($\leq 7$) [145].

dMDD Participants: Individuals were selected with primary MDD in a current major depressive episode who met the Diagnostic and Statistical Manual of Mental Disorders-IV-TR [2] criteria for major depressive disorder and whose current HAM-D score was in the moderately-to-severely depressed range ($\geq 19$) [145]. The participants were drug-naïve or had not received psychotropic drugs for at least 3 weeks (8 weeks for fluoxetine) prior to scanning. Effective medications were not discontinued for purposes of the study.

rMDD Participants: Individuals were selected with a past history of MDD in current remission by DSM-IV criteria [2]. Effective medications were not discontinued for the purposes of the study and participants were medication-free and in remission for a minimum of three months prior to scanning.

Individuals were excluded if they had a) serious suicidal ideation or behavior, b) psychosis to the extent that the ability to provide informed consent was in doubt, c) medical conditions or current medications that were likely to influence cerebral blood flow or neurological function including cardiovascular, respiratory, endocrine and neurological diseases, d) a history of drug or alcohol abuse within 1 year or a lifetime history of alcohol or drug dependence [2], e) current pregnancy (as documented by pregnancy testing prior to scanning), f) general MRI exclusion criteria, g) a history of non-response to sertraline or of intolerable or adverse side effects during sertraline treatment. Additional exclusion criteria applied to *control* participants were: a) a

current or past history of axis I psychiatric conditions, b) a first-degree family member with current or past history of mood disorder.

Individuals beyond age 50 were excluded from the study to reduce the biological heterogeneity encompassed by the MDD criteria, and to reduce the variability of the BOLD signal [146]. Participants whose first major depressive episodes arose after other major medical or psychiatric conditions were also excluded, since their functional imaging results generally differ from those reported in primary MDD [24].

### 3.1.3 Antidepressant Drug Treatment

Ten dMDD participants underwent treatment with the antidepressant drug sertraline hydrochloride (50 mg/d for 3 days and then titrated to 100 mg/d as tolerated). Each person completed a pre-treatment fMRI scan and follow-up post-treatment scan after eight weeks of pharmacotherapy. Ten HC participants completed the fMRI task before and after the same time interval to control for test-retest and other non-specific order effects. Under the supervision of the study psychiatrist, the sertraline dose was increased or decreased as clinically indicated. Participants received a constant dose for at least four weeks prior to the post-treatment fMRI scan. The mean (SD) dose was 105 (50) mg/d (range, 50-200 mg/d) at the time of the post-treatment scan. Participation in the antidepressant treatment was voluntary and not required for enrollment in the study. An interim analysis between HC and dMDD groups was performed to validate the fMRI task prior to the start of the treatment portion of the study. Following the task validation, all dMDD participants who enrolled in the study were presented with the treatment option. Individuals who declined treatment were still eligible to participate in the first fMRI scan.

### 3.1.4 Patient Monitoring and Withdrawal Criteria

Successful antidepressant drug treatment was not discontinued for participants with MDD to be included in the study. Upon entering the study, participants were permitted to remain unmedicated for up to two weeks for the purpose of obtaining the fMRI scan in the unmedicated condition. Although individuals with dMDD who expressed serious suicidal ideation at entry were excluded, participants were

monitored by a health care professional during the two-week time period to monitor suicidal ideation and the potential for worsening of depressive symptoms.

While no suicidal ideation developed for the participants of this study, the plan for participants who became suicidal or developed clinically serious exacerbations of their clinical condition involved exclusion from further participation and an evaluation of the need for more intensive management or inpatient hospitalization either at the National Institutes of Health or under the care of their own physician. Following the post-treatment scan, dMDD participants were referred to treatment trials being offered by the NIMH. Participants who were either ineligible or unwilling to participate in such protocols were referred to a private mental health professional (e.g. psychiatrist). Participants were treated as clinically appropriate and stabilized in the transition between finishing the study and being discharged from the NIMH clinic for a period of up to three months.

During the treatment period of the study, participants were evaluated on a weekly basis for clinical progress, side/adverse effects, compliance, and development of clinical manifestations such as suicidal ideation or hypomania. During the latter part of the treatment period dMDD participants who were psychiatrically stable and clinically improved were evaluated biweekly. Participants were asked to contact the study psychiatrist before discontinuing the sertraline trial. Abrupt discontinuation of sertraline may have resulted in adverse effects such as depressed, anxious, irritable or fluctuating mood, restlessness, fatigue, insomnia, tingling or prickling sensations on the skin, dizziness, or headache. Participants were provided with contact information in case of emergency through which they could receive medical care from a physician at any time.

### 3.1.5  Human Subjects Protection

*3.1.5.1  Consent Process*

All volunteers asked to participate in the study were informed that no immediate personal or medical benefit would be derived from participation.  fMRI was described as a research tool with the potential to benefit individuals with similar illnesses in the future, but at the present time was not helpful for indicating treatment,

diagnosis, or prognosis. Participants were also informed of all potential risks involved in their participation. They were reminded that participation was completely voluntary and they could withdraw from the study at any time. Informed consent was documented using specific forms for each study group, reviewed and approved by the National Institute of Health Combined Neuroscience Institutional Review Board. The research was conducted under protocol 04-M-0002 entitled "The Functional Neuroanatomy of Emotion Regulation in Major Depressive Disorder". Informed consent was obtained by the principal investigator or a designee. Participants were required to read the informed consent form and the investigator additionally described all aspects of the study procedure. The consent documents included a payment schedule for participation in the study. All subjects were compensated for their time commitment for completing the interviews, neuropsychological testing, ratings scales, physical exam, fMRI scans, and follow-up visits during antidepressant treatment.

### 3.1.5.2   *Evaluation of Benefits and Minimization of Participation Risks*

The research study consisted primarily of the performance of non-invasive brain imaging procedures. Participants underwent MR imaging, which involved laying still for up to 90 minutes, but no other risks or discomforts. No known hazards exist for the exposure of magnetic waves during MR imaging. However, there is a potential risk of heart rhythm disturbances in patients with previous heart rhythm abnormalities or with certain types of pacemakers and a substantial risk to persons who have metallic objects inside their bodies.

The potential risks related to MRI were minimized in the following manner: 1) Claustrophobia from MRI, due to having one's head confined in a relatively small space, was reduced by explaining the nature of the MRI scanner in detail to all participants prior to their enrollment in the study. Participants who had a history of significant claustrophobia were not entered into the study and if it occurred, the study was terminated at the subject's request. 2) A possible history of any intraocular, intra-aural, intracranial, or intrathoracic metal excluded the subject from the study. 3) A radiology technologist was present throughout the MRI scan and a physician was available in the vicinity of the MRI facility for medical emergencies. 4) Pregnant women were excluded from the study because of unknown effects on the fetus.

Participant confidentiality was maintained by keeping all clinical records in locked filing cabinets and password-protected computer files. MRI scan files were identified by a number, and associated names were kept in a confidential file accessible to only specified personnel with legitimate scientific interests. Image files that included experimental details were maintained in filing systems accessible only to the physicians and other technical staff working in the MRI laboratory.

In summary, the fMRI, clinical and psychological assessments involved in the study posed no more than minimal risk to participants. The treatment phase of the study with sertraline involved a minor increase over minimal risk due to the delay of treatment by up to two weeks for the depressed individuals described above. However, sertraline had the potential to provide benefit to the depressed participants by providing treatment for major depression. By increasing understanding of pathophysiology, the research study proposed the possibility to improve treatment modalities, diagnostic capabilities, and classification systems and also to aid in de-stigmatizing psychiatric illness and convincing noncompliant persons that medications were likely to be helpful. The importance of the knowledge to be gained from this study clearly exceeded the associated potential risks.

## 3.2   BACKWARD MASKING TASK DESIGN

A novel fMRI task was developed using the technique of backward masking and a slow-event related design to assess neurophysiological responses to emotional face stimuli presented below the level of conscious awareness. In the scanner, participants were shown two neutral target face stimuli at the beginning of each of four 10-minute task runs. They were instructed to remember the target faces for the duration of the run. When the run began, faces appeared on the screen in pairs of two, displaying a masked face for 26 ms immediately followed by an unmasked face for 107 ms to inhibit conscious detection of the first face. Participants were unaware of the masking component of the task. They were asked to use a button box with their right hand to make a decision as quickly as possible about each face that appeared on the screen. If the face they perceived was a target face, they pressed the "1" (top) button. If the face was not a target, they pressed the "2" (bottom) button. The faces displayed neutral, sad or happy expressions and participants judged whether or not the face was a target based on the identity of the original two neutral target faces, not

the depicted emotional expression. Prior to entering the scanner, participants confirmed their understanding of the task by performing an abbreviated version of the task using flash cards.

By design, an emotional face stimulus was presented in the masked position and followed by a neutral stimulus for the sad-neutral (SN), happy-neutral (HN) or neutral-neutral (NN) face pairings. In addition, a neutral face stimulus was presented in the masked position and followed by an emotional face stimulus for the neutral-sad (NS) and neutral-happy (NH) face pairings. A sad or happy face stimulus in the masked position was never also presented in the unmasked position (i.e. the identity of the emotional face was different). Within a single trial, the *identity* of the masked face was never the same as the identity of the unmasked face, but the two face stimuli always depicted the same gender. The gender for all stimulus pairings was balanced across runs. The SN, HN, NS, and NH stimulus pairings were each presented eight times each and the NN pairings were presented 16 times each within a single run in a pseudo-randomized, mixed-trial design. Each run used different target faces and emotional face stimuli from distinct actors. The data from the four runs were combined so that each stimulus pairing was presented a total of 32 times for pairs that included an emotional face and 64 times for pairs including only neutral faces. A 10-13s interstimulus interval was selected to allow the hemodynamic response to return to baseline prior to the next stimulus pair presentation. For participants who performed the task before and after an 8-week treatment interval, unique emotional face stimuli were used for each scanning session.

The backward masking task and behavioral response collection was programmed and controlled via E-Prime on a Monarch Hornet computer with a cathode ray tube monitor at 75 Hz and a cloned projection display that was time-linked to the image acquisition in the scanner. The face images were projected onto a screen in the scanner that was easily visible to subjects in the scanner gantry using a mirror system. Presentation time accuracy for the masked and unmasked face images were verified using a photodiode and oscilloscope. Face stimuli for the backward masking task were obtained from the NimStim Set of Facial Expressions [147] and modified to change the images from color to black and white, to display only the face (excluding clothing and hair) and to align the images at the level of the eyes to maintain consistency between the images.

Figure 1. Example images from the backward masking task design. Neutral face placeholders are shown instead of neutral faces, due to restrictions on the publication of images from the NimStim Set of Facial Expressions.
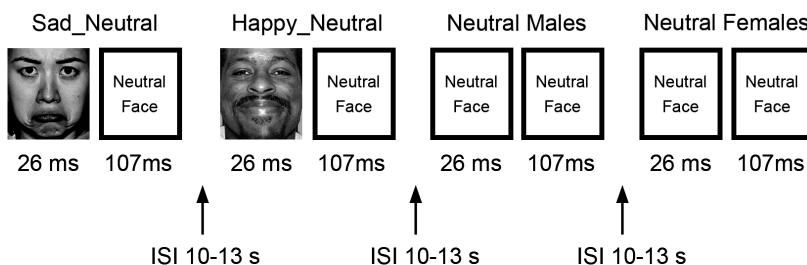


## 3.3 PSYCHIATRIC AND NEUROPSYCHOLOGICAL ASSESSMENTS

Study participants completed a battery of interviewer-based and paper and pencil rating scales for psychiatric and neuropsychological assessment (listed below). Data obtained from the assessments were analyzed using SPSS version 14.0 statistical software. Analysis of variance (ANOVA) and t-tests were used to compare differences in scores (e.g. on the Hamilton Depression Rating Scale) between dMDD, rMDD and HC participants, as well as differences before and after anti-depressant treatment in dMDD participants (or the equivalent time period for HC participants). Additional analyses were conducted to assess whether these scores correlated with changes in the hemodynamic response to emotional stimuli obtained from the functional imaging analyses in individuals with dMDD.

### 3.3.1 Interviewer Assessment Scales

*Structured Clinical Interview for DSM (SCID):* The SCID is a structured interview used to determine major DSM Axis 1 diagnoses in adults [143].

*Family Interview for Genetic Studies (FIGS):* The FIGS is a semi-structured assessment designed to evaluate family history of psychiatric illness [144].

*Hamilton Depression Rating Scale (HAM-D):* The HAM-D is a clinician-administered rating scale designed to assess the severity of depressive symptoms [145].

### 3.3.2  Self-Report Assessment Scales

*Automatic Thoughts Questionnaire (ATQ):* The ATQ is a self-report rating scale designed to measure the frequency of negative thoughts [148].

*Thought Control Questionnaire (TCQ):*  The TCQ is a self-report rating scale designed to assess an individual's ability to control negative thinking [149].

*State-Trait Anxiety Inventory (STAI):* The STAI is a self-report rating scale used to assess state-dependent and trait characteristics of anxiety [150].

*The Inventory of Depressive Symptomatology (IDS):* The IDS is a self-report rating scale used to assess an individual's view of their depression [151].

*Edinburgh Handedness Inventory (EDI):* The EDI is a self-report laterality scale used to estimate the degree of right or left hand dominance during everyday activities [152].

### 3.3.3  Neuropsychological Assessment Scales

*Wechsler Abbreviated Scale of Intelligence (WASI):*  The WASI is a 15 minute 2-subtest index of estimated general intelligence normed for ages 6 – 89 [153].

*Cambridge Neuropsychological Test Automated Battery (CANTAB):*  The CANTAB is a computer-based cognitive assessment system consisting of 22 neuropsychological tests, administered to subjects using a touch screen computer.

### 3.4   BEHAVIORAL DATA ACQUISITION AND ANALYSIS

Participants were debriefed following completion of the fMRI scan and asked about their experience of performing the task in the scanner. Reaction time (RT) and accuracy data for detection of each face stimulus as "target" or "non-target" were recorded via the E-Prime software used to present the backward masking task. Each participant response was classified into one of four categories according to target detection accuracy: correction detection, correct rejection, incorrect detection (false alarm rate) and incorrect rejection. Behavioral accuracy data were analyzed using SPSS version 14.0 statistical software. ANOVA and t-tests were used to explore differences between groups and response categories to target faces presented in the masked position and to explore differences in reaction time to target masked-sad and masked-happy faces.

## 3.5 FUNCTIONAL AND ANATOMICAL MRI DATA ACQUISTION

Images were collected on a General Electric 3.0 Tesla scanner (GE Signa, Milwaukee, Wisconsin) with an 8-channel phased-array head coil. The parameters for the echo planar imaging (EPI) included: continuous axial slices = 39, echo time (TE) = 20ms, repetition time (TR) = 2000 ms, flip angle = 90°, matrix size = 64 x 64, field of view = 22 cm, and voxel dimensions = 3.4 x 3.4 x 3.0 mm$^3$. A total of 290 fMRI images were acquired in each of four 10-minute runs during the backward masking task. Four images were discarded from the beginning of each run to allow for steady-state tissue magnetization. The parameters for the anatomical images included: a fast spoiled gradient echo (FSPGR) sequence, TR = 780 ms, TE = 2.7 ms, flip angle = 12°, FOV = 22 cm, matrix= 224 x 224, 128 axial slices, 1.2 mm thick, in-plane resolution = 0.98 mm$^2$.

## 3.6 FMRI DATA PROCESSING AND ANALYSIS

Functional imaging analyses were performed using the general linear model within the SPM5 statistical imaging software (Wellcome Trust Center for Neuroimaging, London, England). Each participant's whole brain EPI data were realigned, co-registered to the anatomical image, and normalized to fit the Montreal Neurological Institute (MNI) standard brain template and smoothed with a Gaussian filter (8 mm, full-width at half-maximum). Motion artifacts were modeled into the analysis as regressors to correct for movement. An fMRI run was excluded from the analysis if the subject showed movement of more than one-half voxel (1.5 mm) translation or 1.25° rotation. Data from a minimum of three runs were included for each participant. Following the statistical analyses, coordinates were transformed from MNI to the stereotaxic array of Talairach and Tournoux [154]. Anatomical localization was performed using the Mai et al. [155] and Talairach and Tournoux [154] stereotaxic atlases.

### 3.6.1 Paper I

Single-subject t-contrast maps were generated by comparing the difference between conditions (e.g. SN-HN: masked-sad versus masked-happy faces, SN-NN: masked-sad versus masked-neutral faces, and HN-NN: masked-happy versus masked-neutral faces). At the group level, *beta*-weight values obtained from the single-subject

analyses were evaluated for significant differences within an amygdala region-of-interest (ROI) using "small volume correction". The reported results remained significant after applying a false-discovery rate error correction or consisted of a cluster of ≥10 contiguous voxels at a threshold of p<0.05 (uncorrected). An exploratory brain analysis was performed *post-hoc* for regions outside the amygdala and results included regions with a cluster of ≥10 contiguous voxels at a threshold of p<0.001.

In Experiment 1, dMDD and HC participants were compared using two-sample t-tests to evaluate the difference in the hemodynamic response of the amygdala between groups for masked-sad versus masked-happy faces. *Post-hoc* t-tests also evaluated differences in the amygdala response to SN-NN and HN-NN. Finally, the hemodynamic response between dMDD and HC participants was compared for unmasked-sad versus unmasked-happy faces. *Beta*-weights were extracted at the peak voxels within the left and right amygdala region-of-interest.

In Experiment 2, rMDD participants were compared to dMDD and HC participants. A repeated-measures ANOVA was used to analyze hemodynamic differences across conditions (SN, HN, NN) and groups (dMDD, rMDD, HC). Independent t-tests were performed *post-hoc* and *beta*-weights were extracted at the peak voxel within a cluster to characterize specific interaction effects.

In Experiment 3, ten dMDD participants were rescanned after 8 weeks of sertraline treatment and 10 HC subjects were rescanned following the same time interval. Paired t-tests were used to compare the hemodynamic response of the amygdala in dMDD participants before and during treatment for each emotional face condition. A time x group (MDD, HC, pre- vs. post-) ANOVA was conducted to evaluate differences in the response to SN-NN across the treatment interval.

### 3.6.2  Paper II

Single-subject t-contrast maps for HC and MDD were generated by comparing the difference between the emotional conditions (SN, HN, NN) and baseline (crosshair). A 2x3 (group x emotion) ANOVA was performed to analyze *regional* hemodynamic differences across conditions. Within the group x emotion model, t-tests were performed to characterize the specific contrasts that accounted for

the interactions (i.e. SN-HN, SN-NN, and HN-NN). Eigenvariate values were extracted from each significant cluster's peak voxel. The reported results remained significant at a height threshold for the peak voxel in a cluster at $p_{uncorrected} \leq 0.001$ and a minimum cluster size of k=23 voxels, as determined using the AlphaSim program within the Analysis of Functional Neuroimages (AFNI) software. Regions that remained significant after applying this correction were noted in the tables with an asterisk. To maintain continuity with the Paper I results that reported on the amygdala region-of-interest analysis, additional regions were included in the tables that remained significant at $p_{uncorrected} \leq 0.001$ with a minimum cluster size of 10 voxels.

### 3.6.3  Paper III

A paired samples t-test was used to evaluate *regional* hemodynamic response differences between pre-treatment and post-treatment fMRI scans for the SN-HN contrast. The significance threshold was set at $p_{uncorrected} \leq 0.001$ for the peak voxel within each cluster, with a minimum cluster size of 10 voxels, to provide continuity with Papers I and II. To correct for multiple comparisons, a minimum cluster size of k=23 voxels was applied, as determined using the AlphaSim program within AFNI. These regions were noted with an asterisk in the Paper III tables. *Beta*-weights were extracted from the peak voxel within the cluster-level corrected region identified in the SN-HN contrast and compared across conditions.

Additional *post-hoc* t-tests were performed to evaluate regional differences in the hemodynamic response to SN-NN and HN-NN, independently of the SN-HN comparison. The reported results included regions with a minimum cluster size of k=23 voxels in conjunction with a peak voxel t-value of $p_{uncorrected} \leq 0.001$.

Independent t-tests were performed for the SN-HN contrast to evaluate differences between dMDD and HC subjects after the 8-week treatment interval. Results were reported in parallel with the SN-HN contrast described above for only dMDD participants. An amygdala region-of-interest analysis was performed using the "small volume correction" option within SPM5 (p<0.05, minimum k=10 voxels).

# 4 RESULTS AND DISCUSSION

## 4.1 RELATIONSHIP BETWEEN AMYGDALA RESPONSES TO MASKED FACES AND MOOD STATE AND TREATMENT IN MDD (PAPER I)

### 4.1.1 Characteristics of the Study Participants

No significant differences were found between the HC, rMDD and dMDD groups with respect to gender composition, mean age and mean intelligence scores.

Of the 22 dMDD participants, 4 were diagnosed with a chronic, single episode of MDD and 19 with recurrent MDD. 13 had no co-morbid psychiatric diagnoses, while 5 were diagnosed with MDD and social anxiety disorder (SAD), 3 with MDD and panic disorder, 1 with MDD, SAD and dysthymic disorder, and 1 with MDD, post-traumatic stress disorder (PTSD) and simple phobia. 13 participants were naïve to drug treatment. The mean age of onset of depressive symptoms for the dMDD group was $16.7 \pm 6.0$ years.

Of the 16 rMDD participants, 5 were diagnosed with a past history of a single episode of MDD in full remission and 11 with recurrent MDD in full remission. 13 participants had no co-morbid psychiatric diagnoses, while 1 was diagnosed with rMDD and past alcohol abuse, 1 with rMDD, SAD in remission and past alcohol abuse, and 1 with rMDD and PSTD in remission. 3 participants were naïve to drug treatment. The mean age of onset for the rMDD group was $18.3 \pm 4.3$ years.

For the subset of 10 dMDD participants who participated in the sertraline treatment portion of the study, 1 was diagnosed with a chronic, single episode of MDD and 9 with recurrent MDD, 8 had no co-morbid psychiatric diagnoses, 1 was diagnosed with MDD and SAD, and 1 with MDD, PTSD and simple phobia. 4 were treatment naïve. The mean age of onset was $18.6 \pm 5.6$ years.

### 4.1.2 Psychiatric Assessment Scales

For the 22 dMDD, 16 rMDD and 25 HC participants, the mean (SD) HAM-D score was $24.0 \pm 6.3$, $1.0 \pm 1.4$ and $0.0 \pm 0.2$, respectively. dMDD participants scored

greater than HC participants on the HAM-D, ATQ, IDS-SR, STAI, and 3 subscales of the TCQ (all p<0.001). dMDD participants scored greater than rMDD participants on the HAM-D, ATQ, IDS-SR, STAI (all p<0.001), and 3 subscales of the TCQ (all p<0.05). rMDD participants scored higher than HC participants on the HAM-D (p<0.005), ATQ (p<0.005), IDS-SR (p<0.05), and STAI-Trait scale (p<0.001).

For the subset of 10 dMDD participants who underwent treatment, dMDD participants significantly decreased their scores on the HAM-D from the pre-treatment (24.8 ± 5.8) to the post-treatment condition (6.4 ± 6.0) (p<0.001). Additionally, scores decreased in the dMDD-post vs. dMDD-pre condition on the ATQ, IDS-SR, STAI-Trait scale (all p<0.001) and STAI-State scale, and 2 subscales of the TCQ (all p<0.05). The mean HAM-D score was 0.0 (0.0) for the HC participants in both the pre- and post-treatment conditions.

### 4.1.3  Antidepressant Pharmacotherapy Efficacy

Nine of the 10 dMDD participants reached the clinical definition of a treatment responder by the end of the eight-week period (HAM-D score reduction of $\geq 50\%$). Seven out of 10 entered full remission from their symptoms during treatment (HAM-D score $\leq 7$). Despite the significantly decreased HAM-D scores, the dMDD-post scores remained higher than rMDD participants on the HAM-D and IDS-SR (p<0.01) and ATQ, STAI and 2 subscales of the TCQ (all p<0.05).

### 4.1.4  Behavioral Results

*4.1.4.1  Reaction Time*

A significant difference in reaction time was revealed between HC, rMDD and dMDD participants for sad faces in the masked face position (p<0.001). *Post-hoc* tests showed rMDD participants responded faster to masked-sad faces than both dMDDs (p<0.05) and HCs (p<0.001). Consistent with and in support of the neuroimaging results shown in the amygdala (see section 4.1.5), dMDD participants responded faster to target masked-sad faces than HCs (p<0.05) and HCs responded faster to target masked-happy faces compared to target masked-sad faces (p<0.001).

*4.1.4.2   Task Efficacy*

Participants were debriefed upon completion of the fMRI task and questioned about their subjective experience in the scanner. No participant reported conscious awareness of the backward masking procedure (i.e. they did not endorse seeing 2 faces) during the fMRI scan. An objective measure of each participant's awareness involved an examination of the accuracy data obtained during the backward masking task. Participants did not differ in the accuracy of their response to a target face in the masked position (24.4 % correct detection)  versus when no target face was presented in either face position (25.4% incorrect detection or false-alarm) (p=0.88).   These results provide evidence that the experimental task was successful at inhibiting conscious perception of the masked face stimuli.

## 4.1.5   fMRI Results

The following results highlight significant hemodynamic response differences within an amygdala region-of-interest analysis to masked-sad vs. masked-happy faces (SN-HN), masked-sad vs. masked-neutral faces (SN-NN) and masked-happy vs. masked-neutral (HN-NN) faces between dMDD, rMDD and HC participants, as well as the effect of sertraline treatment on the amygdala response to emotional face stimuli.

In Experiment 1, the left and right amygdala hemodynamic response to masked-sad vs. masked-happy faces (SN-HN) was significantly greater in dMDD vs. HC participants ($t_{45}$=3.00, p<0.005 and $t_{45}$=2.80, p<0.005, respectively) and remained significant following correction for multiple comparisons (p<0.05, bilaterally) (Figure 2a). Figures 2b-d illustrate the magnitude of the difference between groups for SN-HN and corresponding differences in SN-NN and HN-NN at the peak voxel coordinate for the SN-HN comparison. Specifically, the bilateral amygdala response to SN-HN and SN-NN was greater in dMDD participants than HCs (p<0.005). In contrast, the left amygdala response to HN-NN was greater in HCs than dMDDs (p<0.01), replicating previous findings that report an increased amygdala response to masked-happy faces relative to either fixation or neutral faces [113,114] An exploratory whole brain analysis for SN-HN showed a greater response in the hippocampus ($t_{45}$=3.91, p<0.001) in dMDDs than HCs and in contrast, a greater response in the thalamus in HCs than dMDDs ($t_{45}$=3.52, p<0.005). An additional whole brain analysis revealed no significant

difference between groups in the amygdala for unmasked-sad vs. unmasked-happy faces (NS-NH).

Figure 2. (a) Activation map illustrating a significant difference between dMDD and HC participants to masked-sad faces vs. masked-happy faces (SN-HN). Arrows refer to the left amygdala (x= -26, -7, -16) and right amygdala (x= 26, -1, -12). (b-d) *Beta-weight* contrasts for the peak voxel coordinates in the SN-HN comparison.
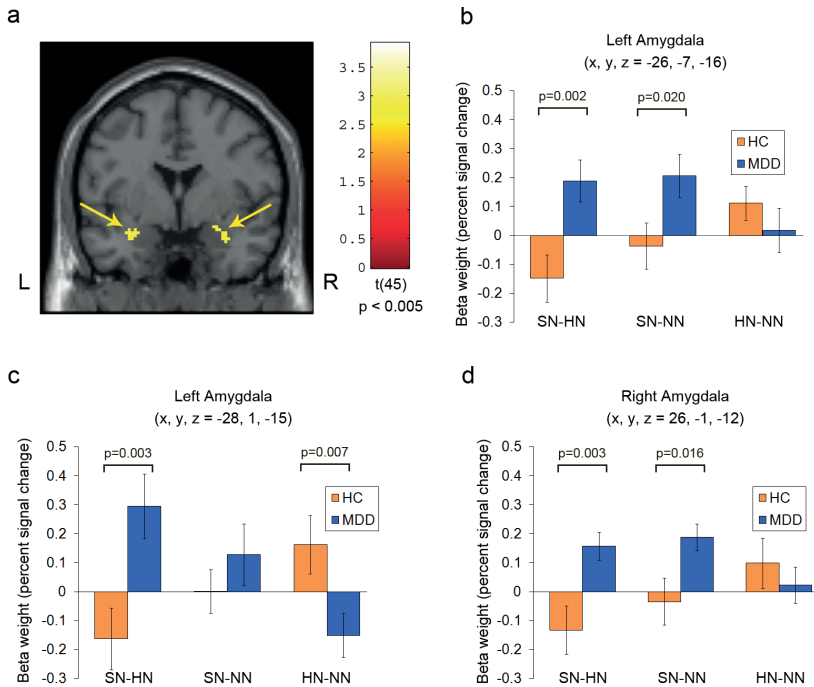


These amygdala findings are consistent with evidence that the amygdala is selectively tuned toward the detection of biologically salient information [52]. The amygdala receives projections from both the sensory cortices that facilitate explicit perception and subcortical cortical structures that allow for rapid non-conscious assessment of stimulus characteristics [53,60]. This rapid response system can detect information that is novel, threatening, rewarding or socially significant in the amygdala, including faces. Our results show that non-conscious presentations of emotional information show differential amygdala response biases dependent upon the social significance of the stimuli and current mood-state (i.e. either healthy or currently depressed). The lack of an amygdala response difference in unmasked faces suggests

that presenting emotional faces below the level of conscious awareness may be advantageous in the identification of processing biases in the amygdala. This method may provide an enhanced sensitivity for identifying differences in response patterns between groups to emotionally salient information.

The main behavioral correlation analysis revealed a significant inverse relationship between depression severity (HAM-D score) and the hemodynamic response of the amygdala, such that the response to HN-NN decreased as depression severity increased (r=-0.45; p<0.05; Figure 3). This result provides evidence that the amygdala response to non-consciously presented stimuli can be influenced by the severity of the current mood-state. In addition, the amygdala response showed a significant inverse correlation with reaction time, such that the hemodynamic response to SN-HN increased as reaction time to target masked-sad faces decreased (r=-0.53; p<0.05).

Figure 3. The correlation between depression severity and the right amygdala response to masked-happy versus masked-neutral faces in dMDD participants (HN-NN).
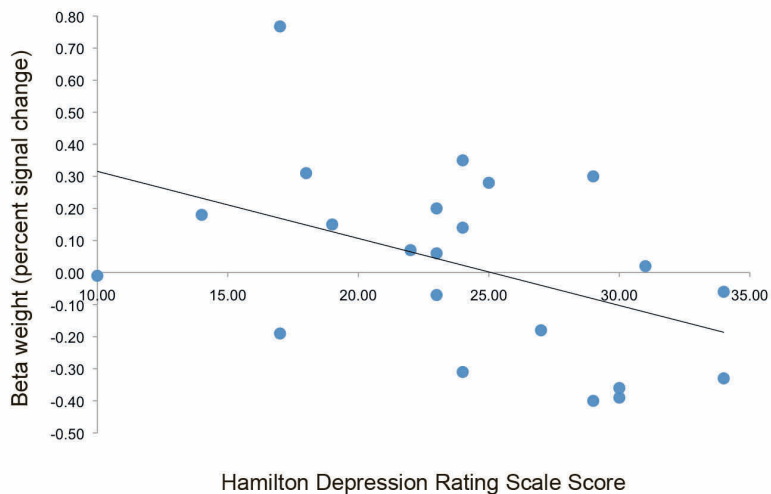


In Experiment 2, an ANOVA revealed a significant condition (SN, HN, NN) by group (dMDD, rMDD, HC) interaction in the left amygdala (Figure 4a). The left amygdala hemodynamic response to masked-sad vs. masked-happy faces was greater in the dMDD and rMDD participants compared to HC participants (Figure 4b). There

were no differences shown between dMDD and rMDD participants. These results suggest evidence of a negative emotional processing bias that persists independently of the current mood-state in major depressive disorder. This "illness-congruent" finding for individuals in remission may suggest a potential biomarker of relapse vulnerability and likelihood for the recurrence of clinically significant depressive symptoms. Interestingly, rMDD participants showed increased trait anxiety and automatic negative thought patterns compared to HC participants, symptoms that appear endophenotypic in the development of MDD [156].

Figure 4. (a) Activation map illustrating a significant difference between groups by emotion in the left amygdala. Arrow refers to the left amygdala (x= -26, y= -5, z= -16). (b) *Beta*-weight contrasts for the peak voxel in the SN-HN comparison.
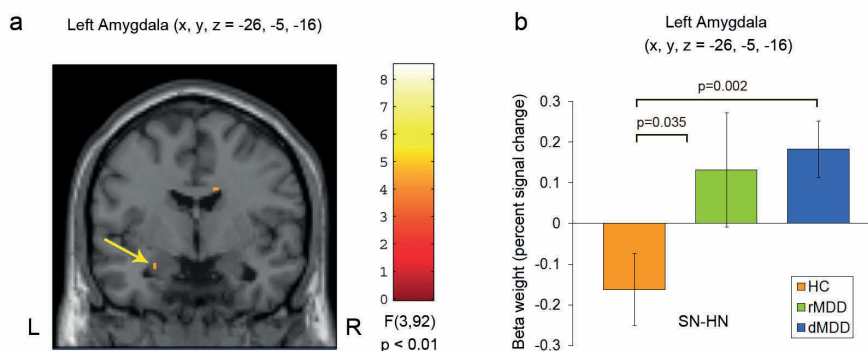


In Experiment 3, dMDD participants showed a significant decrease in the right amygdala hemodynamic response to masked-sad versus masked-neutral faces ($t_9$=3.26; $p<0.01$) following antidepressant treatment (Figure 5a) with a concomitant significant increase in the left amygdala response to masked-happy faces versus masked-neutral faces ($t_9$=2.59; $p<0.05$) (Figure 5b). Across the treatment interval, an ANOVA revealed a significant reduction in the amygdala response to SN-NN in dMDD participants ($p<0.05$), but no reduction in the HC participants ($p=0.17$).

Previous studies have shown a reduction in the hemodynamic response to unmasked sad [99] or fearful faces during treatment [129,157]. However, this study was the first to show, to our knowledge, that non-conscious processing biases were affected by antidepressant treatment in a reciprocal pattern, such that a decreased response to

masked-sad faces was combined with an increased response to masked-happy faces. These results provide support for the hypothesis that a primary therapeutic mechanism of antidepressant treatment may involve the normalization of negative processing biases [131,133,134].

Of note, 9 out of 10 of the treatment study participants were considered treatment responders. However, an exaggerated response to masked-sad faces has been reported in persistently depressed patients receiving antidepressant medication [88]. This finding suggests that the reduction of negative processing biases in the amygdala may depend on treatment effectiveness and may provide support for the maintenance of antidepressant treatment, considering the increased response to masked-sad faces reported in the thesis work for MDD patients in remission.

Figure 5. Activation maps and coordinating *beta*-weight contrasts at the peak voxel within each cluster illustrating a signifcant difference in dMDD participants before vs. after eight weeks of antidepressant treatment in the (a) right amygdala for masked-sad versus masked-neutral faces and (b) left amygdala for masked-happy versus masked-netural faces.
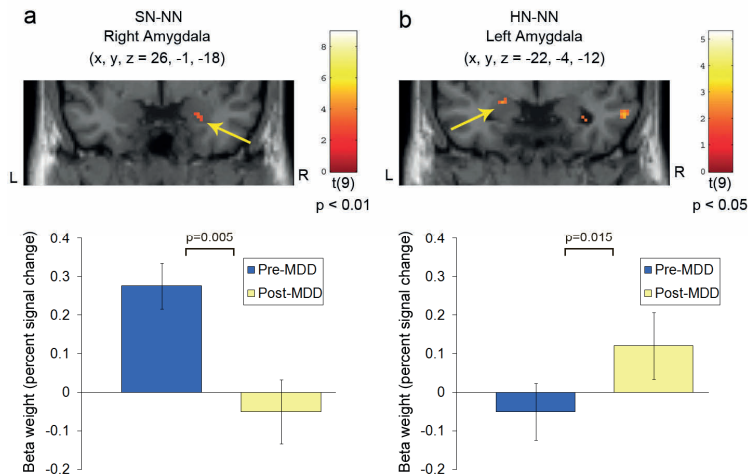
## 4.2 THE EXTENDED FUNCTIONAL NEUROANATOMY OF EMOTIONAL PROCESSING BIASES FOR MASKED FACES IN MDD (PAPER II)

### 4.2.1 fMRI Results

A whole-brain group (dMDD, HC) x emotion (SN, HN, NN) ANOVA revealed a significant interaction in the left anterior insula ($F_{2,90}$=9.63, p<0.001), left rostral superior temporal gyrus (rSTG; $F_{2,90}$=7.62, p=0.001) and left hippocampus ($F_{2,90}$=7.34, p=0.001). Within this model, t-tests provided an evaluation of the specific contrasts that accounted for the interactions.

For the SN-HN contrast, the hemodynamic response was greater in dMDD versus HC participants in the left hippocampus ($t_{90}$=3.74, p<0.001, Figure 6a), left amygdala ($t_{90}$=3.20, p=0.001) and right amygdala/anterior inferotemporal cortex ($t_{90}$=3.28, p<0.001). For the SN-NN contrast, the hemodynamic response was greater in dMDD versus HC participants in the left and right rSTG ($t_{90}$=3.84, p<0.001 and $t_{90}$=3.19, p=0.001, Figure 6b) and right anterior orbitofrontal cortex (OFC; $t_{90}$=3.28, p=0.001, Figure 6c). The hemodynamic response was greater in HC versus dMDD participants in the left inferior parietal cortex ($t_{90}$=3.52, p<0.001) and right frontal polar cortex ($t_{90}$=3.43, p<0.001). For the HN-NN contrast, the hemodynamic response was greater in dMDD versus HC participants in the left anterior OFC ($t_{90}$=3.49, p<0.001), left anterior insula ($t_{90}$=4.42, p<0.001), left pregenual anterior cingulate cortex ($t_{90}$=3.35, p<0.001), left and right rSTG ($t_{90}$=3.29, p=0.001 and $t_{90}$=3.28, p=0.001), right ventral thalamus ($t_{90}$=3.27, p=0.001) and right postcentral gyrus ($t_{90}$=3.18, p=0.001). The hemodynamic response was greater in the HC versus dMDD participants in the left middle occipital gyrus ($t_{90}$=3.43, p<0.001).

Finally, *post-hoc* analyses of the relationship between the neuroimaging results and clinical assessment scores revealed an inverse correlation between the hemodynamic response to HN-NN and depression severity in the right rSTG and left anterior insula. In dMDD participants, the response to masked-happy faces decreased as depression severity (HAM-D score) increased (r=-0.43, p<0.05 and r=-0.51, p<0.05, respectively).

Figure 6. Activation maps and coordinating eigenvariate contrasts at the peak voxel within each cluster illustrating a significant difference between dMDD and HC participants in the (a) left hippocampus for masked-sad versus masked-happy faces (b) left rostral superior temporal gyrus for masked-sad versus masked-neutral faces and (c) right anterior orbitofrontal cortex for masked-sad versus masked-neutral faces.
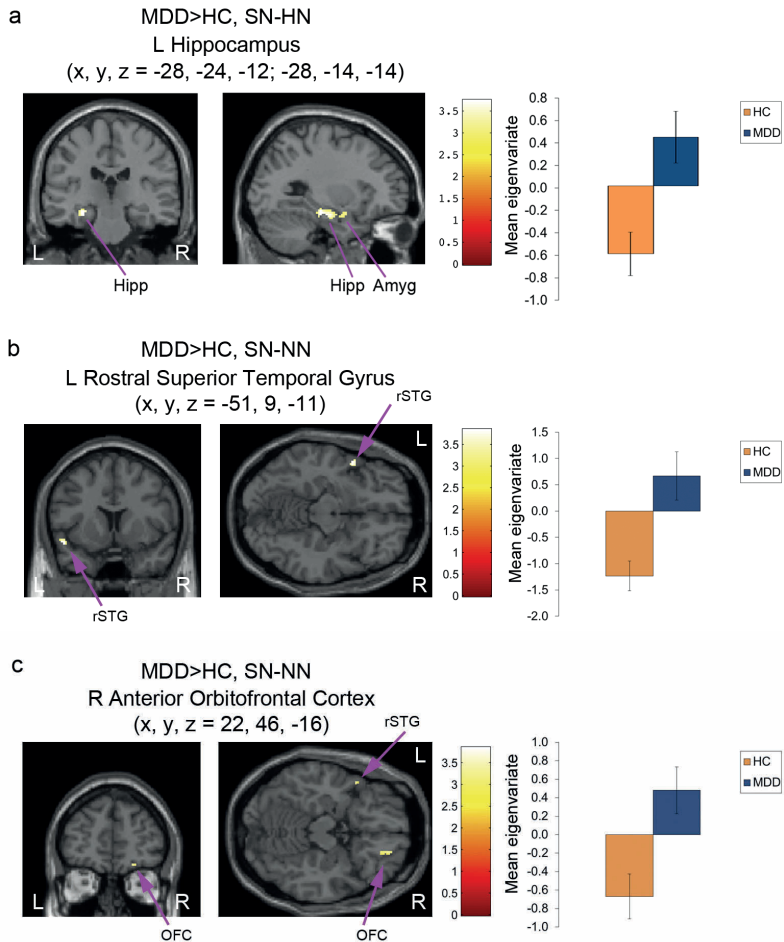


The whole brain group analysis of SN-HN confirmed our original region-of-interest findings in the amygdala from Paper I, although the peak coordinates were slightly different. The hippocampus was also implicated in a separate whole brain

analysis in Paper I. Projections from the hippocampus to the amygdala convey information about environmental context during emotional processing and lesions of the hippocampus interrupt this neurotransmission [158,159]. The hippocampus may be important in setting the context for the negative emotional processing biases shown in depression, driving the amygdala to differentially respond to sad versus happy stimuli.

The group differences in the amygdala, hippocampus, superior temporal gyrus and right anterior orbitofrontal cortex showed both an increased hemodynamic response to masked-sad faces vs. masked-happy or masked-neutral faces in depressed individuals combined with a decreased hemodynamic response to masked-sad faces in healthy controls (Figure 6a-c). Projections from these regions to the amygdala convey information regarding context, sensory integration and stimulus salience to modulate behavioral, emotional and visceral responses [8,12,159,160]. In contrast, the superior temporal gyrus, left anterior orbitofrontal cortex, insula, pregenual anterior cingulate cortex, thalamus and postcentral gyrus showed an increased hemodynamic response to masked–happy faces in dMDD combined with a decreased hemodynamic response in the healthy controls. This pattern is opposite to that shown in the amygdala. Several of these regions form part of the visceromotor network, which has been shown to influence the outflow of amygdala response to emotional information and tune cortical responses to sensory stimuli [8,12,77].

In combination, these findings provide evidence of a network of structures known to participate with the amygdala in the evaluation of the emotional salience of a stimulus. In addition, the shared neural circuitry between MDD and HC individuals suggests these areas may be differentially processing stimuli dependent upon the emotional valence. This network of projections integrates limbic and sensory input and may function to maintain an automatic positive processing bias in healthy individuals and a negative processing bias in depressed individuals.

## 4.3   CHANGES IN THE NEURAL CORRELATES OF IMPLICIT EMOTIONAL FACE PROCESSING DURING ANTIDEPRESSANT TREATMENT IN MDD (PAPER III)

### 4.3.1   Behavioral Results

#### 4.3.1.1   Reaction Time

No differences in reaction time to masked-sad versus masked-happy target faces were revealed between HC and dMDD in the pre-treatment fMRI scan or in either group between scans.

#### 4.3.1.2   Task Efficacy

Participants were debriefed upon completion of the fMRI task after both the pre- and post-treatment scans and questioned about their subjective experience in the scanner. No participant reported conscious awareness of the backward masking procedure. Response accuracy data obtained during the backward masking task revealed no difference in the accuracy of a participant's response to a target face in the masked position (22.8% correct detection) versus when no target face was presented in either face position (22.5% incorrect detection or false-alarm) (p=0.83).

### 4.3.2   fMRI Results

Paired t-tests revealed the hemodynamic response to masked-sad versus masked-happy faces (SN-HN) was greater in the baseline condition compared to the post-treatment condition in the right pregenual anterior cingulate cortex ($t_9$=7.79, p<0.001, Figure 7), left caudal superior temporal gyrus ($t_9$=6.37, p<0.001) and left anterior inferotemporal gyrus ($t_9$=5.17, p<0.001). *Post-hoc* t-tests in the pregenual anterior cingulate region showed the hemodynamic response to SN-NN was greater in the pre-treatment versus post-treatment condition (p<0.001, Figure 7). In contrast, the hemodynamic response to HN-NN was greater in the post-treatment versus pre-treatment condition (p<0.05, Figure 7).

Numerous studies have reported findings that the pregenual anterior cingulate cortex response changes following antidepressant treatment (e.g. [122,161-163]), however

our results extend these findings to non-consciously presented emotional stimuli. Specifically, we showed that the decrease in response to masked-sad versus masked-happy faces was attributable to both a reduction in the response to masked-sad faces and an enhancement in the response to masked-happy faces (Figure 7).

Figure 7. Activation map and coordinating *beta*-weight contrasts at the peak voxel within the pregenual anterior cingulate cortex for the SN-HN contrast, illustrating a significant difference between the dMDD pre- versus post-treatment conditions.



Right Pregenual Anterior Cingulate Cortex
(x, y, z = 14, 42, -5)
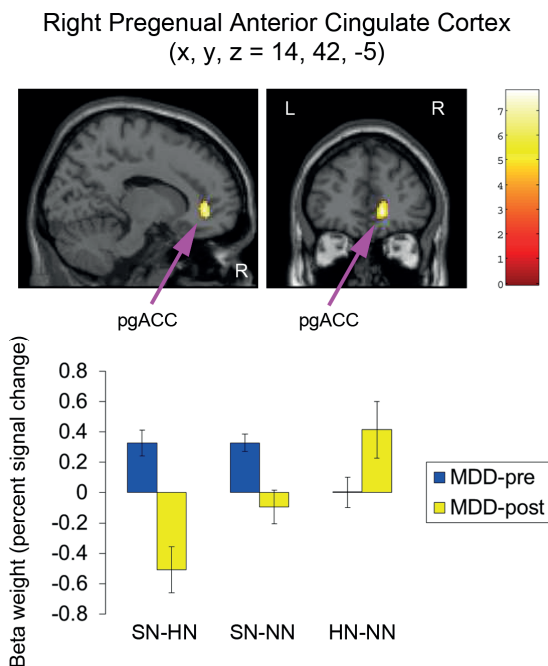
Exploratory *post-hoc* analyses for the SN-NN contrast before versus after the antidepressant treatment period revealed a greater hemodynamic response in the pre-treatment condition in several regions of the sensory and visceromotor networks. Regions of the visceromotor network share extensive reciprocal connections with the amygdala [8,12]. Some of the regions within these networks include the pregenual anterior cingulate ($t_9$=8.95, p<0.001), bilateral medial thalamus ($t_9$=4.64, p=0.001 and $t_9$=4.29, p=0.001), anterior and posterior insula ($t_9$=4.88, p<0.001 and $t_9$=5.46, p<0.001), ventrolateral prefrontal cortex ($t_9$=4.21, p<0.001), and medial and lateral orbitofrontal cortex ($t_9$=5.06, p<0.001 and $t_9$=4.75, p=0.001). Many of these regions participate in the

evaluation of the salience of emotional stimuli and modulation of our experiences and behavior [12].

Independent t-tests of the dMDD participants across the treatment interval and HC participants across the same time interval for the SN-HN contrast revealed a greater hemodynamic response in the dMDD participants relative to controls in the pre- versus post-treatment condition in the pregenual anterior cingulate cortex ($t_{18}$=3.98, p<0.001), posterior cingulate cortex ($t_{18}$=3.72, p=0.001), temporopolar cortex ($t_{18}$=3.65, p=0.001) and amygdala ($t_{18}$=2.95, p<0.005). In contrast, dMDD participants showed a greater hemodynamic response compared to HC participants in the post- versus pre-treatment condition in the lateral frontal polar cortex ($t_{18}$=4.65, p<0.001).

In conclusion, antidepressant treatment with sertraline was associated with differential hemodynamic responses to non-conscious emotional stimuli in a network of regions of that share extensive anatomical connections with the amygdala. Of particular interest, these changes reflect a shift in the pattern of hemodynamic activity in the pregenual anterior cingulate cortex and areas of the orbital and medial prefrontal network shown prior to treatment. These results were in parallel with our amygdala ROI findings observed in Paper I, in which the response to masked-sad faces decreased following treatment. In contrast, the opposite pattern was observed for masked-sad faces following treatment in the lateral frontal polar cortex, a region shown to modulate depression severity (reviewed in [3]) and found to show an increased response in HC individuals toward masked-sad faces in Paper II. These findings provide further evidence to support the hypothesis that a primary therapeutic mechanism of antidepressant treatment may involve an alteration in negative processing biases toward the positive direction in major depressive disorder [164,165].

# 5  SUMMARY AND CONCLUSIONS

The studies presented in this thesis investigated the role of functional neuroanatomical correlates of mood-congruent processing biases toward negative information associated with major depressive disorder and differences in neural response patterns under antidepressant treatment. We developed a novel backward masking technique to evaluate the differential processing of non-conscious emotional stimuli in patients with current major depressive disorder, patients with major depressive disorder in full remission and healthy volunteers.

In Study I, our results showed behavioral and neurophysiological evidence that negative emotional processing biases occur automatically, below the level of conscious awareness, in patients with major depressive disorder and that these biases persist independently of mood-state in the amygdala. Both unmedicated currently depressed and remitted depressed individuals showed an increased hemodynamic response to masked-sad versus masked-happy faces. As well, depressed individuals responded faster than healthy volunteers to masked-sad faces presented outside conscious awareness. In contrast, healthy volunteers showed an increased hemodynamic response to masked-happy faces versus masked-neutral faces in the amygdala. The negative emotional processing bias shown in depressed individuals resolved while a positive bias developed during selective serotonin reuptake inhibitor antidepressant treatment.

In Study II, our results identified several cortical and subcortical regions, in addition to the amygdala, that showed automatic negative mood-congruent processing biases in unmedicated currently depressed individuals versus healthy controls. An increased hemodynamic response during exposure to masked-sad faces versus masked-happy or masked-neutral faces was demonstrated in such regions as the hippocampus, inferotemporal cortex, superior temporal gyrus, orbitofrontal cortex, thalamus and pregenual anterior cingulate cortex, regions known to have substantial connections with the amygdala.

In Study III, our results expanded on the antidepressant treatment findings from Study I and identified several regions of the medial and orbital prefrontal cortical networks associated with the amygdala that showed differential processing of negative

emotional information under antidepressant treatment. Most notably, the pregenual anterior cingulate response to masked-sad versus masked-happy faces decreased following antidepressant treatment. Similar to our amygdala findings, a shift in the pattern of results was observed such that the hemodynamic response decreased to masked-sad faces while the hemodynamic response increased to masked-happy faces.

These studies showed for the first time in unmedicated individuals with dMDD that mood-congruent processing biases present in the amygdala toward emotional stimuli presented *below the level of conscious awareness* involve a resolution of the negative emotional processing bias and the development of a normative positive processing bias under antidepressant treatment.

Taken together, the presence of non-conscious mood-congruent processing biases in the amygdala and network of regions associated with amygdala may function to influence the salience of social stimuli and contribute to the maintenance of dysfunction in social perceptions and interactions associated with major depressive disorder. These studies provide important information regarding the underlying neurocircuitry associated with automatic processing biases and implicate several regions influenced by antidepressant treatment. This knowledge of the neural processes associated with the emotional dysregulation in major depressive disorder may hold the potential to direct future therapeutic approaches for the treatment of mood disorders with abnormalities in emotional processing.

# 6  FUTURE PERSPECTIVES

## 6.1  FUTURE DIRECTIONS

Additional studies are needed to explore the neural mechanisms that function to produce changes in the processing of emotional stimuli and to assess whether these changes may provide a sensitive and specific biomarker of antidepressant treatment response and vulnerability to relapse. Developmental studies are needed to determine if the processing biases extant in MDD may represent a potential endophenotype and to explore the relationship of this finding with the emergence, maintenance and recurrence of major depressive episodes throughout the lifespan. These studies would also assist in establishing whether the present findings are mood-state dependent or instead reflect trait-like biases that precede or persist across mood episodes. Furthermore, additional studies are needed to address limitations of the current studies by expanding the paradigm to include other mood disorders, such as bipolar disorder, or to anxiety disorders that often occur co-morbidly with depression, such as social anxiety disorder, as well as to assess the effects of other antidepressant drug classes (e.g. dual-reuptake inhibitors). The relatively small number of participants included in the treatment portion of the study may also be expanded, allowing for explorations into possible gender differences in emotional processing biases and an evaluation of the processing biases shown in individuals who do and do not respond to antidepressant treatment.

## 6.2  BACKWARD MASKING TASK DESIGN MODIFICATION

The original version of the backward masking task included a slow event-related design in order to allow the hemodynamic response to return to baseline and reduce overlap of the neural response between conditions. While the design showed robust responses to emotional stimuli, it did not include an explicitly modeled baseline measure. The backward masking task has recently been modified and updated to a rapid event-related design to allow for improved modeling of an explicit baseline measure. The new design also permits more trials to be completed in a single session and less time in the scanner, thereby reducing the likelihood of motion artifact. The new design has been piloted and extended with promising findings at the Laureate Institute for Brain Research on a state of the art GE Discovery MR750 scanner and coil

system that allows for twice the signal-to-noise ratio of the scanner used to acquire the original results.

## 6.3 INTERNAL FUNDING SUPPORT

The following ongoing study protocols have been funded by the Warren Foundation at the Laureate Institute for Brain Research involving extensions of the thesis work using the backward masking task paradigm (Principal Investigator: Wayne Drevets, M.D., Co-Investigators: Teresa Victor, Jonathan Savitz, Ph.D., Julie Frost-Bellgowan, M.S.):

1. Functional neuroanatomical networks underlying emotional processing biases to affective stimuli in mood and anxiety disorders

    The aims of this research are to 1) investigate the pathophysiology of mood and anxiety disorders including major depressive disorder, bipolar disorder and social anxiety disorder, 2) establish the neural networks involved in emotional information processing to conscious and non-conscious stimuli, 3) determine specific endophenotypes related to emotional processing in individuals at risk for developing major depressive disorder, 4) determine the effect of genetics, immune system and endocrine system dysfunction on emotional processing of facial stimuli, 5) differentiate trait-like versus mood-state dependent neural response abnormalities, and 6) identify effective antidepressant treatments directed at neuroanatomical regions involved in mood and anxiety disorders

2. Neurophysiological correlates of emotion processing in mood disorders during development and aging

    The purpose of this study is to investigate the functional neuroanatomical networks underlying emotional processing biases across the lifespan beginning in childhood in individuals with a primary mood disorder (major depressive disorder or bipolar disorder), healthy controls and high risk controls with a first-degree relative with a mood disorder or personal history of a childhood anxiety disorder. The study aims to: 1) establish the neural networks involved in emotional information processing and 2) determine specific endophenotypes of mood disorders by assessing differences among "at risk" individuals who do, and do not, develop mood disorders. These studies

may help to inform new methods for early detection, intervention and possibly prevention of mood disorders.

## 6.4   EXTERNAL FUNDING SUPPORT

The work performed for this thesis has resulted (in whole or in part) in several successful grant applications awarded to investigators at the Laureate Institute for Brain Research. Each of these grants includes the backward masking task paradigm as the functional neuroimaging component of the study:

1.  Oklahoma Center for the Advancement of Science and Technology (OCAST) Oklahoma Health Research Program: Principal Investigator- Wayne Drevets, M.D., Lead Investigator- Teresa Victor. The goal of this grant proposal is to extend the findings obtained in this thesis work and establish whether the neurophysiological correlates of the emotional processing biases may provide a biomarker to sensitively and specifically predict the response to antidepressant treatment using SSRIs in MDD. The results hold the potential to identify individuals who will be more likely to improve and respond to SSRIs prior to treatment and facilitate the development of more effective antidepressant pharmacotherapies by improving the power of clinical trials.

2.  Brain and Behavior Research Foundation (formerly NARSAD, the National Alliance for Research on Schizophrenia and Depression): Principal Investigator- Jonathan Savitz, Ph.D. The goal of this grant was to evaluate whether the negative emotional processing bias shown in these studies using the backward masking paradigm is an illness-dependent trait or endophenotype that predisposes individuals to develop major depressive disorder. A "high-risk" group of healthy individuals with first-degree relatives with MDD was compared to a currently depressed MDD group and a group of healthy controls with no first-degree relatives with psychiatric illness.

3.  National Institute of Mental Health RO1: Dimensional Approaches to Research Classification in Psychiatric Disorders Award. Principal Investigator- Wayne Drevets, M.D., Co-Investigator- Teresa Victor. The goal of this grant is to elucidate the relationship between anhedonia, inflammation, gene expression

and the function of positive valence systems to establish a pathophysiology-based phenotype within mood disorders. In addition, the grant seeks to develop a biomarker signature composed of multiple measures that sensitively and specifically classify individuals with mood disorders and predicts treatment outcomes.

4. National Institute of Mental Health Parent K01: Mentored Research Scientist Development Award. Principal Investigator- Jonathan Savitz, Ph.D. The goal of this grant is to identify the biological pathways through which inflammation affects brain structure and function in major depressive disorder and through the knowledge of these molecular targets guide the development of next-generation antidepressant medications.

# 7  ACKNOWLEDGEMENTS

This thesis project was a labor of love for the scientific process and the quest to contribute to the many unanswered questions regarding the application of neuroscience research to mental illness. I would like to express my sincere appreciation and gratitude to everyone who has supported and encouraged me throughout this research endeavor. In particular, I would like to thank:

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Jessica Gordon, Earle Bain and Linda Mah, for sharing cubicle space with me in the basement of Building 1 during "the early years". Thank you for your friendship and willingness to accept a new graduate student into the lab with open arms.
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