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Characterizing Depression Neurobiology through Treatment Outcomes

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Characterizing Depression Neurobiology through Treatment Outcomes

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Abstract

Characterizing Depression Neurobiology using Treatment Outcomes
By Callie L. McGrath

Neurobiological variability is a critical issue in studies of depression pathophysiology with profound implications for treatment selection and outcome. We hypothesize that pretreatment brain metabolism can distinguish patients remitting to psychotherapy and antidepressant medication, categorizing them into metabolism based subtypes. We approach neurobiological variability through this lens of treatment response with three different imaging approaches: (1) resting-state FDG-PET to determine treatment subtypes, (2) multivariate analysis of FDG-PET to relate subtypes to behavior and (3) resting-state fMRI to determine network connectivity differences between subtypes. We identify two clinically relevant subgroups: insula activity greater than whole brain mean (remission to escitalopram/nonresponse to CBT) and insula activity less than whole brain mean (remission to CBT/nonresponse to escitalopram) and show that these insula metabolism based subgroups differently impact network dynamics. Anterior insula variance is not the only neurobiological variance that should be incorporated into models of depression neurobiology. We examine metabolic predictors of non-response to multiple treatments and explore the network dynamics of metabolic outcome predictors. Taken together, these studies define distinct depression subgroups at a network level with direct implications for antidepressant treatment selection in individual patients. Finally, we refine models of depression neurobiology by incorporating variability related to treatment outcomes, contributing to a model with potential for direct clinical linkages.

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CHAPTER 1: INTRODUCTION

Preamble

This dissertation aims to advance and refine neurobiological models of depression by evaluating neurobiological variability driven by differences in treatment outcome-based patient subgroups. Available neurobiological models vary, but most include some combination of regional abnormalities in major depression, anatomical connections between emotion, attention, cognition and motor systems, or changes in brain activity in response to depression treatments. All published neurobiological models incorporate findings from univariate comparisons of brain activity and/or structure in depressed patients compared with controls, and some models incorporate interactions between regions, but no published neurobiological models directly address possible biological subgroups. This exclusion of subgroups means models don't accommodate the "consistent inconsistencies" seen throughout depression research. While identifying biomarkers to guide treatment selection for individual patients and improving circuit models have not been strongly linked previously, they are complementary research goals. By dividing patients into subgroups based on their response to standard first-line antidepressant therapies, we can test if such groups contribute meaningful variability at the brain level by identifying brain regions that differ between clinically relevant groups. This both improves our understanding of depression neurobiology and directly incorporates the biological heterogeneity seen in depression into a neurocircuitry model with clear potential to impact clinical decision making.

This introduction will discuss depression's broad impact, known heterogeneity in depression, current brain-derived neural network models of depression, previous attempts to accommodate illness heterogeneity using symptoms, and how neurobiological differences between patients responding to psychotherapy or antidepressant medication provide an opportunity to both incorporate additional potential sources of biological heterogeneity into neural circuitry models of depression and improve their clinical usefulness.

Depression's Broad Impact

Major depressive disorder (MDD) negatively impacts both individuals and society. The twelve-month incidence of mood disorders in the United States approximates 6 percent with lifetime incidence approaching 15 percent (Birnbaum et al., 2010; Judd et al., 2000; Kessler et al., 2003). Suicide risk in depression is a key concern. Appraisals of the lifetime risk of suicide related to an affective disorder range from 6 to 15% (Guze & Robins, 1970; Rihmer, 2001). Depression debilitates people, ranking as a leading cause of years lived with disability (Murray & Lopez, 2013; World Health Organization, 2004). Large losses of productivity and treatment expense make depression fiscally costly at both the individual and societal levels. Each year, the direct and indirect medical and non-medical costs associated with depression in the US are estimated to exceed $80 billion (Greenberg et al., 2003).

Heterogeneity in Depression

Major depression is a neuropsychiatric disorder characterized by disruptions in mood, cognitive, homeostatic, and motor functions. Persistent mood disruption

is a key clinical diagnostic criterion of major depression, but the nature of the mood disruption and the disruption of other systems varies between patients.

Diagnosis and Clinical Heterogeneity

Depression etiology is unknown and therefore diagnosis of a major depressive episode is based on patient report and clinical evaluation; there are no biology-based tests for diagnosis. In the US, MDD is diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria. The studies reported herein use diagnostic criteria based on the DSM-IV-TR (American Psychiatric Association, 2000)[1]. Diagnosis of a major depressive episode occurs when at least five of the following symptoms have been present during the same 2-week period, representing a change from previous functioning (American Psychiatric Association, 2000)[2].

1. Depressed mood most of the day, nearly every day, as indicated either by subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful)
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated either by subjective account or observation made by others)

---

1 The DSM-V was released on May 18, 2013, superseding the DSM-IV-TR, however patient enrollment was completed before the release of the DSM-V, using the diagnostic guidelines in the DSM-IV-TR. Changes from the DSM-IV-TR to DSM-5 (American Psychiatric Association, 2013a) are relatively minor, limited to format, mixed episode definitions and bereavement (American Psychiatric Association, 2013b).

[2] Additional criteria include: symptoms do not meet criteria for a mixed episode; symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning; symptoms are not due to the direct physiological effects of a substance or general medical condition; or symptoms are not better accounted for by bereavement.

3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day
4. Insomnia or hypersomnia nearly every day
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
6. Fatigue or loss of energy nearly every day
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or specific plan for committing suicide

Because diagnosing a major depressive episode depends on meeting 5 of the 9 criteria (with all patients endorsing either item 1 or 2) the clinical manifestation of a major depressive episode varies, both between patients and even between episodes in the same patient (Oquendo et al., 2004; Young, Fogg, Scheftner, & Fawcett, 1990). Based on the DSM criteria, there are 227 possible symptom constellations (Harald & Gordon, 2012). Consider two patients: Patient A endorses depressed mood, weight loss, psychomotor agitation, fatigue, and worthlessness. Patient B endorses a markedly diminished pleasure in all

activities, psychomotor retardation, diminished ability to concentrate, suicidal ideation, and hypersomnia. Both patients meet criteria for a major depressive episode, but their experience of the illness diverges. Mood disruption is a common component of depression diagnosis across patients, but disruptions of motor, cognitive, and homeostatic functions can vary widely. Given this range of symptoms without the context of a clearly defined biology, it is understandable, if not expected, that studies investigating depression often show inconsistent results.

Vulnerability, Resilience and Neurobiological Variability

Variance in neurobiological findings echoes the clinical heterogeneity seen in major depression. Differences in methods and patient recruitment may account for some variance in findings, however, little evidence supports depression criteria as defining a distinct biology or grouping similar biological conditions into one disorder (Antonijevic, 2006, 2008; Halbreich, 2006; V. Krishnan & Nestler, 2010). More likely, variance reported in neuroimaging findings reflects this lack of a unifying biology- at least some of the variance is likely driven by true differences between patients. The mechanisms by which the brain adapts to its environment can vary, and depression may represent maladaptive processes in the attempt to maintain homeostasis. Emotions, including negative emotions, like low mood and anxiety can have utility in responding to environmental circumstances (Nesse, 1998). While low mood can be useful, when it becomes excessive, prolonged or expressed in inappropriate situations, it transitions from potentially adaptive to pathological (Nesse, 2000).

The time course of depression onset is gradual. While clinicians draw boundaries for practical use, it is not always clear when normal sadness transitions into a major depressive episode (Wakefield & Schmitz, 2013). Diagnosis requires a minimum of 2 weeks of symptoms. This suggests that the true onset of depression is unknown. A stressor often precipitates a depressive episode (Hammen, 2005; Kendler et al., 1995; Kendler, Thornton, & Gardner, 2000; Kessler, 1997). Potentially, depression may develop after a stressful event as mechanisms responding to that stressful event fail (Mcewen, 1998). The brain's ability to adapt to changing circumstances has been described in many ways- behavioral flexibility (Reynolds & Zahm, 2005), degeneracy (Price & Friston, 2002), allostasis (Karatsoreos & McEwen, 2011). Of these concepts, allostasis is the most directly applied to neuropsychological disorders. Allostasis describes the active process of adaptation to potential threats to an organism's survival and changes in their environment in order to maintain homeostasis and promote survival (Peters & McEwen, 2012). Changes in the environment can be framed as stressors, and how an individual's brain adapts to stressors is relevant to whether they are vulnerable or resilient to depression. Allostatic systems can be either overworked, fail to shut off after the stressful event is over, or fail to adequately respond to a stress, over taxing other systems (Mcewen, 1998). Effects of stress on the brain are measurable in both human and animal models. Stress alters the neural circuitry underlying cognition, decision making, anxiety and mood resulting in increased or decreased expression of those behaviors/behavioral states (McEwen, Eiland, Hunter, & Miller, 2012). Both adaptive and maladaptive cellular changes in response to stress occur in the hippocampus, prefrontal cortex

and amygdala (McEwen, 2005; Radley et al., 2008; Shansky, Hamo, Hof, McEwen, & Morrison, 2009). Recent studies show functional implications of such stress-induced structural remodeling. In rats, chronic variable stress decreases dendritic spine density, regresses dendritic morphology in the medial prefrontal cortex, and decreases the number of excitatory synapses in the anterior portion of the bed nuclei of the stria terminalis. These structural changes reduce limbic forebrain inhibition of the hypothalamus-pituitary-adrenal (HPA) axis under acute stress, effectively increasing the stress response (Radley, Anderson, Hamilton, Alcock, & Romig-Martin, 2013). Brain activity changes in response to stress are also measurable in humans. Chronic forms of stress, particularly childhood maltreatment, have been linked with volume decreases, principally in the hippocampus but also in the corpus callosum, insula, orbitofrontal cortex, anterior cingulate gyrus, and caudate (Dannlowski et al., 2012; Frodl, Reinhold, Koutsouleris, Reiser, & Meisenzahl, 2010; Teicher et al., 2004; Vythilingam et al., 2002). Functional changes in response to more acute stressors have also been identified. In depressed patients, recent life stressors alter function in the orbital frontal cortex, ventrolateral prefrontal cortex, subgenual cingulate and nucleus accumbens during negative word processing (Hsu, Langenecker, Kennedy, Zubieta, & Heitzeg, 2010).

Many factors influence how the brain responds to stressors and impact whether depression develops. Such vulnerability/resilience factors include genetic vulnerabilities, personality, developmental insults and both acute and chronic environmental stressors. Personality is one vulnerability/resilience factor that influences network activity. Personality trait measures correlate with brain

activity during emotion and cognition tasks (Canli et al., 2001; Keightley et al., 2003; Moresco et al., 2002). Compared with non-depressed healthy controls, depressed individuals display increases in neuroticism, negative emotionality, negative affectivity, harm avoidance, self-criticism, dependency, and perfectionism, as well as reduced extraversion, positive emotionality, positive affectivity, novelty seeking and conscientiousness (Bagby, Psych, Quilty, & Ryder, 2008). Models of personality differ on the nature of the relationship between personality and depression. Models have suggested that personality traits predispose a person to depression (Vulnerability Model), depression interacts with how personality is expressed (Pathoplasty Model), depression leads to personality changes (Complication/Scar Model), depression represents increases along an already present dimension (Spectrum Model), or a shared etiological factor gives rise to personality features and depression (Common Cause Model) (Bagby et al., 2008).

Neuroticism is one dimension of personality that shows a strong relationship with depression and underlying genetic risk. Genetic factors related to MDD influence neurobiology, both directly and resulting from interactions with environmental factors. For example, carriers of the short allele of the serotonin transporter gene (5-HTTLPR) show reduced gray matter volume in the rostral cingulate and amygdala, regions import for negative emotion processing. Further, short 5-HTTLPR allele carriers show a functional decoupling of cingulate and amygdala activity (Pezawas et al., 2005). The short allele conveys an increased risk for depression but is also associated with neuroticism and has neurobiological impact in healthy populations (Homberg & Lesch, 2011).

Variance in Neuroimaging Studies

To accommodate heterogeneity in MDD symptoms, vulnerability/resilience factors and timing of onset, we can frame the regional dysfunction seen in untreated depression as a mixed state of etiological abnormalities and compensatory mechanisms that play both adaptive and maladaptive roles (Mayberg, 2003). Potentially evolution of new symptoms may result from further failed adaptation attempts. Variability in depression (both clinical and neurobiological) is likely related to this combination of different vulnerability/resilience factors and adaptation/maladaptation to the environment over time.

Given the many possible combinations of risk/resilience factors combined with an individual's behavioral flexibility in response to their environment, it is unsurprising that neuroimaging studies show variability when comparing depressed patients with healthy controls. Dorsolateral prefrontal cortex activity exemplifies a well-replicated finding that shows equally well-replicated variability. Studies comparing unipolar MDD patients with healthy controls consistently identify differences in dorsolateral prefrontal cortex activity (specifically BA9/BA46). A meta-analysis of dorsolateral prefrontal cortex findings shows inconsistencies in the laterality and the direction of findings (Fitzgerald et al., 2006). The most common finding is a left-sided decrease, with right-sided and bilateral decreases, as well as increased activity also reported. Other brain regions identified by patient versus control comparisons also vary in reproducibility, including ventral lateral prefrontal cortex, orbital frontal cortex, medial frontal cortex, anterior cingulate, posterior cingulate, amygdala, anterior

hippocampus, anterior temporal lobe, superior temporal gyrus, insula, basal ganglia, caudate, lingual gyrus and thalamus (Fitzgerald, Laird, Maller, & Daskalakis, 2008; Mayberg, 2003; Pandya, Altinay, Malone, & Anand, 2012). This variance in differences across individual regions has led to the development of neural models of depression. Neural network models integrate widely distributed brain regions to map the coordinated activity of brain regions as a system (Holtzheimer & Mayberg, 2009). Because emphasis is placed on how regions function together rather than how a particular region functions in isolation, network models are better able to accommodate variability in findings. A successful model will accommodate variability while maintaining the most simplistic schema possible.

Development of Neural Models of Depression

Despite inconsistencies in neuroimaging results, much progress has been made in developing neural circuit models of MDD. Different objectives produce different models. Models have been based on regional abnormalities, both structural and functional, anatomical circuits, experimental work, conceptual frameworks and combinations of these features.

Evolution from Individual Regions to Systems

Prior to multi-region or systems model development, early depression work focused on regional abnormalities. Lesions caused by stroke and other neurological damage in the prefrontal cortex and striatal regions result in major depression (Starkstein & Robinson, 1996). When comparing depressed patients with healthy controls, both patients who have a neurological disorder with co-morbid depression and patients diagnosed with depression as a primary mood

disorder show functional abnormalities in frontal and limbic regions (Mayberg, 1994). Curiously, lesions to limbic regions that show functional or volumetric abnormalities, such as the cingulate, amygdala and hippocampus do not result in primary depression (LeDoux, 2000). Potentially, functional abnormalities (particularly in regions where lesions do not result in mood disorders) represent a failed compensation for a primary problem, but what that driver or primary process/problem might be is unknown. Depression may result from a "lesion" and failed adaptation or failure to return to homeostasis. Models may be improved by assuming a primary process as foundation for depression, and heterogeneity of symptoms resulting from compensation to that primary process. The inability of individual lesions to explain development of depression led researchers to integrate individual abnormalities into a broader systems perspective. It is generally accepted that depression does not result from dysfunction of a single brain region (or neurotransmitter system). Rather, depression is conceptualized as a systems-level disorder that affects distinct pathways that are functionally integrated. Accordingly, contemporary MDD neural models have shifted from focusing on individual structural or individual functional anomalies to instead emphasize dysfunctional interactions between multiple regions. Most depression models include similar regions, with the general consensus emphasizing the importance of mood regulation and the interaction between cognitive and limbic regions.

Systems Neurocircuitry Models

Systems neurocircuitry models can be subdivided into models with an anatomical basic, models with a conceptual basis, and data-derived models.

Models with an Anatomical Basis

Early definitions of depression neuroanatomy used models of cortical-striatal-pallidal-thalamic function. The cortical-striatal-pallidal-thalamic circuit as described by Alexander et al. in 1986 (Alexander, DeLong, & Strick, 1986) proposes a functional segregation of parallel circuits connecting the basal ganglia and cortex. Many models find a basis in this work, using it as a framework to incorporate abnormalities. Understanding of the complexity of this system has grown over time. Cross talk between systems is now recognized. Different striatal regions interface via an ascending spiral between regions, providing an anatomical mechanism to integrate limbic, cognitive and motor information in the midbrain (Haber, Fudge, & McFarland, 2000).

Models with a Conceptual Basis

Phillips et al (2003a, 2003b) provide a conceptual framework of emotional regulation, which can be extended to psychiatric disorders. Phillips et al.'s emotional regulation is centered on three stages: identification and appraisal of a stimulus, producing an affective state in response to a stimulus, and regulating the produced affective state (Phillips, Drevets, Rauch, & Lane, 2003a). Phillips et al. divide key neural structures hypothesized to underlie these three stages of emotion processing into two systems. A primarily ventral system consisting of the amygdala, insula, ventrolateral prefrontal cortex, orbitofrontal cortex, ventral anterior cingulate, thalamus, ventral striatum and brainstem nuclei and a primarily dorsal system comprised of the dorsolateral prefrontal cortex, dorsomedial prefrontal cortex, dorsal cingulate and hippocampus. The ventral system identifies emotional significance, produces affective state, and regulates

autonomic responses while the dorsal system is important for executive function, including selective attention, planning and effortful regulation of the resulting affective states. Potentially a reciprocal relationship occurs between these two systems. Labeling a stimulus as emotive and the resulting production of an affective state may depend on activity levels in these ventral and dorsal systems. Extending this conceptual model of emotion perception to incorporate dysfunction in major depression (Phillips, Drevets, Rauch, & Lane, 2003b), Phillips et al suggest that volume reductions in the ventral system combined with increased activity in these regions may result in a restricted emotional range, biased by the amygdala towards the perception of negative emotions. In the dorsal system, structural and functional impairments associated with executive function and effortful regulation of emotional behavior may exacerbate this issue of ventral stream biasing, resulting in depressed mood and anhedonia. Notably lacking from this model of emotion perception is a link to many of the motor and somatic symptom clusters that is commonly present in major depression.

Data-derived models

Limbic-cortical dysregulation

Experimentally-based models, such as Mayberg's limbic-cortical dysregulation model, incorporate studies of brain function and interaction in the context of an anatomical framework. The limbic-cortical dysregulation model of depression is based on experimental evidence of brain activity changes during normal sadness and depression abnormalities that resolve with treatment (Mayberg, 1997). During the induction of normal sadness, brain activity increases in limbic structures and decreases in cortical structures. Similarly to this 'sadness' pattern,

depressed patients show corresponding sustained increases in limbic activity and decreases in cortical activity (Mayberg et al., 1999a). A more recent version of this model segregates regions with demonstrated functional or structural abnormalities by their proposed network function (Mayberg, 2003). Regions are segregated by system, including mood state, attention, gating, and autonomic functions. Further, based on studies of changes with treatment, regions that may be targeted by different treatment types are incorporated. For example, patients who respond to antidepressant mediation show changes in limbic regions that are associated with autonomic/circadian type functions.

Seminowicz et al (2004) examined clinical variability related to the limbic cortical dysregulation model by using path analysis on a compressed version of the limbic-cortical dysregulation model. A representative 7-region model consisting of lateral prefrontal cortex, anterior thalamus, anterior cingulate, subgenual cingulate, orbital frontal cortex, hippocampus, and medial frontal cortex was tested and found to be stable across three groups of depressed patients from different study sites. Path differences differentiated treatment outcome groups. Limbic-cortical connections between lateral prefrontal cortex, subgenual cingulate, orbital frontal cortex and hippocampus differentiated drug between treatment responders and non-responders. The path connections between the hippocampus and lateral prefrontal cortex as well as path connections between the orbital frontal cortex and medial frontal path differentiated between CBT and pharmacotherapy responders (Seminowicz et al., 2004). Path analysis supported this 7-node version of the limbic-cortical dysregulation model and showed that baseline activity varies between clinically

defined treatment outcome groups. Our approach will aim to expand the full limbic-cortical dysregulation model more directly.

Other groups also support dysfunction of this limbic-cortical circuit, although they may reframe it slightly. For example, Anand's prefrontal-amygdalar-pallidostriatal-mediothalamic mood regulating circuit (Anand et al., 2005a, 2005b) involves changes in the same limbic and cortical regions.

Meta-analytic models

A recent meta-analysis of PET and fMRI findings presents a model of depression that incorporates the salience of negative information (J. P. Hamilton et al., 2012). In this model, over activity in the pulvinar nucleus of the thalamus primes anatomically connected regions important to the salience network, particularly the amygdala, dorsal anterior cingulate, and insula for a potentiated response to negative stimuli. Compounding this, nigrostriatal relays fail to propagate information to the dorsal striatum (specifically the caudate) and dorsolateral prefrontal cortex, limiting the ability of these regions to reduce the impact of negative stimuli. Hamilton et al posit overactive pulvinar as primary in major depression and suggest a system of a reaction. What their framework lacks is the idea that different adaptive states may occur in response to such over activity, and these adaptive states may be just as key to depression as a primary insult. Most data incorporated in this meta-analysis is derived from fMRI studies of depression. Beyond this meta-analysis, fMRI measured dysfunction in resting state networks provide another framework for building depression models.

Resting-state BOLD fMRI Network Studies

Neural circuitry frameworks incorporating resting state networks have focused on 3 data-derived regional 'networks' representing areas with consistent covariance patterns in subjects at rest. These 'networks' - default mode, salience and executive –are named based on task patterns activating these regions (J. P. Hamilton, Chen, & Gotlib, 2013). The hypothesized function of each of these networks can be linked with aspects of depression symptomology. The default mode typically plays a role in normal self-referential processing (van Buuren, Gladwin, Zandbelt, Kahn, & Vink, 2010), but in depression this network is suspected to play a role in rumination, which can be conceptualized as aberrant self-directed processing. During self-reflective processing of negative stimuli, MDD patients fail to deactivate parts of the default mode network that are "turned down" during such tasks in healthy controls (Sheline et al., 2009). These data support the hypothesis that the default mode network fails to disengage in depressed patients, leading to rumination. During ruminative self-focus in depressed patients, default network structures show increased activation (Cooney, Joormann, Eugène, Dennis, & Gotlib, 2010).

The executive network, normally responsibly for cognitive control (Seeley et al., 2007), is suspected to play a role in emotional disinhibition in depression. Interest in the executive network is driven by evidence of the role of the dorsolateral prefrontal cortex (DLPFC) in depression. DLPFC regions that are abnormal during the resting state and regions that respond to negative stimuli are not overlapping, but both regions are components of the executive network (J. P. Hamilton et al., 2013).

The salience network, normally responsible for the identification of salient information, likely has a role in emotional over-activity in depression. Individual components of the salience network, including the amygdala, anterior insula, and dorsal anterior cingulate, have all been indicated as dysfunctional either at rest or during processing of negative stimuli (J. P. Hamilton et al., 2013).

Studies are starting to integrate data across functional networks. A study by Sheline et al (Sheline, Price, Yan, & Mintun, 2010) shows increased connectivity across default mode, salience and executive networks in the dorsal medial prefrontal cortex (termed the dorsal nexus) in depressed patients. Depressed patients also show a greater dominance of default mode network activity relative to task positive network activity that is associated with increased rumination (J. P. Hamilton et al., 2011). The anterior insula, a key feature of the salience network, appears to be responsible for switching between default mode and task positive networks.

Most depression neural circuitry models attempt to provide a framework that links reported abnormalities in brain activity and structure with abnormal systems. There is overlap of the involved regions across models but how relationships between regions are incorporated and what regions are featured prominently vary. Each model takes a different approach and whether the approach is biased towards anatomy, functional interactions, network dynamics, or conceptual frameworks, no current models directly incorporate heterogeneity in pretreatment biology.

Neuroimaging Variability Related to Treatment Outcomes

Some individual experiments do directly exploit depression's inherent neurobiological heterogeneity. Studies correlating treatment outcome with brain activity directly assess variability, although these analyses are not usually framed from the perspective of understanding variability. Instead, such studies are framed as identifying treatment predictors. Underlying a region's ability to predict differential outcomes is a difference in brain activity between patients. While individual studies incorporate variability, heterogeneous findings from individual experiments are generally left out of neurocircuitry models. Therefore, the next step is to directly incorporate heterogeneity into models, ideally in a clinically meaningful way. Many studies have shown that brain activity varies between patients in ways that can be linked to treatment outcomes. By focusing on variance related to treatment outcomes to refine neurocircuitry models, the goal of a more refined model that is clinically relevant can be accomplished. Toward this goal, we will divide patients into subtypes by linking treatment specific outcomes with neuroimaging data. This approach to patient subgrouping can benefit from the long history of subtyping attempts in major depression. Previous studies have tried to link clinical, behavioral, and/or demographic data to stable subtypes, but biological support has been limited for such divisions and so far there has been no successful linkage between depression subtypes and clinical outcomes. The next section will review previous approaches for subtyping and outline how treatment-outcome based subtyping may improve neural circuitry models in a clinically relevant way.

Strategies for Subgrouping MDD Patients

Subgrouping has been guided by the premise that dividing patients into distinct groups should decrease heterogeneity of findings; however there is no consensus on the best criteria for defining depression subtypes. Approaches for dividing MDD patients into subtypes vary. Researchers have considered strategies ranging from behavior to illness characteristics with varied success, particularly when considering supporting biological evidence. Recent approaches for dividing patients into subgroups can be categorized by five types: symptoms, etiology, time of onset, gender-based, and treatment resistance (Harald & Gordon, 2012), however none of the proposed groupings have consistently demonstrated differential biology of cause and treatment. Some subtypes have biological correlates while others lack supporting biological evidence. Symptom and etiology based-frameworks have the strongest links with biological measures and were an intuitive first approach to understanding heterogeneity.

Symptom-based Frameworks

Subtypes based on clinical syndromes have the longest history as well as the strongest links to biology. Currently, symptom subtypes (syndromes) are subdivided into melancholia, psychotic depression, atypical depression, and anxious depression. Symptom based divisions have historically been the most accessible and are therefore the most prevalent. One commonly attempted division that has been reiterated by many approaches is some version of endogenous versus reactive depression (Akiskal, 1995). While such divisions haven't been helpful for diagnosis, there is some face validity. In modern

depression terminology, the endogenous vs. reactive subtype is best approximated by melancholic vs. atypical depressive subtypes.

Melancholic depression has the most support as its own distinct entity from major depression, with approximately 20 percent of patients classified as having depression with melancholia (Day & Williams, 2012). Melancholia is characterized by blunted emotional response, non-reactive mood, pervasive anhedonia, psychomotor disturbances (retardation or agitation), vegetative and cognitive signs of interrupted sleep (early insomnia, early awakening), loss of appetite, reduced libido, diurnal variation, and impaired concentration and working memory (Parker et al., 2010; M. A. Taylor & Fink, 2008). Biological correlates of melancholia include hypercortisolemia, psychomotor disturbance, and specific sleep patterns, including measurable disturbances in rapid eye movements (REM) (Antonijevic, 2008; Armitage, 2007; Buyukdura, McClintock, & Croarkin, 2011; Dinan & Scott, 2005; Gold & Chrousos, 2002; Leventhal & Rehm, 2005; Parker et al., 2010; M. A. Taylor & Fink, 2008). Whether melancholia is a distinct neurobiological construct is unknown, but some neuroimaging abnormalities have been reported (Day & Williams, 2012).

Atypical depression is often poised opposite of melancholic depression. Atypical depression is characterized by mood reactivity with the additional criteria of two or more of the following: weight gain/increased appetite, hypersomnia, leaden paralysis, or long-standing vulnerability to rejection. Atypical depression is estimated to occur in 15–20 percent of depressed patients.

Psychotic depression is major depression marked by delusions or hallucinations. In additional to psychotic features, over-valued feelings of worthlessness and

guilt, severe psychomotor disturbances, and deficits in attention, psychomotor speed, executive function and memory have been associated with psychotic depression. Biological correlates of psychotic depression share similarities with melancholic depression, both present with hypercortisolemia and reduced REM latency. Additionally, patients with psychotic depression show reduced serum dopamine-beta-hydroxylase activity. Studies report enlarged cerebral ventricles and paralimbic abnormalities (Keller, Schatzberg, & Maj, 2007; Rothschild, 2003).

Previous work towards defining melancholic and atypical subgroups set the stage to look for biological markers that discriminate treatment outcome. Symptom-based subgroups presented the first suggestions that different patients may do better or worse on different treatments. Melancholic patients often respond to electroconvulsive therapy (ECT) and atypical patients often respond to monoamine oxidase inhibitors (MAOIs). There is no underlying neurobiological subtyping of atypical versus melancholic patients, and no direct biological indicator of treatment. A better biological link could have been made by directly studying the biology of patients responding and not responding to MAOIs or patients responding and not responding to ECT. Response to treatment itself may be a better starting point for a biological discriminator.

Why Refocus on Treatment-based Subgroups?

Depression can be divided many different ways, but the current approaches to subtyping have not improved clinical outcomes and made minimal impact on neural circuitry models of depression. Most previously explored subgroups show no definitive evidence of a unique biology based subtype that can be linked with

treatment outcome. Dividing patients into subgroups can improve our understanding of depression pathology, but approaches need to balance knowledge of pathology with clinical practicality. One issue with previous studies is that the subtype comes first, and an underlying biology and any relationship to treatment outcomes are investigated separately. This approach has resulted in limited success and no biology linked with both a subtype and treatment outcome. A treatment-based subtyping will allow for direct linkage of biology to treatment outcome. This practical approach will reduce noise and increase signal, balancing both practicality and pathology. Previous studies showing differential brain activity related to different treatments support the potential for the existence of such subtypes. Within the framework of depression as a mixture of etiology, adaptation, and maladaptation, compensatory brain activity may differentiate treatment-specific 'subtypes'.

Different Brain Activity Changes with Different Treatments

Treatment-specific subtypes would not only improve MDD models by incorporating depression heterogeneity, but they would also provide foundation for clinical applications of neural circuitry models. By defining biology of subtypes susceptible to specific treatment types, patients could potentially be assigned a treatment based on their neurobiology, improving clinical outcomes. A clinically relevant first step is to contrast differences between standard initial treatments. Either an evidence-based psychotherapy or an antidepressant medication in the form of a serotonin-selective re-uptake inhibitor (SSRI) are the APA recommended first-line treatments for a major depressive episode (American Psychiatric Association, 2010). Psychotherapies and SSRI's are

equally effective in treating a mild to moderate major depressive episode (DeRubeis et al., 2005; DeRubeis, Gelfand, Tang, & Simons, 1999; Hollon et al., 1992), but both result in remission in less than half of patients. Results from the Nemeroff et al. nefazadone/CBASP study further support equal response rates across antidepressant drug and psychotherapy treated patients, but this study also suggests that psychotherapy may be essential in the treatment of patients with early trauma. In a randomized clinical trial of nefazodone, CBASP (Cognitive Behavioral Analysis System of Psychotherapy) or their combination, patients with a history of early life trauma showed better response with psychotherapy alone than nefazodone alone (Nemeroff et al., 2003). While this study did not suggest a biological correlate and has not been replicated, it is an example of a defined subgroup being better benefitted by a specific class of treatment while on the whole patients generally responded equally to antidepressant mediation and psychotherapy treatment classes.

Standard Treatments and their Mechanisms of Action

Evidence-based psychotherapies and SSRI's have different proposed mechanisms of action (DeRubeis, Siegle, & Hollon, 2008). Evidence suggests CBT and anti-depressants, specifically paroxetine, mediate remission through different neural mechanisms (Goldapple et al., 2004). Patients treated with CBT showed increased hippocampus and dorsal mid-cingulate (BA 24b/c) metabolism and decreased dorsolateral prefrontal cortex (BA 9/46), ventrolateral prefrontal cortex (11/47), superior and inferior medial frontal (9/10/11), posterior cingulate, inferior parietal (BA 40), and inferior temporal cortex (BA 20) metabolism from pre- to post-treatment. These pre-post treatment metabolic changes were

contrasted post-hoc with treatment changes with paroxetine. CBT and paroxetine treatments share a pre-post treatment decrease in metabolism in the ventral prefrontal cortex (BA 47). Changes in dorsal mid-cingulate, ventromedial frontal and posterior cingulate metabolism were unique to CBT treatment. CBT and paroxetine treatments show inverse metabolic changes in dorsolateral prefrontal cortex, inferior parietal cortex, and hippocampus. Changes in subgenual cingulate (BA 25), insula, brainstem, and cerebellum metabolism were unique to paroxetine treatment. These two treatment classes remedy depression through different alterations in brain metabolism, and each is effective for some individuals and not others. Potentially, the necessary metabolic changes produced by these different treatment classes can only be induced in patients with an amenable initial brain state. In addition to the variability seen in activity changes with response to different treatments, pretreatment brain activity is also variable. Such variability is seen is studies relating treatment outcome to pretreatment brain activity.

Single Treatment Outcomes Relate to Variability in Brain Activity

The best replicated brain-based predictor of treatment outcome is activity in the rostral anterior cingulate (Pizzagalli, 2011). Rostral anterior cingulate, specially Brodmann Area 24a/b, was first reported in an inpatient group of unipolar patients as hypometabolic in antidepressant medication non-responders when compared with controls and hypermetabolic in antidepressant medication responders (Mayberg et al., 1997). Over activity in the rostral cingulate as a predictor of response to single pharmacological interventions has been replicated with various antidepressants and various neuroimaging methods (Little et al.,

2005; Milak et al., 2009; Pizzagalli et al., 2001; Saxena et al., 2003). While activity in the rostral cingulate is the best-replicated finding, it is by no means the only finding. Additional regions of the cingulate have been related to outcomes, with high activity in the subcallosal cingulate (Brodmann area 25) linked with treatment non-response (Konarski et al., 2009). Activity in other brain regions has been variably reported as predictive of single treatment outcomes, including mesiotemporal cortex, dorsal prefrontal cortex, ventral prefrontal cortex, anterior cingulate, posterior cingulate, insula, parietal cortex, temporal cortex, occipital cortex, basal ganglia, thalamus, cerebellum, and midbrain (for review, see Milak et al. 2009). A recent meta-analysis of predictive neural biomarkers of clinical response to various treatments for depression identified medial prefrontal cortex and anterior cingulate, particular the pregenual anterior cingulate, as regions where increased activation was associated with positive response to treatment. Increased activation in the putamen extending into the right caudate and the right anterior insula extending into the inferior frontal gyrus was associated with decreased likelihood of response to treatment (Fu, Steiner, & Costafreda, 2012). This line of research has been driven by the specific goal of improving clinical outcomes using measurable structural or functional alterations in the brain, and as such, current approaches have not extensively explored the neuroscience implications of treatment predictors. A more comprehensive approach would both identify regions that predict treatment outcome and inform depression neural circuit models.

Overview of Dissertation Goals:

Despite much progress in MDD research since the advent of neuroimaging, "[w]e still lack a cogent, comprehensive and therapeutically useful model of brain function and dysfunction in this disorder." (J. P. Hamilton et al., 2013). Current models take an integrative network approach. However, such models do not directly account for sources of heterogeneity seen in MDD. This dissertation hopes to refine MDD neurocircuitry models by testing the utility of subgrouping patients using unambiguous recovery to standard treatments. By directly integrating neurobiological variability as up and down brain states into current models, we can improve our understanding of network dynamics in major depression as well as move towards a more clinically relevant network conceptualization. Experiments contained within this dissertation work towards these goals:

- Chapter 2: Defines two brain-based subtypes through treatment outcome to cognitive behavior therapy or escitalopram.
- Chapter 3: Explores anterior insula metabolism's relationship with whole brain network dynamics.
- Chapter 4: Identifies baseline brain metabolism associated with two treatment non-response and explores related network dynamics.
- Chapter 5: Collates experimental evidence in chapters 2 through 4 into expanded neurobiological models of depression.

CHAPTER 2: DEFINING CBT AND ESCITALOPRAM RESPONSE SUBTYPES[3]

Overview of Treatment Prediction Research

Over the past several decades, a number of studies have contributed to the clinical goal of improving treatment outcomes by developing a marker that can guide treatment selection. Many approaches- clinical (Quitkin et al., 1993), immune (Irwin & Miller, 2007), inflammatory (Müller, Myint, & Schwarz, 2011), endocrine (Arana, Baldessarini, & Ornsteen, 1985), genetic (D'Empaire, Guico-Pabia, & Preskorn, 2011; Huezo-Diaz et al., 2009; Ising et al., 2009) and neuroimaging/EEG (Conway et al., 2012; DeBattista et al., 2011; Kennedy et al., 2007; Konarski et al., 2009; Leuchter, Cook, Marangell, et al., 2009; Siegle et al., 2012) - have been applied in pursuit of a treatment outcome predictor.

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) clinical trial is the largest published predictive study to date. Unfortunately, the study was unsuccessful in its primary goal of determining which of several treatments is the most effective "next step" (Gaynes et al., 2009). Demographic and clinical factors were associated with outcome, but not with selection of one treatment over another. STAR*D showed that a simple demographic or clinically based biomarker was unlikely, and further highlighted the poor outcomes to currently

[3] Parts of Chapter 2 are reprinted from JAMA Psychiatry, 70/8, CL McGrath, ME Kelley, PE Holtzheimer III, BW Dunlop, WE Craighead, AR Franco, RC Craddock, HS Mayberg, Toward a Neuroimaging Treatment Selection Biomarker for Major Depressive Disorder, 821-829, Copyright 2013, with permission from the American Medical Association

available treatments. After 4 different treatment strategies were applied, approximately 1/3 of patients did not achieve remission.

For a patient presenting with MDD, an antidepressant medication or evidence-based psychotherapy is currently recommended as first-line treatment (American Psychiatric Association, 2000, 2010; Kennedy, Lam, Parikh, Patten, & Ravindran, 2009; National Collaborating Center Mental Health, 2009). However, fewer than 40% of patients achieve remission with initial treatment (Gaynes et al., 2009; Holtzheimer & Mayberg, 2011), and choosing the "wrong" initial treatment has significant individual and societal costs due to continued distress, risk of suicide, loss of productivity, and wasted resources associated with two to three months of an ineffective treatment (Dunlop et al., 2011; Kessler et al., 2006). Given the public health consequences of inadequately treated depression, a clinical or biological marker to guide initial treatment selection for MDD could have major health and economic impact (Kapur, Phillips, & Insel, 2012).

Neuroimaging Markers to Guide Treatment

In other areas of medicine, identification of markers to guide treatment has significantly improved clinical outcome. For example, in cancer (Saijo, 2012) and heart disease (Welch, Yang, Reeder, & Gersh, 2012), biomarkers are currently used to optimize initial treatment selection as well as guide treatment modifications with disease progression. Despite extensive research, no clinically useful marker to guide treatment selection for depression has emerged. Measures of neural activity have been investigated in attempts to both predict and understand the mechanism underlying treatment outcomes. Previous neuroimaging studies have suggested that pre-treatment brain activity patterns

can predict efficacy, but those studies have generally focused on a particular treatment (Conway et al., 2012; Siegle et al., 2012). For example, the well replicated higher rostral cingulate and/or lower subgenual cingulate activity has been associated with greater improvement with antidepressant medications (Konarski et al., 2009; Mayberg et al., 1997; Pizzagalli et al., 2001), sleep deprivation(Wu et al., 1999) and cingulotomy (Dougherty et al., 2003), but all in studies of a single treatment. High activity in the rostral cingulate remains the best replicated indicator of treatment response (Pizzagalli, 2011), but has not been tested prospectively.

Work using task-based fMRI to predict cognitive behavior therapy response also shows promise (Siegle et al., 2012; Siegle, Carter, & Thase, 2006). Patients benefiting from CBT showed low-sustained reactivity in the subcallosal cingulate and high-sustained reactivity in the amygdala in response to emotional stimuli. Early change studies using EEG indicate whether a patient should continue on current treatment or switch between buproprion and venlafaxine, two antidepressant medications with different mechanisms of action (Cook et al., 2013; Leuchter, Cook, Gilmer, et al., 2009), but this effect requires at least 2 weeks of treatment before predictive change in EEG can be measured. Comparisons of different treatments have thus far identified markers of response and nonresponse, but not pretreatment patterns that differentiate among the treatments tested (Brody et al., 2001; Ketter et al., 1999; Konarski et al., 2009). Further, imaging studies demonstrate that medications and psychotherapy have differential effects on distinct brain regions, (Goldapple et al., 2004; Kennedy et al., 2007) suggesting that baseline activity may dictate response to one treatment

versus the other. Although no prior imaging study has directly assessed the association of pre-treatment brain activity patterns with *differential* response to different treatments (e.g., medication vs. psychotherapy), these past studies strongly suggest that a neuroimaging-based treatment-specific biomarker can be defined.

In the process of developing a marker to guide antidepressant treatment selection, it is important to consider what qualities such a marker should have. Toward this goal, a non-specific biomarker that predicts improvement regardless of treatment is not useful. Rather, a clinically meaningful and treatment-specific biomarker (TSB) should (a) predict an individual's improvement to a specific treatment, and (b) predict *non-response* to an alternative treatment. Such a biomarker can only be identified in a study that assesses outcome to two or more different treatments.

Experiment 1.1: Defining a Treatment-Specific Biomarker (TSB)

In this study, we measured pre-treatment brain glucose metabolism in patients with MDD randomized to receive a selective serotonin reuptake inhibitor (escitalopram [sCIT]) or cognitive behavioral therapy (CBT) (A. Beck, Rush, Shaw, & Emery, 1979; A. T. Beck, 2005). PET scan measurement of glucose metabolism was selected based on its high reliability and availability combined with its established use for studies of baseline scan patterns in depression and effects of various antidepressant treatments (Bartlett et al., 1991; Brody et al., 1999; Drevets et al., 2002; Goldapple et al., 2004; Kennedy et al., 2007; Kimbrell et al., 2002; Konarski et al., 2009; Little et al., 2005; Mayberg et al., 1997; Milak

et al., 2009; Saxena et al., 2003). Our aim was to define an imaging TSB for these two potential first-line treatments; i.e., a brain activity pattern that distinguishes sCIT remitters from both sCIT non responders and CBT remitters while concurrently distinguishing CBT remitters from both CBT non responders and sCIT remitters. Such a pattern both meets criteria for a treatment-specific biomarker, and would provide the opportunity to explore two biological depression-subtypes based on treatment outcomes.

Methods

Patient Selection

Eligible participants were adult outpatients with a primary diagnosis of MDD as assessed by the Structural Clinical Interview for DSM-IV Diagnoses (SCID-I) (First, Spitzer, Miriam, & Williams, 1996) and confirmed through a psychiatric evaluation conducted by a study psychiatrist. Patients aged 18 to 60 were recruited through the Mood and Anxiety Disorders Program at Emory University via advertisements and clinician referrals (Dunlop, Kelley, et al., 2012). Patients were required to have moderate-severe symptoms of depression, defined as a Hamilton Depression Rating Scale (HDRS) (M. Hamilton, 1960) 17-item score ≥18 at screening and ≥15 at the baseline randomization visit. Exclusion criteria included a current diagnosis of a primary psychiatric disorder other than MDD; a medical or neurological condition that could contribute to depression or that might interfere with response to treatment such as chronic pain syndromes and IBS; current suicidal ideation requiring urgent clinical intervention; comorbid substance abuse within the past 3 months; substance dependence within 12 months prior to the screening visit; current or intended pregnancy or

breastfeeding; use of antidepressants within seven days of the screening visit (five weeks for fluoxetine); current psychotherapy at the time of screening; or receipt of electroconvulsive therapy within six months of the screening visit. Patients were also excluded if they had a lifetime history of failure to respond to ≥6 weeks of treatment with escitalopram (≥10 mg/day), or four or more sessions of CBT for depression. Written informed consent was obtained from all participants with the protocol conducted as approved by the Emory Institutional Review Board and as registered at clinicaltrials.gov (NCT00367341).

Treatment Protocol

Treatment consisted of two phases: an acute treatment phase (Phase 1) and a combination treatment phase (Phase 2). Phase 1 data was used in experiment 1.1, Phase 2 data will be introduced in Experiment 1.2. In Phase 1, patients were randomly assigned (1:1 ratio) to receive a 12-week treatment course of either sCIT (flexibly dosed from 10-20 mg/day) or manual-based, depression-focused CBT (16 one-hour sessions over 12 weeks) (Figure 1). Prior to the study's start, the study statistician prepared a permuted block randomization schedule, with the assignments placed in order and sealed in opaque envelopes. Following acquisition of the pre-treatment PET and MRI scans, patients who continued to meet eligibility criteria were randomized to sCIT or CBT. Escitalopram was started at 10 mg/day, and could be increased to 20 mg/day at or after week 3 if the patient had a HDRS > 7 and was tolerating the medication. Down titration to 10 mg/d was permitted if side effects were intolerable at the 20-mg/day dose. CBT sessions were scheduled twice weekly for the first 4 weeks, followed by weekly sessions for the subsequent 8 weeks. Changes in symptom severity were

assessed using the HDRS conducted by raters blinded to treatment group. Ratings were performed weekly for the first 6 weeks and then biweekly until week 12. Patients who did not remit upon completion of their Phase 1 treatment were offered enrollment in Phase 2 comprising an additional 12 weeks of treatment with combination sCIT + CBT.

Outcome Metrics

Clinical outcomes were defined using the HDRS with the target endpoint being remission (REM), defined as a HDRS score ≤7 at both weeks 10 and 12 of Phase 1 treatment (Rush et al., 2011), to ensure stability of remission beyond a single "good week." Non-response (NR) was defined as a ≤30% HDRS change from baseline to Phase 1 endpoint (Nierenberg et al., 2000). Partial responders (change in HDRS >30% but not achieving remission) and dropouts were not included in the analyses for this report in order to avoid potential dilution of either the remission or the non-response groups.

Imaging Acquisition

Prior to treatment randomization, brain glucose metabolism was measured using positron emission tomography (PET) (Siemens HRRT, Nashville, TN), using standard methods without arterial blood sampling (Phelps et al., 1979). For each scan, a 10miC dose of 2-[18F]-fluoro-2-deoxy-D-glucose (FDG) was administered intravenously, with a 20-minute 3-D image acquisition beginning 40 minutes after tracer injection. During uptake, patients remained supine, awake, and resting with eyes closed and ears uncovered. Patients were given no explicit cognitive instructions, but were asked to avoid ruminating on any one topic during the 40- minute FDG uptake period (D'Empaire et al., 2011). Raw emission

images were corrected for injected dose and attenuation (using Cs-137, 6 minute transmission scan), reconstructed, and smoothed to an in-plane resolution of 4.0 mm full width at half maximum. A high-resolution T1-weighted structural magnetic resonance imaging (MRI) scan was separately acquired for spatial normalization procedures and anatomical reference (Siemens Tim Trio 3T whole body scanner, 3D MP-RAGE optimized at TE/TR=5/35, matrix=256×208×196, 1mm isotropic resolution).

Image Preprocessing

Attenuation corrected PET images were co-registered to corresponding T1-weighted MRI anatomical images using a six degrees of freedom linear transform and subsequently written into standard space using a non-linear transform calculated from the T1-weighted image (DARTEL (Ashburner, 2007), SPM8; Welcome Department of Imaging Neuroscience, London, England; http://www.fil.ion.ucl.ac.uk/spm/). Four patients had no anatomical scan and were normalized using a study specific FDG-template. Spatially normalized images were smoothed with an 8mm FWHM Gaussian kernel and corrected for differences in whole brain global mean activity (Kennedy et al., 2007). Relative glucose metabolic rates were used for all analyses.

Image Analysis

A two-way analysis of variance (2x2 ANOVA) with treatment (sCIT, CBT) and outcome (REM, NR) was performed to identify a putative sCIT-CBT remission TSB using the baseline pretreatment FDG PET scans (analyses performed with AFNI and SPSS; statistical threshold $p < .001$ uncorrected, and a minimum cluster volume of 100 voxels, 0.34mL). With this approach, a main effect of

remission would identify brain regions associated with remission to treatment independent of randomization group; i.e., a non-specific biomarker. The treatment X outcome interaction would identify brain regions where the CBT treatment effect (REM, NR) was distinguished from the sCIT treatment effect (REM, NR). Average normalized glucose metabolism values were extracted from clusters identified by the ANOVA (mean cluster activity) for further analysis. *Post-hoc* analyses of the extracted regions from the ANOVA interaction were used to refine selection of a potential TSB pattern by examining the effect sizes (ES) of the group differences for each region. We defined a region as a true TSB if it differentiated both the REM-NR differences (by treatment) and the sCIT-CBT differences (by outcome); thus, there were four comparisons of interest to consider when evaluating each ROI as a stratification tool for treatment recommendation. Given the limited sample size, we report these comparisons using ES, rather than statistical significance, in order to quantify their actual potential use as an eventual TSB. The two-group effect size can be interpreted as the difference in metabolic activity between specified groups in units of standard deviation (Cohen, 1988). Because each region had a different magnitude of glucose metabolic activity and variation, each individual value was standardized using a z-score with regional z-score means plotted to illustrate the nature of the regional interaction effects. As these data are already standardized to the level of variation, there are no “error bars” in the related graphs.

To further assess the generalizability of findings identified in this restricted analysis to the full sample of study completers, metabolic activity was correlated with percentage change in HDRS within each treatment group to determine if the

putative biomarkers identified in the ANOVA showed the predicted general pattern in the full cohort of phase 1 treatment completers.

Results

Clinical Effects

Eighty-two patients were randomized to treatment; however two patients received a change in their psychiatric diagnosis during the trial, and they were not included in the analyses. This resulted in 41 patients randomized to CBT and 39 to sCIT. Sixty-five patients completed Phase 1; 63 of the completers (79% of the total sample) had baseline FDG-PET scans available for analysis. Phase 1 remission rates were similar for both treatments: CBT= 12/33 (36.3%) and sCIT= 12/30 (40.0%) (Figure 1, Table 1). Non-response rates were also similar for both treatments: CBT= 9/33 (27.3%) and sCIT=6/30 (20.0%). Only patients with both unambiguous outcomes and usable PET scans were included in the primary analysis: 12 CBT REM, 11 sCIT REM, 9 CBT NR and 6 sCIT NR. There were no statistical differences in age, gender, demographic or illness characteristics between randomization groups (sCIT vs. CBT). There were also no baseline demographic differences among the treatment-specific Phase 1 outcome groups (Table 1). However, CBT non-responders had higher baseline anxiety ratings (HAMA total).

Neuroimaging Results

Treatment X Outcome ANOVA. There was no significant main effect of remission: i.e., no treatment *nonspecific* biomarker was identified. Significant Treatment X Outcome interaction effects were demonstrated for six regions: right anterior insula, right inferior temporal cortex (Brodmann Area, BA

20), left amygdala, left premotor cortex (BA 6), right motor cortex (BA 3), and the precuneus (BA 7) (Table 2, Figure 2).

Post-hoc Analyses of Extracted Regions of Interest (ROI):

The average effect size (ES) of each region for the various contrasts are shown in Table 2 in order of cluster size from the ANOVA. Average effect size was used to rank the ROIs in the order of their potential utility as a discriminator. Only the insula and precuneus showed differences larger than one standard deviation in all four contrasts, with the insula showing the largest average difference across all four comparisons. These findings indicate metabolic activity of the right anterior insula is the most viable TSB candidate (Table 2; Figure 3). Further, the anterior insula was the only region that showed relative hypometabolism in one group (region/whole brain <1.0) and hypermetabolism in the other (region/whole brain >1.0) adding support for potential uses as a treatment stratification tool and for defining brain-based subtypes.

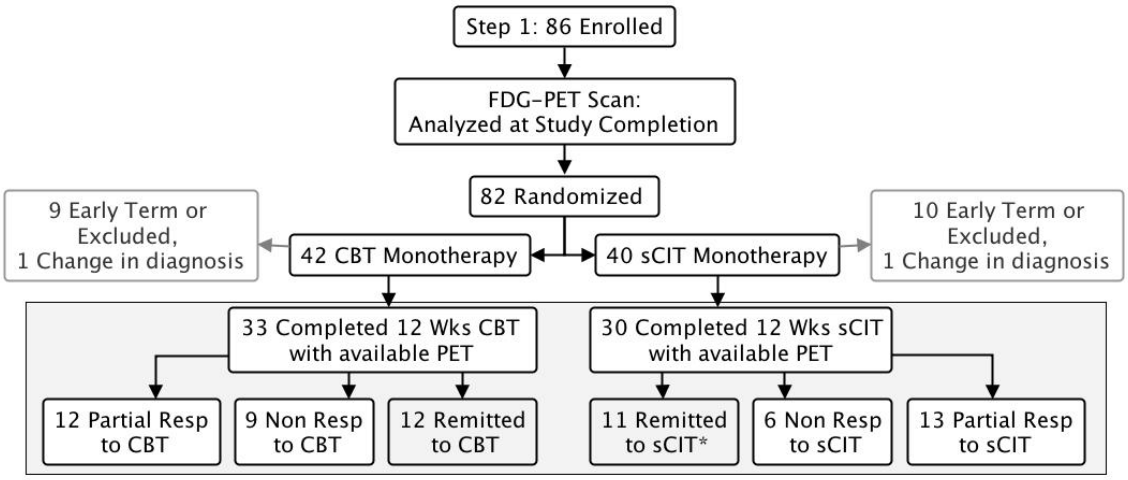

Figure 1: Phase 1 Study Design and Outcomes:
Outcome groups defined by Hamilton depression ratings scale (HDRS) scores. Remission was defined as HDRS ≤ 7, Partial Response as HDRS decrease >30% but not achieving remission. Non-response as HDRS decrease ≤ 30%. Treatment abbreviations: CBT = Cognitive Behavioral Therapy, sCIT = escitalopram. A total of 12 patients achieved remission with sCIT, but only 11/12 patients has usable PET scans.

Table 1: Group comparisons on clinical characteristics -mean (SD) or count (%)

	CBT		Escitalopram		
Variable	Non-responders (n=9)	Remitters (n=12)	Non-responders (n=6)	Remitters (n=12)*	Sig test of group *treatment Interaction†
Age	45.4 (8.8)	42.5 (10.8)	40.3 (5.2)	39.8 (6.3)	0.665
Gender (male)	3 (33.3%)	7 (58.3%)	2 (33.3%)	5 (41.7%)	0.628
Race (white)	7 (77.8%)	8 (66.7%)	5 (100%)	8 (72.7%)	0.345
Yrs of Education	14.7 (1.7)	15.6 (1.6)	15.4 (1.7)	16.2 (2.1)	0.940
Age of onset MDD	28.4 (12.3)	28.7 (11.2)	24.0 (11.6)	25.5 (10.7)	0.877
3 or more lifetime episodes	4 (50.0%)	4 (33.3%)	2 (33.3%)	4 (33.3%)	0.623
Duration of current episode (wks)	124.5 (118.5)	257.3 (308.8)	299.8 (620.0)	135.3 (266.2)	0.201
# previous AD trials in current episode	1.2 (1.5)	1.2 (1.0)	1.5 (1.4)	1.0 (1.1)	0.590
Melancholic subtype	4 (44.4%)	5 (45.5%)	3 (50.0%)	6 (50.0%)	0.976
Current anxiety disorder	3 (33.3%)	2 (16.7%)	1 (16.7%)	5 (41.7%)	0.166
Baseline HDRS 17	19.9 (3.8)	17.9 (2.7)	18.0 (2.1)	19.3 (3.7)	0.133
Baseline HAMA total	18.9 (7.6)	12.8 (2.8)	13.3 (2.3)	15.3 (3.0)	0.009
BDI total	19.2 (4.6)	18.7 (7.2)	20.0 (3.8)	21.5 (7.7)	0.642

p-values for continuous outcomes from 2 way ANOVA;
for categorical outcomes from test of the homogeneity of the OR (Breslow-Day)
* only 11/12 sCIT remitters had usable PET scans

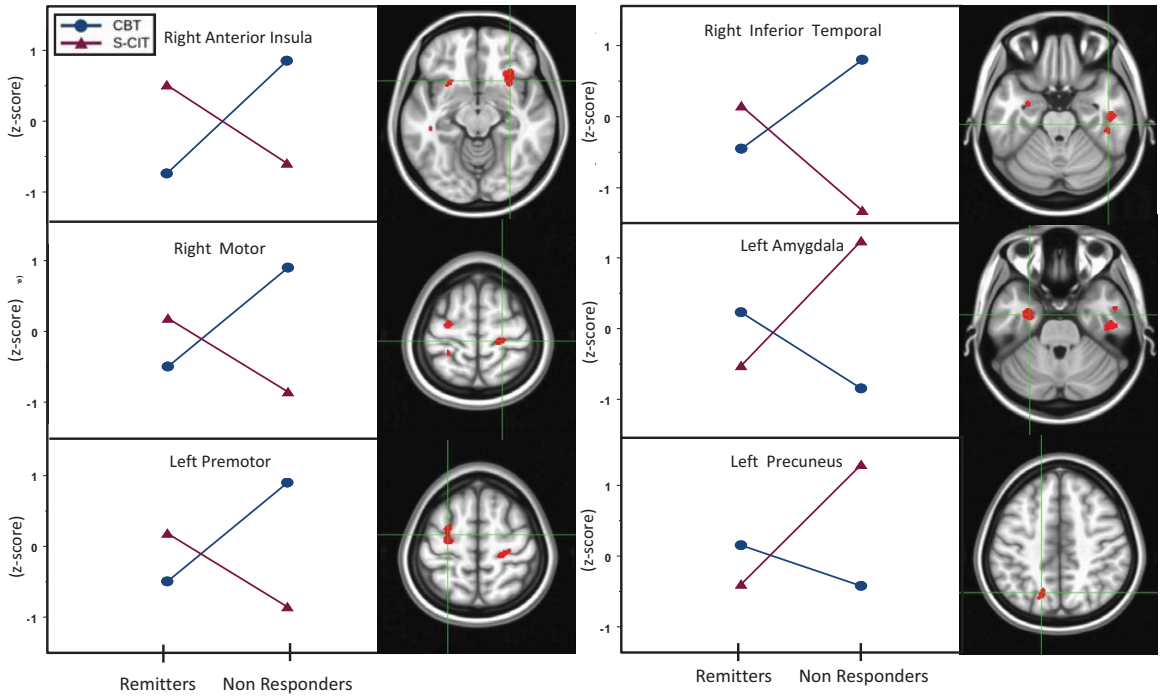

Figure 2: Potential Treatment Specific Biomarker (TSB) candidates
Mean regional activity values for Remitters and Non-responders segregated by treatment arm are plotted for the six regions showing a significant treatment x outcome ANOVA interaction effect. Regional metabolic activity values are displayed as region/whole brain metabolism converted to z-scores. Regions match those shown in Table 2.

Table 2: Treatment by outcome interaction results and post-hoc analyses of extraction ROIs

				Effect Sizes*				
Region	**MNI coordinates (peak) x y z**	**Side**	**cluster size † in voxels**	REM-NR to CBT	REM-NR to sCIT	CBT-sCIT in REM	CBT-sCIT in NR	Avg. marg. ES
Anterior Insula	30.0 24.0 -13.5	R	529	1.69	1.17	1.52	1.33	1.43
Motor cortex (BA 3)	42.0 -33.0 -25.5	R	469	1.23	1.45	2.09	0.59	1.34
Amygdala	-27.0- 7.5 -27.0	L	233	0.98	1.61	1.89	0.69	1.29
Premotor cortex (BA 6)	-27.0 1.5 58.5	L	233	1.40	1.03	1.75	0.68	1.22
Inferior Temporal (BA 20)	25.5 -27.0 60.0	R	147	0.61	1.78	1.80	0.58	1.19
Precuneus (BA 7)	-18.0 -67.5 43.5	L	101	1.18	1.27	1.37	1.08	1.23

† whole brain 2-way ANOVA with p<0.001 uncorrected; voxel size 1.5mm x 1.5mm x 1.5mm

** Effect Size = |mean difference/pooled SD|*

REM-NR = mean difference remitters – non-responders (remission effect)

CBT-sCIT = mean difference CBT-sCIT (treatment effect)

Abbreviations: CBT: Cognitive behavior therapy; MNI: Montreal Neurological Institute; ROC: Receiver operating characteristic; ROI: Region of interest; sCIT: escitalopram; Avg. Marg. ES: Average Marginal Effect Size

Assessment of the Insula TSB Across the Full Sample:

There was a significant correlation between baseline insula activity and percent change in HDRS scores in both the CBT and sCIT groups. A positive correlation was shown for the CBT group ($r=0.55$, $df=31$, $p=0.001$; Figure 3). In contrast, the sCIT-treated patients showed an opposite but less strong correlation ($r=-0.31$, $df=28$, $p=0.094$). Both correlations are consistent with the more restricted findings in the binarized remitter/non-responder analyses. Correlations with baseline activity and percent change in HDRS scores in both the CBT and sCIT groups in all six candidate regions are reported in Table 3.

Although not a primary planned analysis, the presence of multiple regions identified in the ANOVA suggests that a combination rather than a single TSB might be more accurate in discriminating the groups. Although underpowered, a principal component analysis (PCA) was performed using the 6 identified ROIs. All regions loaded on one factor, which did not provide superior internal consistency to insula alone (data not shown).

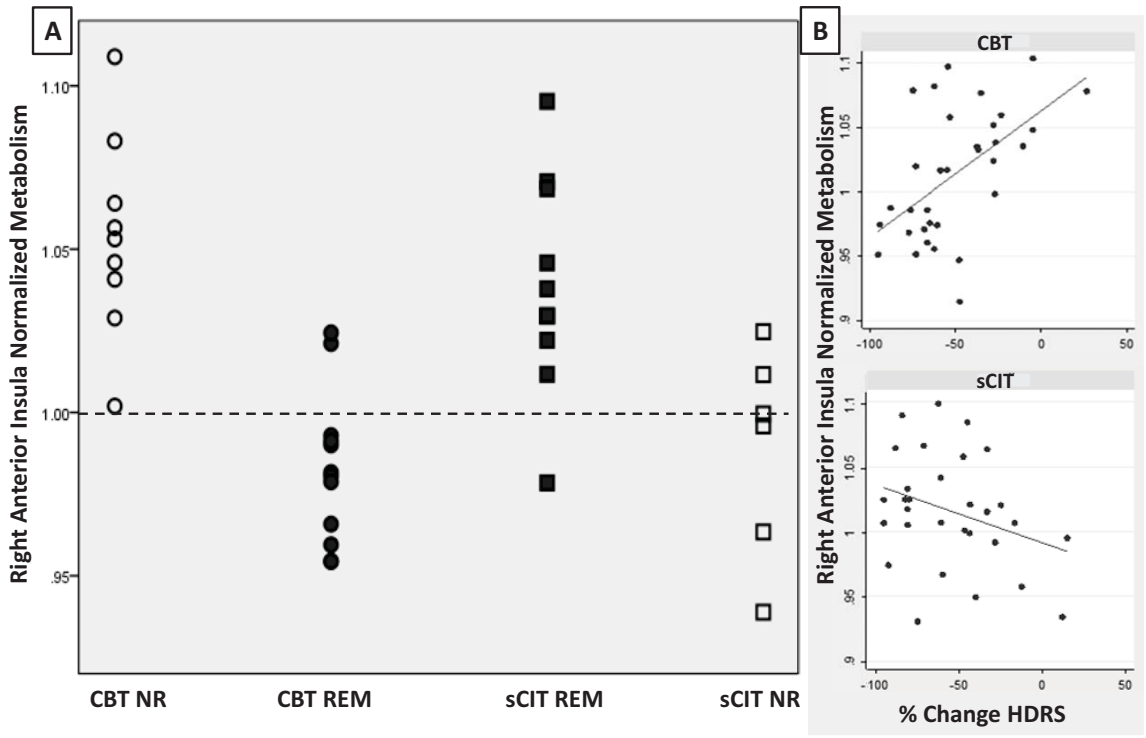

Figure 3: Right Anterior Insula as the Optimal TSB Candidate
Expanded view of findings. A: Scatter plot of insular activity from individual subjects in the remitter and non-responder groups. Note: The anterior insula is the only region where the interaction subdivides patients into hypermetabolic (region/whole brain mean >1.0) and hypometabolic (region/whole brain mean < 1.0) subgroups. B: Correlations of insula activity with percentage change in HDRS in the full cohort of CBT and sCIT treated subjects.

Table 3: Correlation of baseline metabolism in candidate regions with percent change in HDRS in sCIT and CBT treated groups

Region	CBT Treated		sCIT Treated	
	R	***p***	***R***	***p***
Right anterior insula	0.55	0.001	-0.31	0.094
R inf temporal	0.34	0.023	-0.44	0.015
L amygdala	-0.43	0.013	0.57	0.001
L premotor	0.43	0.012	-0.39	0.045
R motor	-0.22	0.225	0.51	0.004
Precuneus	-0.44	0.010	0.38	0.041

Discussion

This 12-week randomized study of two first-line treatments for MDD identified two FDG-PET defined brain pattern subtypes that differentially predicted remission to either CBT or sCIT. Among the six identified cortical and limbic regions, anterior insula metabolism best discriminated treatment outcome: insula hypometabolism was associated with remission to CBT and poor response to sCIT, while insula hypermetabolism was associated with remission to sCIT and poor response to CBT. These data suggest that insula metabolism alone (relative to each person's whole brain mean metabolism) may serve as a pre-treatment biomarker to guide initial treatment selection (medication vs. CBT) for a patient presenting with a major depressive episode. To validate the insula TSB, a prospective replication study in which patients are treated according to brain type will be required. That said, this forced-choice analytic strategy establishes a potential stratification algorithm for managing MDD patients based on brain state rather than patient or professional preference, anticipating the real-world decision making process faced by clinicians; namely, choosing a first treatment that will most likely lead to remission while also avoiding a treatment that is likely to fail.

A role for the anterior insula in major depression is well established. The insula is crucial in mediating the translation of visceral experiences to subjective feeling states (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004). Additionally, anterior insula activity is linked to behaviors relevant to depression including interoception, emotional self-awareness, decision-making, and cognitive control (Craig, 2009; Critchley, 2005; Farb, Segal, & Anderson, 2012). The anterior

insula is extensively connected to various frontal, limbic and brainstem regions, including the anterior cingulate cortex, amygdala, and hypothalamus (Augustine, 1996). Volume reductions of the anterior but not posterior insula have been described in currently ill as well as remitted MDD patients compared to healthy controls (Takahashi et al., 2010). Changes in insula activity occur with a variety of treatments for MDD, including medication (Kennedy et al., 2001), vagus nerve stimulation (Conway et al., 2006), deep brain stimulation (Mayberg et al., 2005), and mindfulness training (Farb et al., 2007) suggesting a role for this region in mediating antidepressant response and remission more generally (Fu et al., 2012). Notably, past studies have reported both increases (Ketter et al., 1999) and decreases (Kimbrell et al., 2002) in baseline resting state activity relative to never-depressed control subjects. This is consistent with the presence of at least two baseline patterns within the population of depressed patients. Most recently, baseline insula activity has been correlated with response to VNS (Conway et al., 2012). These previous studies taken together with the current findings support anterior insula metabolism as a potential candidate for an imaging TSB.

Of the remaining unselected candidate regions, variance in the amygdala is particularly interesting. The amygdala is included in most models of MDD, and is prominently featured in conceptual and meta-analytic models (J. P. Hamilton et al., 2012; Phillips et al., 2003b). In this study, the amygdala was a better predictor of medication outcomes than CBT response, similar to how the insula was a better predictor for CBT outcomes. The amygdala was not significantly associated with treatment response in a recent meta-analysis, but was reported as a region with significant heterogeneity (Fu et al., 2012).

Contrary to past published studies (reviewed in Pizzagalli 2011), the rostral anterior cingulate did not discriminate the outcome subgroups in either the main effect or interaction analyses. A post-hoc examination of responder/non-responder differences within each treatment arm did reveal a non-significant rostral cingulate activity difference with metabolism in responders greater than non-responders, but solely in the sCIT group (Appendix A). While consistent with past reports, this finding did not meet the TSB criteria defined for the current study: i.e., a region whose activity can differentiate both good and poor outcomes for both treatments.

Critical to the stated aims, remission (rather than response) was the targeted endpoint in this study because the presence of residual symptoms is a known predictor of clinical relapse, even in patients with significant improvement (Judd, Paulus, et al., 2000; Paykel et al., 1995). Because the primary aim of this study was to identify distinct brain patterns that optimally predict remission to each of two specific treatments, patients with unclear treatment outcomes were excluded from the primary analysis (i.e., responders without remission; partial responders). This enriched sample allowed for detection of clear remission and nonresponse signals; as such, these analyses did not attempt to characterize the neurobiological variability of patients with more ambiguous clinical outcomes. This is a commonly used approach when the goal is to develop or test a biological signal for stratifying subjects (Ridker, Hennekens, Buring, & Rifai, 2000; Ridker, 2003). Nevertheless, baseline insula activity did correlate with change in depression severity across all Phase 1 completing subjects, supporting the interpretation that insula activity is a plausible TSB suitable for further testing.

Based on the correlational analysis across all subjects, the data further suggest that the anterior insula TSB may most optimally identify those patients who require CBT.

If confirmed with prospective testing, this putative TSB has both clinical and pathophysiological implications. At present, treatment failure with antidepressant medication often leads to the addition of a second drug and not a categorical switch to an evidence-based psychotherapy (Gaynes et al., 2012). Results here suggest that patients who require CBT have a distinct neurophysiology that differs categorically from patients who require sCIT and knowledge of such may help to improve current clinical practice patterns. Further, using this or any other imaging-based TSB to define patient subgroups provides a brain-based platform to investigate genetic, immune, neuroendocrine and behavioral variations from a new perspective.

While these first results are encouraging, there are several limitations. Clearly there are patients who are not successfully treated with either of these two options, either alone or in combination (Thase et al., 2007). Therefore, our strategy can be best seen as a first-line stratification approach to treatment selection. Future studies, in addition to testing this insula biomarker prospectively, should include a design that works to identify patients resistant to both of these first-line treatments (Mayberg et al., 2005; Rush et al., 2009). Chapter 4, Experiment 3.1 will begin to address this issue.

The lack of a placebo arm could be considered a limitation, but given the randomized design of the study there is no reason to believe that placebo responders would be unevenly distributed between the two groups. Thus, even if

present, placebo effects on remission rates would be expected to be similar in both treatment groups. Although inclusion of a placebo arm might have provided further insights into mediators of improvement during treatment, the absence of a placebo arm does not diminish the potential clinical utility of the identified TSB.

It is also possible that these results are specific to the cohort recruited for this trial. As such, a stratification strategy based on insula metabolism will require prospective testing in a new group of comparably depressed patients. Similarly, additional studies will be required to determine if remitters to other medications have a similar or different TSB from that seen with sCIT, or if remitters to other evidence-based psychotherapies have a similar TSB to that seen with CBT (Dunlop, Binder, et al., 2012; Kennedy et al., 2012). Such studies are critical next steps toward the development of biology-based algorithms to guide treatment selection for MDD at all stages of illness. Patients with anterior insula metabolism above (i.e.,, higher than) and below (i.e., lower than) whole brain mean may represent two biologically distinct depression subtypes. If replicated, the insula TSB defined in this study would provide the first objective marker to guide initial treatment selection for major depression – an important advance in potentially reducing the costs and disability associated with this highly prevalent disorder.

Experiment 1.2: Confirmation of the Anterior Insula TSB- Phase 2 Treatment within Study Replication

For many patients, the Phase 1 treatment they were randomized to did not match the insula biomarker prescribed 'optimal' treatment. In Phase 2, Phase 1 non-

remitting patients were augmented with the treatment they had not yet received, providing the opportunity for a within study replication. To validate the insula as a biomarker, we can evaluate the number of patients who achieve remission after receiving the insula predicted treatment in the Phase 2 follow-up.

Methods

Phase 2 Treatment

Phase 1 non-remitting patients were offered enrollment in the Phase 2 sCIT plus CBT combination therapy stage. Patients receiving sCIT in Phase 1 continued on their current dosage, their treatment augmented with biweekly sessions of CBT for 4 weeks, followed by weekly CBT for 8 weeks. Patients randomized to CBT in Phase 1 received three booster sessions of CBT at monthly intervals, augmented by sCIT dosed as in Phase 1. Similar to Phase 1 remission, Phase 2 remission was defined as an HDRS ≤ 7 at both weeks 22 and 24 of treatment (weeks 10 and 12 of Phase 2).

Imaging

Each patient's baseline anterior insula metabolism was extracted as reported on page 34. Insula subgroups were defined based on mean metabolism in the anterior insula ROI identified in Figure 2. The ROC curve (Figure 4) determined the best threshold to divide patients into insula metabolism-based groups is at a whole-brain mean corrected metabolism equal to 1.0. Importantly, in whole-brain mean corrected data, 1.0 is the mean metabolism of the whole brain. Therefore patients were divided into two anterior insula metabolism subgroups, patients with anterior insula metabolism above whole brain mean (I-AM) and patients with anterior insula metabolism below whole brain mean (I-BM).

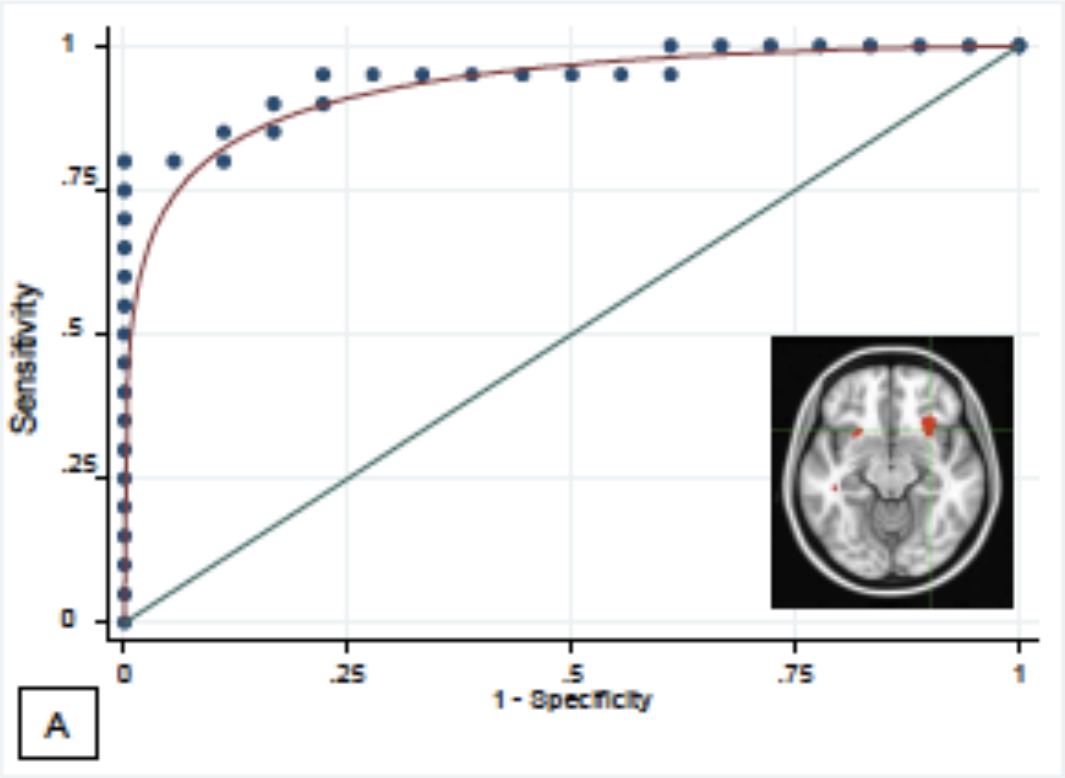

Figure 4: ROC curve
Receiver operating characteristic curve showing sensitivity and specificity of the insula's ability to discriminate between groups. Area under curve (AUC) = 0.94

Results

At the end of Phase 1 of treatment, the 40 non-remitting patients were offered Phase 2 combined treatment. Of these 40 patients, 30 enrolled in Phase 2 treatment, with 27 completing the full 12 weeks of treatment. The phase 2 remission rate was 52.6% (10/19) among patients whose second (augmented) treatment in phase 2 matched the treatment indicated by their baseline anterior insula activity, compared with a 25% (2/8) remission rate in patients whose augmentation treatment was mismatched with the treatment predicted by their anterior insula metabolism. Patients were unevenly distributed between anterior insula subgroups, with 7 I-BM patients and 20 I-AM patients (Figure 5). Of the 6 I-BM patients who were augmented with CBT in Phase 2, 4 of 6 achieved remission (66.7%). The sole I-BM patient augmented with sCIT, achieved remission, however a single data point is too small to drawn conclusions. Seven I-AM patients were augmented with CBT in Phase 2 with only 1 patient achieving remission (14.3%). In the I-AM patients receiving the sCIT augmented treatment 6 of 13 patients achieved remission (46.2%).

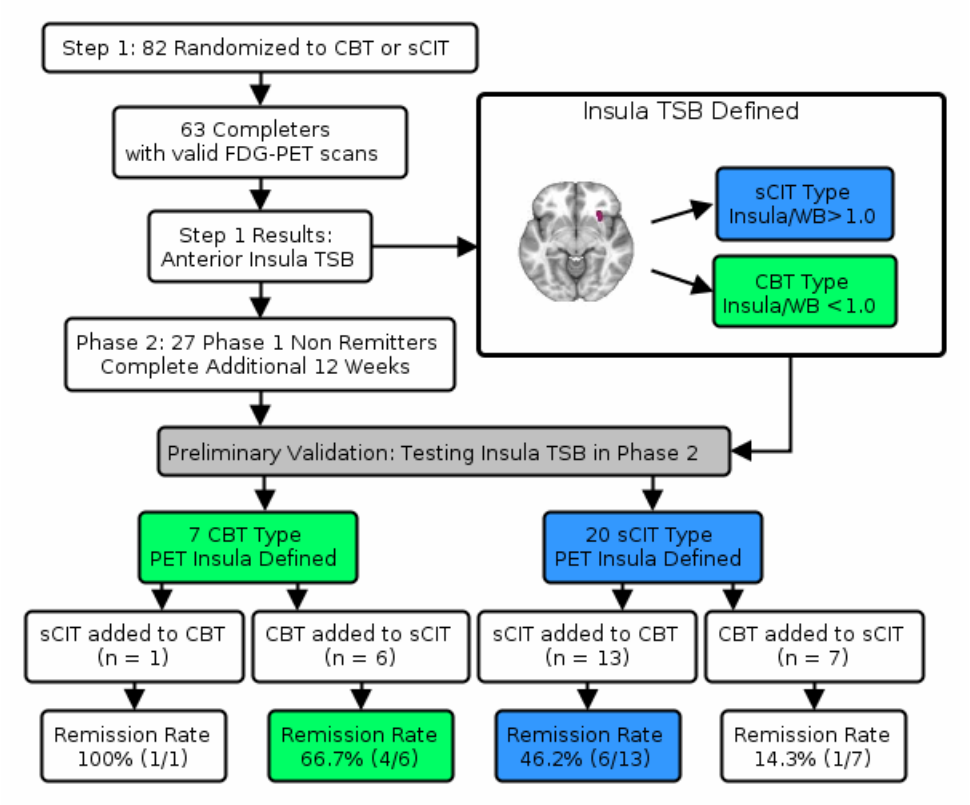

Figure 5: Phase 2 outcomes related to insula activity
Outcomes for patients entering Phase two divided by Anterior insula subtypes. Patients with anterior insula metabolism greater than whole brain mean are referred to as sCIT type (I-AM), patients with anterior insula metabolism less than whole brain mean are referred to as CBT type (I-BM). Twenty-seven patients entered phase 2 after non-remission in phase 1; the anterior insula TSB indicated that at phase 1 baseline, 7 patients had a CBT type and 20 had an sCIT type. Adding CBT treatment to the CBT type patients resulted in a 66.7% remission rate; adding sCIT treatment to the sCIT type patients produced a 46.2% remission rate.
CBT – cognitive behavior therapy; PET – positron emission tomography, sCIT – escitalopram antidepressant medication; TSB – treatment selection biomarker

Discussion

Most patients who achieved remission in Phase 2 received treatment augmentation with the treatment matching their insula predicted remission treatment. A larger number of I-BM patients achieved remission with CBT augmentation, supporting anterior insula metabolism as a stronger predictor of CBT remission than sCIT remission, although sCIT matched patients also did well. Generally, patients receiving an augmentation treatment unmatched to their insula type did poorly, with I-AM patients augmented with CBT reporting a low remission rate of 14.3%. The treatment outcome results of I-BM patients augmented with sCIT are difficult to interpret, as the group consists of a single patient. A true crossover would provide stronger support for the anterior insula as a biomarker, however such a study design was not employed. The second treatment was offered as an augmentation (and not a crossover) primarily for practical and ethical concerns. A crossover design would necessitate a wash out period for each treatment. For CBT, there is no washout period. As a form of psychotherapy, CBT cannot be unlearned once initiated. Patients on sCIT could undergo a washout period to remove the effects of this antidepressant medication, however this would take time and would be potentially harmful to patients. The criteria for entry into Phase 2 treatment was non-remission at both weeks 10 and 12 of treatment, meaning some patients had partially responded to initial treatment. We determined that removing patients from a treatment they were receiving some benefit from to test a second treatment was not reasonable in this case. Further, due to the limited ability to “wash out” CBT, there would be no appropriately controlled comparison group. This choice of augmentation

instead of crossover leads to some limitations in the interpretation of the Phase 2 outcome data. While we associated Phase 2 remission with the addition of the second treatment, some patients may require both CBT and sCIT, however since patients are on a full twelve weeks of treatment without achieving remission, it is likely that the conversion from non-remitter to remitter is due largely to the addition of the second treatment.

To help clarify this issue, two additions could be made to the study. A second scan after Phase 1 treatment but prior to initiation of Phase 2 treatment could be used as a new baseline, and tested for its ability to predict response to added treatment. As an alternative to the crossover design, a third randomization arm could be added where patients receive both CBT and sCIT simultaneously to clarify whether patients remitting on both treatments show a unique pretreatment signature.

Experiment 1.3: External Replication of Anterior Insula TSB in CBT Treated Patients

A small sample of raw FDG-PET images from a previously acquired dataset was available for external replication of the ability of anterior insula metabolism to discriminate between CBT remitters and CBT non-responders. This dataset was previously analyzed to understand changes with successful and unsuccessful treatment across treatments (Kennedy et al., 2007; Konarski et al., 2009). The Kennedy et al. study measured changes with treatment. The primary analysis in the Konarski et al. study compared baseline metabolism in treatment responders to either CBT or venlafaxine with non-responders to either treatment, and supplemental analysis of CBT treated patients did not limit analysis to remitters.

Here we will directly compare anterior insula metabolism between remitters and non-responders to CBT treatment.

Methods

Acquisition of External Replication Dataset

The dataset consists of MDD patients recruited from the Centre for Addiction and Mental Health at the University of Toronto, Toronto, Ont. Participants meeting the DSM-IV criteria for MDD in the context of a current major depressive episode were assessed with the Structured Clinical Interview for DSM-IV, patient version (SCID-IP) and Hamilton Depression Rating Scale, 17 item version (HDRS). Depressed patients with an HDRS-17 item score of 20 or higher with no other Axis I diagnoses were included. Patients were free of antidepressant medication for at least 2 weeks preceding the study and were in good physical health.

Treatment Protocol and Outcome Measures

Patients were randomly assigned to receive either venlafaxine (75–225 mg/d) or CBT for 16 weeks. Venlafaxine treated patients are not included in this analysis. Venlafaxine is a selective serotonin-norepinephrine reuptake inhibitor (SNRI), meaning its mechanism of action is different from that of escitalopram, an SSRI. Due to the confounding differences in mechanism of action between escitalopram and venlafaxine, venlafaxine patients will not be further discussed. Severity of depressive symptoms was assessed using the HDRS-17-item. The endpoint criteria used in the original study was response defined as greater than a 50% change in HDRS, however here we will use remission, defined as an HDRS score less than or equal to 7 (N=5). Non-response is defined as less than a 50% change in HDRS score (N=5).

Imaging Data Collection, Preprocessing and Analysis

Resting-state FDG-PET measurements were collected a maximum of 1 week before treatment initiation. For each scan, patients were injected with a 5-mCi (185-Mbq) dose of 2-[18F]-fluoro-2-deoxy-D-glucose intravenously. Image acquisition began after 40 minutes (PC 2048b; GEMS-Scanditronix, 15 parallel slices; 6.5-mm centre-to-centre interslice distance) with patients supine, awake with eyes closed and ears uncovered. Patients were given no explicit instructions, but were asked to avoid ruminating on any one topic during the FDG uptake period. Emission data was acquired during a 35-minute period (~ 1 million counts per slice; 10-cm field of view). Head movement was minimized using a customized, thermoplastic facemask. Raw images were corrected for attenuation, reconstructed and smoothed to a final in-plane resolution of 7.0 mm at full width at half maximum.

FDG-PET data was processed using SPM8 (www.fil.ion.ucl.ac.uk/spm/). Scans were spatially normalized to an FDG-PET template and spatially smoothed with a Gaussian kernel of 8mm. A region of interest of the anterior insula was constructed from the Harvard-Oxford atlas available through FSL (Desikan et al., 2006), based on the anterior insula region described in Experiment 1.1 (Figure 6A). We chose this expanded ROI to maximize the opportunity for replication given the differences in scanner, resolution, institution and subject recruitment. All voxels within the mask were compared between CBT remission and non-response using a two-sample t-test. Results were considered statistically significant at an FWE corrected $p>0.05$.

Results

Voxel-by-voxel insula ROI t-test

FWE corrected results show lower metabolism in the anterior insula in CBT remitters compared with CBT non-responders (Figure 6B).

Discussion

Consistent with Experiment 1.1, this small cohort of CBT remitters show decreased anterior insula metabolism, despite differences in institution, scanner, data quality and processing steps between the two patient cohorts.

An alternative approach to replicating the anterior insula differences could have been based on directly comparing outcome to CBT between patients with anterior insula activity metabolism above and below whole brain mean. Unfortunately, the differences in data acquisition between the two institutions prevented this type of follow-up analysis. FDG-PET data collected at Emory has full brain coverage. In contrast, the FDG-PET data from the Toronto data set does not have full brain coverage, with the region of coverage varying slightly by patient. This means that threshold at "1" (whole brain mean) is not comparable across the two datasets because the regions that contribute to the calculation of whole brain mean are different between the two datasets.

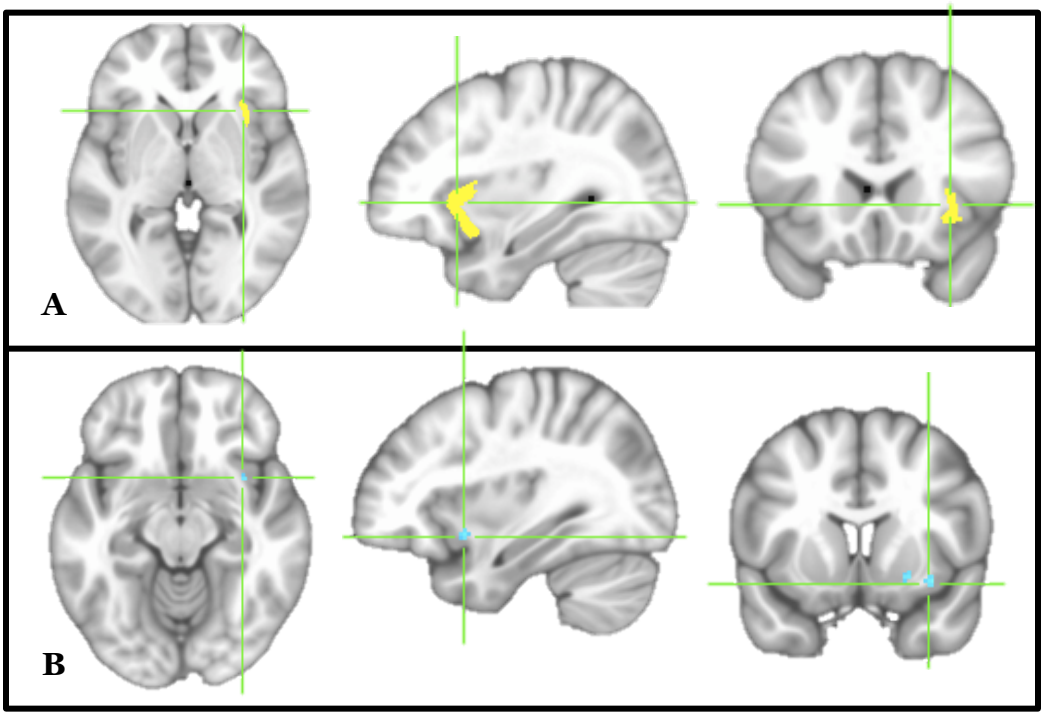

Figure 6: Ant. insula follow-up
Anterior insula sub-region from the FSL atlas. The full insula region was selected, with the posterior boundary determined by the location of the anterior insula finding. A shows the anterior insula ROI. B shows the insula region of statistically significant difference between CBT remitters and non-responders.

Chapter Summary

The results of the three experiments described in Chapter 2 strongly support anterior insula metabolism as indicative of differential outcome to CBT and sCIT treatments in MDD patients. The anterior insula was identified as a potential discriminator of CBT/escitalopram remission in Phase 1 of a randomized treatment trial (McGrath et al., 2013), supported as a discriminator by the Phase 2 treatment outcomes, and replicated in a small sample of previously acquired data. Together, these results suggest that anterior insula metabolism identifies two brain-based depression subtypes directly linked to treatment outcomes. The next step is to explore how anterior insula metabolism fits into the larger perspective- what is its role in the neurobiology of depression?

CHAPTER 3: DYNAMICS OF THE ANTERIOR INSULA

In Chapter 2 we identified a relationship between anterior insula metabolism and differential outcome to treatment with CBT or sCIT. Insula metabolism segregates CBT remission/sCIT non-response patients (anterior insula metabolism below whole brain mean, I-BM) from sCIT remission/CBT non-response patients (anterior insula metabolism above whole brain mean, I-AM). In Chapter 3 we follow up this experiment by investigating differences between anterior insula metabolism subgroups. Specifically, we examine how I-AM and I-BM subgroups differentially relate to network dysfunction in major depression.

Overview of Anterior Insula Function

Many functions are attributed to the anterior insula. In keeping with its anatomical definition as a paralimbic region, the anterior insula integrates limbic and cortical information. The anterior insula is structurally connected with multiple systems, including those underlying sensation, emotion and cognition (Simmons et al., 2012). The insula integrates information from the body, with afferents from the body terminating in both lamina I of the dorsal horn of the spinal cord, and in the nucleus of the solitary tract in the brainstem, then connecting through the thalamus to reach the insula (Craig, 2005). Studies suggest a difference between right and left insula innervation of body inputs, with right insula more closely associated with the sympathetic division of the autonomic nervous system, and left insula more closely associated with the parasympathetic division (Craig, 2005). In the brain, structural connections exist between the anterior insula and many components of the limbic system

(Reynolds & Zahm, 2005) including anterior cingulate (Augustine, 1996), amygdala (Augustine, 1985; Jasmin, Rabkin, Granato, Boudah, & Ohara, 2003; Reynolds & Zahm, 2005), ventral striatum, nucleus accumbens (Reynolds & Zahm, 2005), orbital frontal cortex, and medial prefrontal cortex (D Ongür & Price, 2000) (Figure 7). The anterior insula connects directly with subcortical regions (Chikama, McFarland, Amaral, & Haber, 1997) but also shares a close structural relationship with the posterior insula, which connects to both the thalamus and brainstem. These rich structural connections have widespread functional implications. Anterior insula has been implicated in interoception (Critchley, 2005), emotion (Phan, Wager, Taylor, & Liberzon, 2002), cognition (Huettel & McCarthy, 2004), pain perception (Tracey et al., 2000), inflammation (Hannestad et al., 2012) and homeostasis. Abnormalities in many of these functions have implications for major depression.

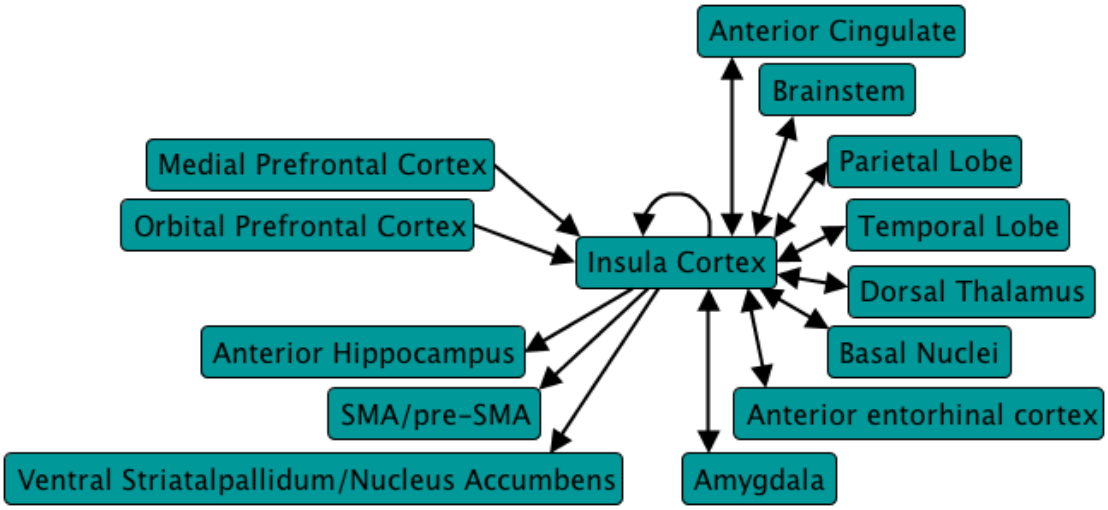

Figure 7: Structural connections of the insula cortex
Organized by regions with reciprocal efferent and afferent, afferent only and efferent only connections. Arrows indicate direction of connection.

Insula Dysfunction and Variance

Lesions in the insula have been linked to disruptions of autonomic functions, perception, body awareness, disgust emotions, mood and willed action, addition behavior, language, and in facilitating smoking cessation (Ibañez, Gleichgerrcht, & Manes, 2010; Suñer-Soler et al., 2012). Studies specifically investigating the neuropsychiatric implications of insula stroke have reported greater frequency of anergia (lack of energy) and under activity as well as tiredness in patients with right insula lesions. In depressed patients, anterior insula activity has been reported as different from healthy controls, and to change with treatment (see Experiment 1.1 discussion of insula findings). While rarely highlighted in individual papers relating brain activity to treatment outcomes, a recent meta-analysis indicates anterior insula metabolism differences between treatment responders and non-responders (Fu et al., 2012). In this meta-analysis, increased activity in the anterior insula was associated with non-response to treatment. The meta-analysis included studies that analyzed multiple forms of treatment, including multiple psychotherapies and anti-depressant medications. This meta-analytic association of low activity with good outcomes is consistent with good CBT outcomes in patients with low anterior insula metabolism as well as a greater number of two treatment non-responders in patients with increased anterior insula activity.

Frameworks for Insula Dysfunction in Depression

Insular cortex integrates cognition, emotion and autonomic control (Critchley, Eccles, & Garfinkel, 2013). These interrelationships provide a foundation for a number of frameworks for approaching insula dysfunction in major depression.

Avery and colleagues argue that somatic disturbances and an altered sense of body awareness implicate the anterior insula in depression. Their recent study shows decreased mid-insula activity in patients during an interoceptive task (compared with controls). The larger the decrease in insula activity, the greater the level of somatic symptoms (Avery et al., 2013) In addition to the mid- insula finding, activity in the ventral anterior insula and ventral-mid insula was correlated with depression severity during the interoceptive task.

A second perspective considering anterior insula dysfunction in depression is related to the insula's role in salience monitoring. By "tagging" salient information, the anterior insula identifies salient information for deeper processing and initiates appropriate control signals. The anterior insula and anterior cingulate together form the basis of the salience network, a network that guides behavior by selecting the most relevant of internal and external stimuli (Menon & Uddin, 2010; Seeley et al., 2007). Functional connectivity of the insula and amygdala to the salience network is altered in depression (Veer et al., 2010).

In a more psychological construct, Paulus and Stein suggest that depression and anxiety are altered interoceptive states. Paulus and Stein (Paulus & Stein, 2010) frame interoception as three pronged. Interoception senses the physiological state of the body (sensations such as pain, temperature, itch, tickle, touch, muscle tension, air hunger, stomach discomfort) (Craig, 2002), represents the internal state in the context of ongoing activities and is associated with motivated action towards homeostatic regulation of the internal state (Craig, 2009). In depression, top-down modulation of interoceptive state is altered by increased and noisy

afferent input and biased by self-referential belief-based states (Paulus & Stein, 2010).

The anterior insula also is important for emotion processing. Anterior insula activity has been indicated in meta-analyses of emotion processing (Phan et al., 2002). The insula is particularly activated by emotion-related tasks involving emotional recall/imagery and emotion tasks with cognitive demand. Philips et al. (2003a) suggest insula as a component of the 'ventral stream', which is important for identifying the emotional significance of environmental stimuli and producing affective states. Strategies for emotion regulation, and their neural correlates can vary. Studies investigating emotional reappraisal have implicated the anterior insula, both in terms of function and structure. Habitual use of expressive suppression over other emotion regulation strategies (e.g., cognitive reappraisal) corresponds with increases in anterior insula volume (Giuliani, Drabant, Bhatnagar, & Gross, 2011).

The anterior insula may link emotion and interoception. Damasio's theory of emotion posits that subjective affective state is derived from interoception (Damasio, 1996). The insula, particularly the anterior insula plays a strong role in interpreting the body state. For example sensations of rapid heart beat may interpreted as feeling fearful (Critchley, 2005). In such a model, insula interoceptive and emotion processes are linked by the insula's function as a body state interpreter. Studies show the anterior insula (and ventral medial frontal cortex) as active when evaluating both emotional and bodily states (Terasawa, Fukushima, & Umeda, 2013).

Chapter 2 presents evidence that differential anterior insula metabolism in subgroups of depressed patients has implications for response to specific treatments. Chapter 3 will determine whether anterior insula activity is altered in both, neither or one anterior insula subgroup, and what the implications of different anterior insula metabolism-based subgroups are for emotional states and network dynamics. The insula may be abnormal, or its relationships with other regions may be abnormal. We explore the anterior insula's role in emotion processing in the same patient cohort. We hypothesize that anterior insula subtypes will show differences in the relationship between brain metabolism and negative affect.

Experiment 2.1: Anterior Insula Subgroups Differentially Modulate Affective State

One aspect of emotion regulation is the resulting mood state. Two dimensions, positive and negative affect, steer self-reported mood (Watson & Tellegen, 1985). Positive and negative affect are not opposite poles of one dimension, instead they are distinctive, orthogonal dimensions. Positive affect is the extent to which a person feels enthusiastic, active and alert. Negative affect is a general dimension of subjective distress and displeasure that subsumes a variant of aversive mood states, including anger, contempt, disgust, guilt, fear, and nervousness. Both dimensions can be measured as either trait or state.

The Positive and Negative Affective Schedule (PANAS) comprises two 10-item mood scales that measure positive and negative affect (Watson, Clark, & Tellegen, 1988). The PANAS provides a means to parse affect in isolation. This measure is a reasonable scale for monitoring the perceived emotional state. Studies have

reported higher negative affect scores and lower positive affect scores in depressed patients (Watson, Clark, & Carey, 1988) but have not looked at the relationship between affective state and brain activity in depression. In healthy controls, differences in trait negative affect impact brain activity with increased trait negative affect corresponding with increased activity in the ventromedial prefrontal cortex, including the subcallosal cingulate (Zald, Mattson, & Pardo, 2002). Here we will analyze the relationship between anterior insula subtypes and brain correlates of mood state in depression.

Methods:

Subject Recruitment.

Recruitment of patients is detailed in Chapter 2.

A single time-point comparison group of 24 healthy volunteers were similarly screened with the additional exclusion criteria of no current or history of MDD. Written informed consent was obtained from all participants with the protocol conducted as approved by the Emory Institutional Review Board.

FDG-PET Acquisition

FDG-PET acquisition is detailed in Chapter 2. The same acquisition protocol was applied in the comparison group of healthy volunteers.

PANAS Scores

This study measured affective state using the positive and negative affect scale (PANAS). The PANAS was administered immediately following the FDG uptake period to capture the coincident affective state at the time of the scans. Subjects endorsed positive and negative affective words on a scale of 1 to 5, with one being very slightly/not at all and 5 being extremely. As the two scales are orthogonal,

positive and negative PANAS items were separately tabulated into positive and negative affect scores (Crawford & Henry, 2004; Watson, Clark, & Tellegen, 1988), and separately divided by the number of tabulated scale items to preserve the 1 to 5 range and interpretation.

FDG-PET Preprocessing

FDP-PET Preprocessing is detailed in Chapter 2.

Anterior Insula Subtyping

In Experiment 1.1, insula metabolism above whole brain mean (I-AM) was associated with remission to sCIT and non-response to CBT. Anterior insula metabolism below whole brain mean (I-BM) was associated with remission to CBT and non-response to sCIT. Of interest in the current experiment is characterizing these two distinct anterior insula states in all patients, including those who did not complete treatment. Mean metabolism was extracted from the anterior insula region as defined in Experiment 1.1 (MNI coordinates x = 30 y= 24 z = -13.5, Figure 3). As defined in Experiment 1.2, the anterior insula subgroups are divided by metabolism greater than 1 (I-AM) or less than 1 (I-BM).

Anterior Insula Metabolism Comparisons between Groups

Anterior insula metabolism was compared separately between each anterior insula subgroup and healthy controls using two independent samples t-tests. We chose to use two separate t-tests (rather than a one-way ANOVA) to compare controls with anterior insula subgroups. Anterior insula subgroups have different metabolisms by definition, and comparing metabolic activity in the anterior insula subgroups to a 3rd group in a one-way ANOVA could artificially boost the statistical significance of the comparison.

Behavioral PLS: Negative Affect, Positive Affect, and Depression Severity.

Partial least squares analysis (PLS) (A. Krishnan, Williams, McIntosh, & Abdi, 2011; McIntosh, Bookstein, Haxby, & Grady, 1996; McIntosh & Lobaugh, 2004) is a multivariate analysis technique used to extract commonalities between brain activity and experimental design. Multivariate analyses allow examination of distributed patterns. Here, behavioral PLS will be applied to analyze associations between behavior (negative mood state) and brain activity (metabolism). The goal of this analysis was to determine 1) the network(s) of regions associated with negative affect and 2) differences in the associated network(s) between anterior insula subgroups. Behavioral PLS uses the covariance between brain activity and behavior to create a new set of variables, called Latent Variables (LV). Deciding which LV's to retain is done through a combination of permutation tests and bootstrapping. The significance of each LV is determined using a permutations test (500 permutations), and the stability is assessed using bootstrapping (100 bootstrapped samples) (McIntosh et al., 1996). The relationship of a given voxel to the LV is expressed as its salience, the weight of the voxel on the LV. Saliences are calculated simultaneously, so there is no indication for the use of multiple comparison correction. Plsgui (http://www.rotman-baycrest.on.ca) implemented in MATLAB was used to perform all PLS analyses. Preprocessed FDG-PET scans were loaded into the plsgui software by anterior insula subtype. Scans were concatenated, skullstripped, and centered. Corresponding negative affect scores were entered for each subject, centered and normalized. Latent variables were then calculated. Permutation tests were considered significant at $p<0.05$. The

same analysis was repeated using positive affect scores. A third behavioral PLS was run with the baseline HDRS scores entered as behavior data to determine if affect state and illness severity were similarly related to brain activity.

Issues with Zero-Inflation

Because some patients show no negative affect during scanning, the negative affect scores are zero-inflated. 27.5% of patients have a total score of 1, which is the minimum positive or negative affect score. To address this issue, we performed two follow-up analyses. First, negative affect scores were binarized into two categories: presence of negative affect (negative affect score greater than 1) and absence of negative affect (negative affect score equal to 1). Whole-brain metabolism was compared between presence/absence groups using a t-test. Second, the negative affect behavioral PLS was re-run using only patients in the presence of negative affect group.

Comparison of Affect Scores between Groups

Positive affect was compared between anterior insula metabolic subgroups and healthy controls with a one-way ANOVA. The skewed distribution of the negative affect data required an alternate analysis. Log transformation of the data did not result in a normal distribution. To accommodate the non-normal distribution the Mann-Whitney U test for two independent samples (a nonparametric test) was performed to compare negative affect between anterior insula subgroups. Healthy controls showed data clustered around 1 (the scale minimum) and were not included in the analysis.

Results:

Insula Subtypes in Patients

In the patients randomized to treatment (N=77 with usable FDG-PET scans), 33 patients showed I-BM and 44 patients showed I-AM. Of these 77 patients, 63 completed Phase 1 treatment and were included in Chapter 2 analyses. In the remaining 14 early terminators/early crossovers, there is no established relationship between anterior insula metabolism and treatment outcome, since these patients did not complete Phase 1 treatment.

Healthy Control Metabolism Compared between Insula Subtypes:

The t-test comparing healthy controls and I-AM patients showed no significant difference (t= 0.045, p>0.964, df=66). The independent samples t-test between healthy controls and I-BM patients showed a significant difference in anterior insula metabolism, but did not pass Levene's test for equality of variances. A follow up non-parametric test (Mann-Whitney U) showed increased anterior insula metabolism in the healthy controls compared with I-BM patients (Standard test statistic 5.883, p>0.000, Figure 8). While patients and controls showed a mean age difference (T=-4.016, p>0.001, df=99 controls N=24, 12 male/12 female; mean age ±SD= 34.13± 7.74, MDD N=77 mean age ±SD=41.74 ±8.22), the mismatch in age is consistent between both depression subgroups.

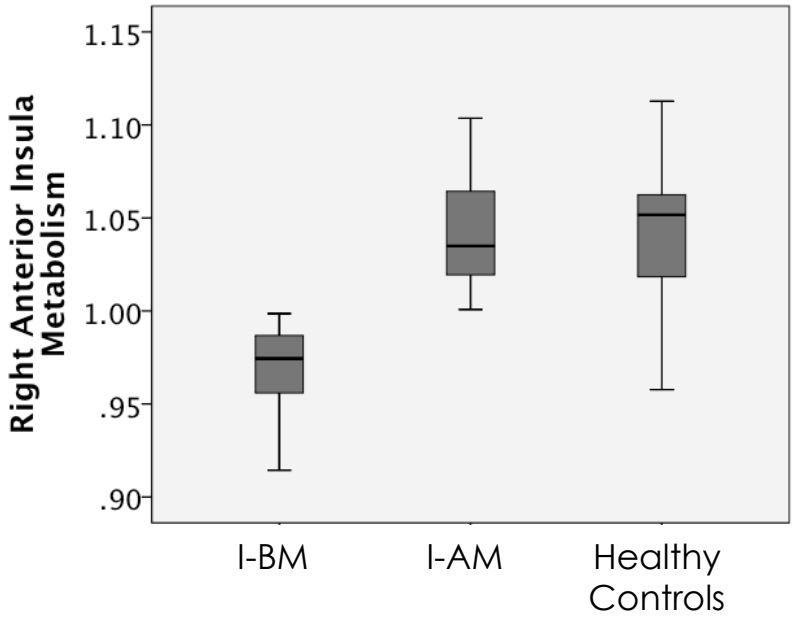

Figure 8: Graph of anterior insula metabolism in anterior insula subtypes and healthy control groups
I-BM, anterior insula metabolism below whole brain mean, I-AM anterior insula metabolism above whole brain mean.

PANAS Scores across groups

The one-way ANOVA comparing positive affect PANAS scores between anterior insula subgroups and healthy controls showed no significant difference (F 1.446, $p>0.230$, Figure 9A). Controls generally do not endorse negative affect, and patients have negative affect scores skewed towards zero. Anterior insula subgroups do not show significant differences in negative affect (Mann-Whitney U test, $p>0.149$, Figure 9B).

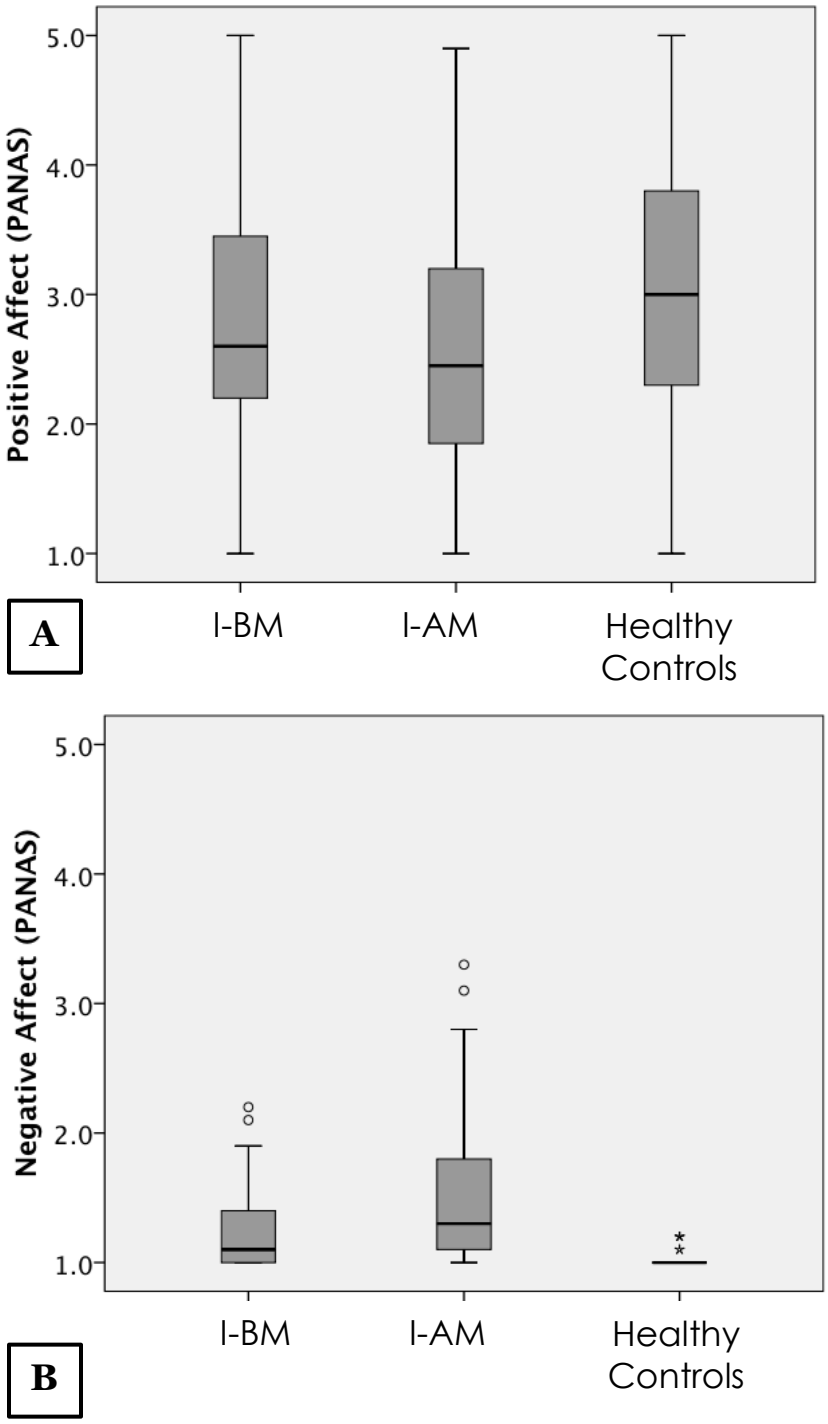

Figure 9: Graphs of PANAS scores by group
A) Boxplots of positive affect scores in patients with anterior insula metabolism below whole brain mean (I-BM) patients with anterior insula metabolism above whole brain mean (I-AM) and healthy control groups. B) Boxplots of negative affect scores in I-BM, I-AM and healthy control groups.

Negative Affect Behavioral PLS Results

Of the two LV's produced, only LV1 was significant (percent crossblock 56.82, p >0.04, LV2 percent crossblock 43.18%, p>0.776) consisting of several regions including insula, amygdala, hippocampus, posterior cingulate, dorsal anterior cingulate, subcallosal cingulate, hypothalamus, thalamus, putamen and brainstem (Figure 10A). Brain saliences loaded negatively in I-AM patients and minimally in I-BM patients meaning that regions in the LV show an association with negative affective in the I-AM patients, but not with the I-BM patients (Figure 10B). Brain regions indicated by the LV were extracted and correlated with negative PANAS scores. Anterior insula, brainstem, amygdala, medial prefrontal cortex, thalamus, premotor cortex and motor cortex (negatively loading regions) positively correlated with negative PANAS, and dorsolateral prefrontal cortex, temporal cortex, and parietal cortex (positively loading regions) correlated negatively with negative affect, but only in the I-AM. There was not a second significant LV showing a relationship common to both brain types or a relationship unique to patients with anterior insula metabolism below whole brain mean.

Presence or Absence of Negative Affect

The results of the whole-brain t-test comparing metabolism between patients endorsing the presence or absence of negative affect show a between group difference in the right subcallosal cingulate/ventromedial prefrontal cortex (MNI center of mass coordinates 12.2, 25.6, -11.5, 120, p<0.001, 120 voxels, Figure 11A). Patients endorsing the presence of negative affect show increased subcallosal cingulate (SCC) metabolism compared with patients who endorse the

absence of negative affect (Figure 11B). In the group endorsing the presence of negative affect, SCC activity correlates positively with negative affect scores (R=0.299, p> 0.025, Figure 11C).

The negative affect behavioral PLS excluding patients with an absence of negative affect largely replicated the results of the full sample (LV 1 percent crossblock 51.56, p>0.058, LV2 percent crossblock 48.44, p>0.645). LV1 brain saliences load positively on I-AM patients, and do not load on I-BM patients (Figure 12A). Most regions implicated in LV1 are produced by both negative affect PLS analyses, although there are some differences. The biggest difference between LV1s resulting from the different negative affect PLS analyses is the absence of the putamen, which appears in LV1 as produced by the PLS using the full sample, but not in LV1 as produced by the PLS using the sample limited to patients endorsing the presence of negative affect (Figure 12B). Further, overall, in the PLS limited to patients endorsing the presence of negative affect, regions are more extensive.

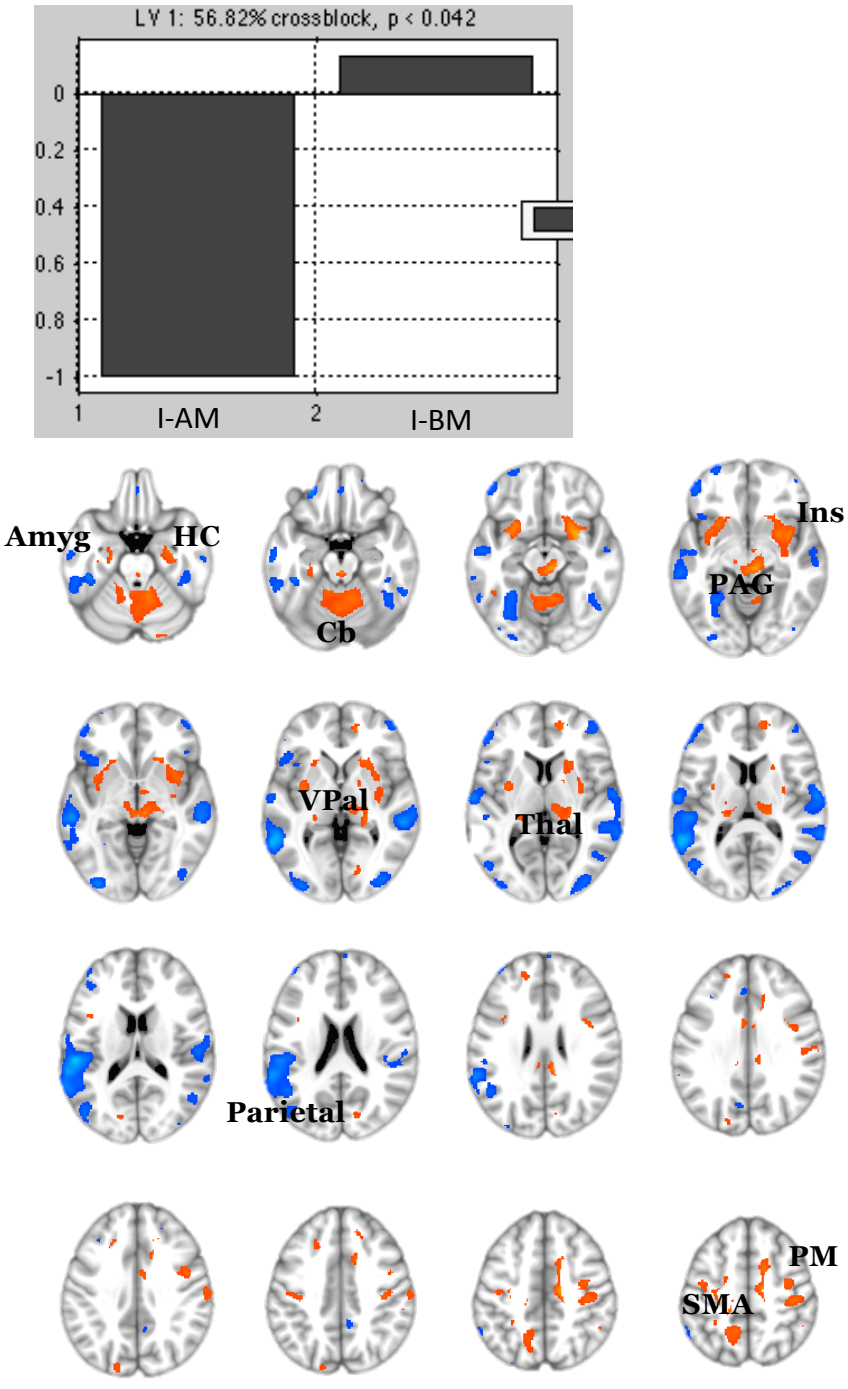

Figure 10: Negative Affect Behavioral PLS
Latent variable 1 (LV1) loads negatively in I-AM patients, and does not load in I-BM patients. Patients with anterior insula metabolism above whole brain mean, I-AM, patients with anterior insula metabolism below whole brain mean, I-BM. Amgy-amygdala, HC-hippocampus, PAG-periaqueductal grey, INS-insula, thal-thalmus, Parietal-Parietal cortex, PM-premotor cortex, SMA-sensory motor association area, V-Pal-ventral pallidum, Cb-cerebellum

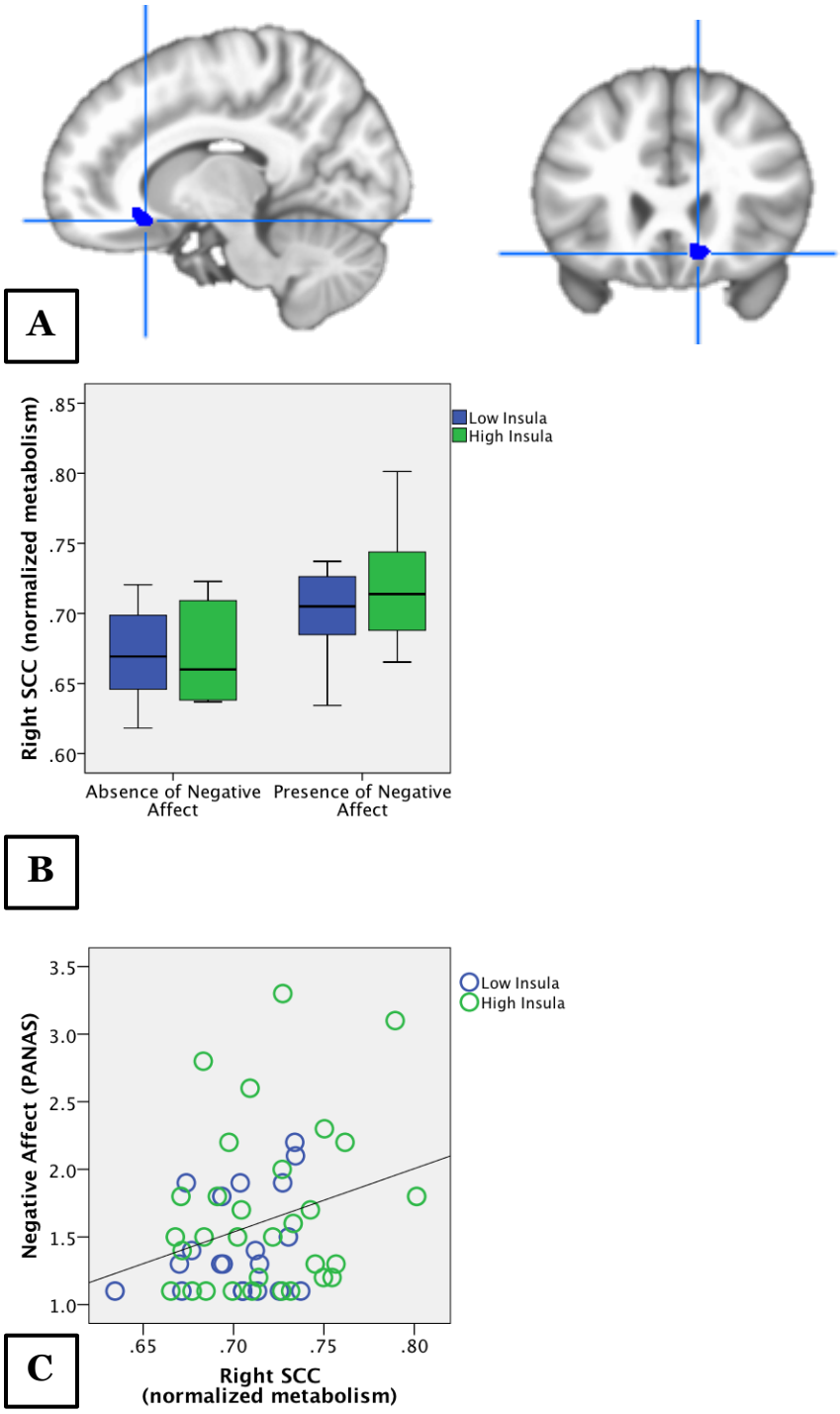

Figure 11: SCC differences between negative affect endorsement groups
A) Right SCC/ventromedial prefrontal cortex region is increased in the presence of negative affect group B) Boxplots of right SCC metabolism in presence and absence of negative affect groups, clustered by I-AM and I-BM type. C) Correlation of Right SCC metabolism with negative affect scores
SCC= subcallosal cingulate, I-AM=patients with anterior insula metabolism above whole brain mean, I-BM= patients with anterior insula metabolism below whole brain mean.

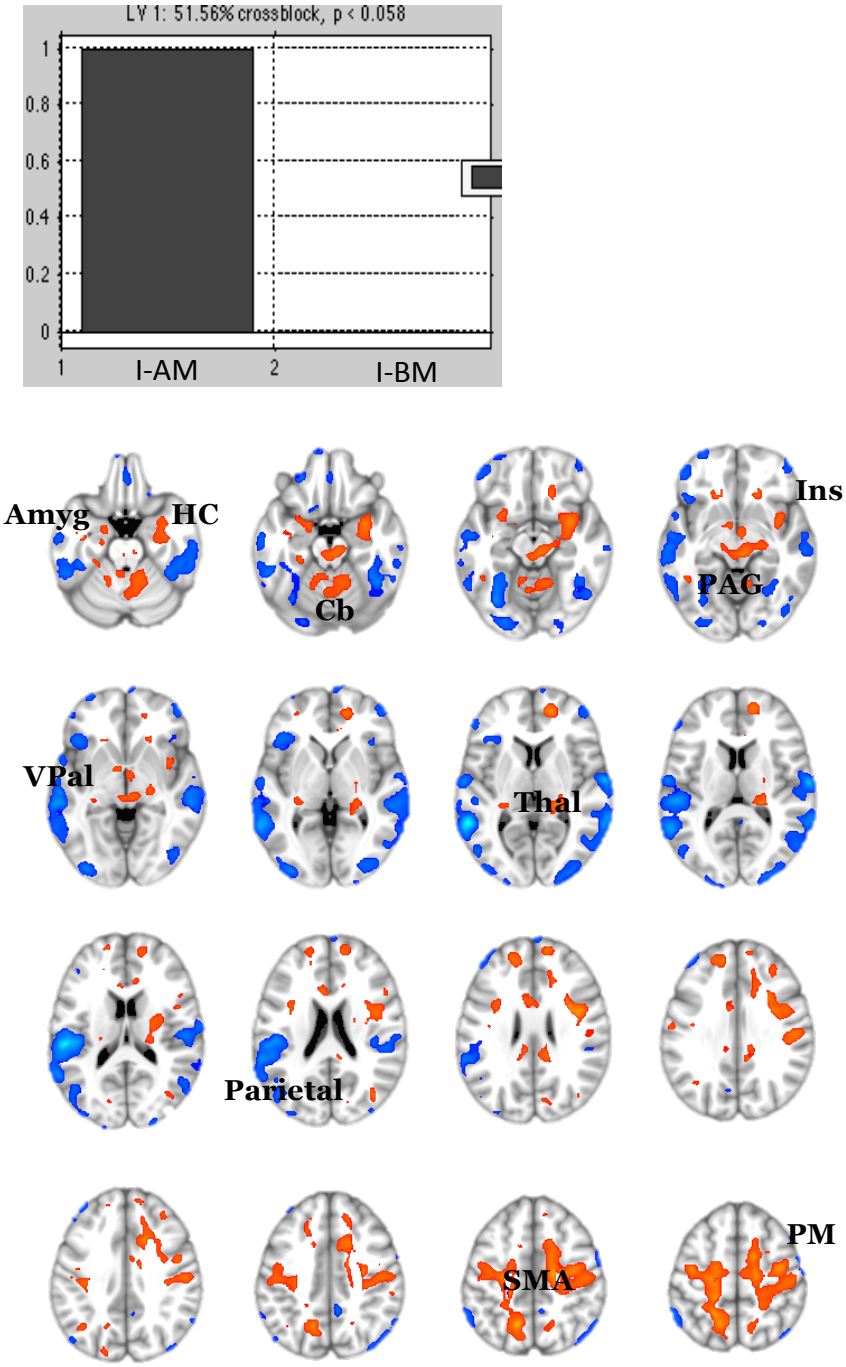

Figure 12: LV1 Negative Affect Behavioral PLS (presence of negative affect only)

Patients endorsing the absence of negative affect were excluded from this follow-up behavioral PLS. Patients with anterior insula metabolism above whole brain mean, I-AM ,patients with anterior insula metabolism below whole brain mean, I-BM. Amgy-amygdala, HC-hippocampus, PAG-periaqueductal grey, INS-insula, thal-thalmus, Parietal-Parietal cortex, PM-premotor cortex, SMA-sensory motor association area, V-Pal-ventral pallidum, Cb-cerebellum

Positive Affect Behavioral PLS Results

Permutation testing of the positive affect behavioral PLS presented no statistically significant (or near significant) LV (LV1 percent crossblock 54.92%, p>0.309, LV2 percent crossblock 45.08%, p >0.276).

HDRS Behavioral PLS Results

Permutation testing of the HDRS behavioral PLS produced no statistically significant LV (LV1 percent crossblock 64.28%, p>0.172, LV2 percent crossblock 35.732%, p>0.695). While permutation testing of LV1 did not reach statistical significance, the pattern of brain saliences is worth examining. In the HDRS behavioral PLS LV1, brain saliences load in a pattern opposite that of the negative affect behavioral PLS LV1, I-AM patients do not load on LV1, and I-BM patients load positively (Figure 13A).

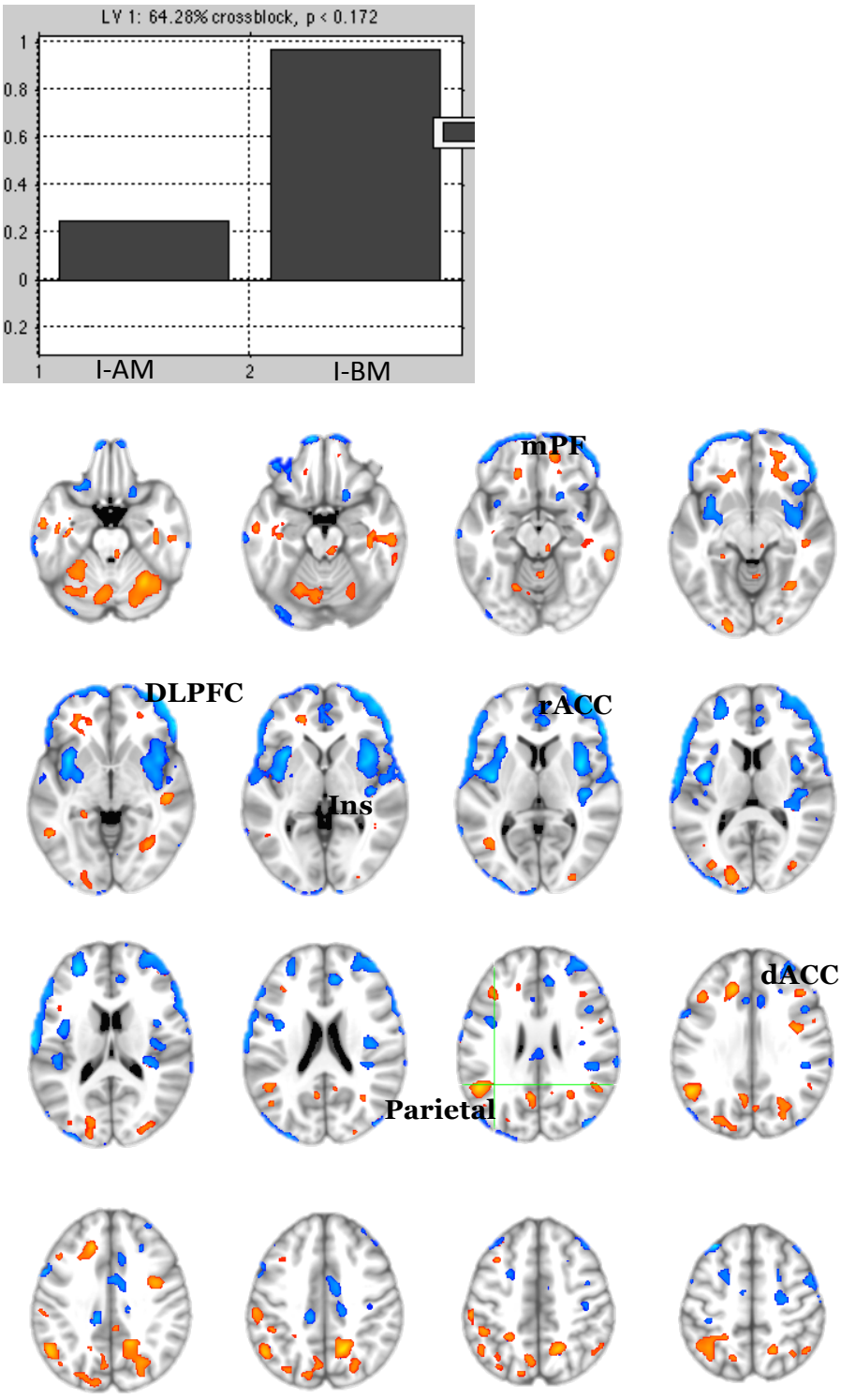

Figure 13: LV1 HDRS Behavioral PLS
Abbreviations: Ins-insula, DLPFC-dorsolateral prefrontal cortex, mPF-medial prefrontal, Parietal-parietal cortex, dACC-dorsal anterior cingulate, rACC-rostral anterior cingulate

Discussion

Negative affect is differentially associated with metabolism in anterior insula subgroups at the network level. Anterior insula metabolism above or below whole brain mean may represent different adaptations for regulating negative affect in depression. Both anterior insula subgroups show similar levels of negative affect, but negative affect is differentially associated with brain metabolism in I-AM and I-BM subgroups. Unique to I-AM patients, negative affect is associated with a pattern of activity distributed among both cortical and subcortical regions. In I-AM patients, metabolism in anterior insula, brainstem, amygdala, thalamus, medial prefrontal cortex, premotor cortex, motor cortex positively correlates with negative affect, and metabolism in dorsolateral prefrontal cortex, parietal cortex and temporal cortex negatively correlates with negative affect. The relationship of these regions with negative affect in the I-AM patients is consistent with early versions of the limbic-cortical dysregulation model of depression (Mayberg, 1997; Mayberg et al., 1999b).

The limbic-cortical dysregulation model of depression divides regional abnormalities into functional units, based on the framework that over activity in the limbic system reciprocally interacts with under activity in the cortex to mediate negative mood state. Similar to the functional segregation of regions in the model, decreased metabolism in dorsolateral prefrontal and parietal cortices is associated with negative affect. Further, anterior insula shows increased metabolism associated with negative affect. Thalamus and brainstem correlate positively with negative affect, unlike the limbic-cortical dysregulation model where they integrate cortical and limbic interactions. The strong positive

correlation of negative affect with brainstem activity is of particular interest. In human imaging studies, increases in brainstem activity, particularly in the periaqueductal gray (PAG), are associated with both pain and negative emotion (Buhle et al., 2013). In addition to the regions commonly seen in both the negative affect PLS results and the limbic cortical-dysregulation model, midline prefrontal and premotor/motor regions also share the pattern of increased activity associated with increases in negative affect. Most consistent with the limbic-cortical dysregulation model, increased subcallosal cingulate metabolism is associated with negative affect in both anterior insula subgroups.

Patients endorsing negative affect have higher subcallosal cingulate metabolism compared with patients not endorsing negative affect. Increased subcallosal cingulate activity associated with negative affect is consistent with previous studies correlating trait negative affect scores with brain activity (Zald et al., 2002) and activity changes during transient sadness (Keightley et al., 2003; Mayberg et al., 1999b). Patients with higher state negative affect may have difficulty down regulating SCC activity, regardless of patient type. The task of being at rest in the scanner is inherently a neutral state, however many patients experience intrusive negative thoughts and feelings. Based on results here, while both groups show variable success in attenuating negative feelings and thoughts during the resting state, anterior insula subgroups take different strategies to regulate such negative feelings. Patients with low SCC may be demonstrating an adaptation at the time of the scan, with I-AM and I-BM employing different strategies to decrease moment-to-moment negative affect. While the I-BM group did not show an association of brain activity with negative affect, anterior insula

metabolism is abnormal in the I-BM patients compared with healthy controls. We speculate that decreased anterior insula metabolism represents one adaptive approach attempting to down regulate negative affect in the I-BM patients while the reciprocal interaction of dorsal-cortical and ventral-limbic regions may attempt to down regulate negative affect in the I-AM patients. The network of brain regions that translates visceral changes into emotion in healthy persons-amygdala, insula, anterior cingulate, brainstem -overlaps with the network of regions associated with negative affect in I-AM patients (Critchley et al., 2005). One interpretation is that in I-BM patients, the translation of body state into affect is interrupted, resulting in a lack of significant correlation between brain activity and negative affect. This interpretation is further supported by differences in insula metabolism between the I-BM patients and healthy controls.

In addition to uncovering univariate differences in SCC activity, dividing patients based on whether they experience the presence or absence negative affect can help resolve issues of zero-inflation in the negative affect behavioral PLS. Regions in LV1 of the negative affect behavioral PLS in the full sample and in the sample restricted to patients endorsing the presence of negative affect are largely overlapping. Besides the subcallosal cingulate, no region showed a significant difference between absence/presence groups when directly compared. Together these analyses indicate that the absence of negative affect group is not systematically biasing the negative affect behavioral PLS results.

Positive affect showed no significant association with brain metabolism. Positive affect may be a less consistent measurement across depressed individuals, and more importantly positive affect is not intrusive during the resting state.

HDRS also showed no overall significant association with brain metabolism, however there was a non-significant association between HDRS and metabolism in the I-BM group. Both I-AM and I-BM groups show similar behavioral scores (HDRS), but a potentially different association with brain metabolism. This difference may reflect a second behavioral dimension where the relationship between brain and behavior can differentiate between I-AM and I-BM groups. This trend LV pattern is generally non-overlapping with the negative affect LV, meaning that disease severity and emotional state (specifically negative affect) do not share the same relationship with brain activity.

I-AM and I-BM groups represent two potential depressive subtypes that link to treatment outcomes and may relate to underlying network differences in emotion processing. These differences in emotion processing may represent different basic adaptive responses to the primary abnormality of depression. Both anterior insula subgroups reported elevated negative affect, but the brain activity correlating with the endorsement of negative affect differs between groups. The differences in ability to respond to treatment between the two groups may relate to fundamental group differences in network dynamics related to emotion processing.

All MDD patients are ill, with certain patterns being adaptive and others, maladaptive. We suggest that brain regions that show a difference from controls in a given subgroup that is beneficial to future treatment response may represent regions of compensation/adaptation. For example, patients with anterior insula metabolism below whole brain mean are abnormal compared with healthy controls, but the lower the activity in the anterior insula, the better patients do

when treated with CBT. Possibly anterior insula metabolic subgroups do not represent unique etiological types, but rather different compensation strategies that are best addressed using different treatments.

Experiment 2.2: Anterior Insula Metabolism Modulates Anterior Insula Functional Connectivity

A complementary approach to explore network dynamics of the anterior insula is to use functional connectivity analysis of resting-state functional magnetic imaging (rs-fMRI) measures. In healthy controls, the insula's functional connectivity is clearly divided by insula subregion. Previous studies of anterior insula functional connectivity show anterior insula functionally connected with pregenual (rostral) cingulate, anterior mid-cingulate and posterior mid-cingulate, the cerebellum, the inferior frontal gyrus/BA9, other parts of the insula, the inferior parietal lobule/BA40, right fusiform/BA37, right angular gyrus/BA39, right paracentral/inferior parietal gyrus/BA2/BA40 (K. S. Taylor, Seminowicz, & Davis, 2009). Other studies replicate functional connectivity of the anterior insula with the cingulate cortex, and show anterior insula connectivity with middle and inferior temporal cortex and other limbic regions that play a role in emotional functions (Cauda et al., 2011). Anterior insula functional connectivity is lateralized, with ventral anterior insula more strongly connected with the anterior cingulate cortex on the right side, and with the frontal cortex on the left side. Functional connectivity of the anterior insula has shown to be altered in major depression (J. P. Hamilton et al., 2011; Veer et al., 2010). Potentially some of these cortical and limbic functional connections vary with differences in anterior insula metabolism. In Experiment 2.2 we will compare right anterior

insula seed to whole brain functional connectivity between I-AM and I-BM groups to determine what functional connectivity relationships with the anterior insula are related to anterior insula metabolism subgroups.

Methods

Subjects

MDD patients were recruited as in Chapter 2. Of the 77 patients with available PET data, only 45 have corresponding resting-state fMRI data.

Anterior Insula Metabolism

Right anterior insula metabolism was extracted as in Experiment 1.1 Methods, page 34. Anterior insula subgroups are as defined on page 69 (anterior insula metabolism above or below whole brain mean, I-AM/I-BM).

Magnetic Resonance Image (MRI) Acquisition

In addition to the T1 structural MR images collected prior to treatment randomization (Experiment 1.1 methods, page 33), resting-state functional magnetic resonance (rs-fMRI) images were acquired using the following parameters: T2*-weighted echo-planar, TR/TE/FA=2920ms/35ms/90, zSAGA, 64x64 matrix, 220 mm FOV, slice thickness = 3.4x3.4x4mm, 30 slices, eyes open, cross hair fixation. The zSAGA (Heberlein & Hu, 2004) sequence was chosen to minimize sinus-cavity related artifacts in the medial and orbital frontal cortices.

Functional MRI Image Pre-Processing

Resting-state fMRI images were preprocessed using AFNI (Cox & Hyde, 1997) and FSL (Smith et al., 2004) software as follows. DICOM images, both EPI and T1 were converted to NIFTI format. The first TR was removed and EPI images were time shifted so that all slice timing was the same using the Fourier method. Next, each EPI volume was registered to the base volume. The anatomical image

was skullstripped, segmented into gray matter, white matter and CSF and a dilated whole brain mask was created. Transforms between the EPI and T1, and EPI and T1 to MNI space were computed using FSL's FLIRT and FNIRT (Andersson, Jenkinson, & Smith, 2010; M Jenkinson & Smith, 2001; Mark Jenkinson, Bannister, Brady, & Smith, 2002). EPI images were tissue segmented. White matter, CSF and local slice by slice white-matter signal (Jo, Saad, Simmons, Milbury, & Cox, 2010) regressors were created, and time series were calculated for each. EPI data was despiked and each voxel time series was scaled to have a mean of 100. EPI data was then detrended. Six degrees of motion (roll, pitch, yaw, ds, dl, dp), CSF, white matter, and slice by slice white matter were regressed out of the EPI data. Data was bandpass filtered through a range of 0.01 to 0.1 hertz. MNI transformation was applied to the EPI. Data was blurred to an 8mm Gaussian kernel. Careful quality control was applied at every preprocessing step.

Anterior Insula Seed-based Functional Connectivity

The anterior insula seed region for the functional connectivity analysis was based directly on the right anterior insula finding from Experiment 1.1 (see Figure 2). Mean activity in this right anterior insula seed was extracted from each subject's fMRI data, at each time point. Mean activity in the anterior insula seed was then correlated voxel-by-voxel in the whole brain for each subject, resulting in a time course correlation for each subject. Time course correlates were then z-scored using Fisher's method for statistical comparisons.

Statistical Analysis

An independent samples t-test compared whole brain anterior insula functional connectivity between anterior insula metabolism subgroups using AFNI. Z-scored time courses were extracted from each region that met statistical significance (p 0.001, uncorrected).

Results

Five regions show a significant difference in anterior insula functional connectivity between anterior insula metabolism subgroups. Functional connectivity with the anterior insula is greater in the I-AM patients compared with the I-BM patients in the left thalamus, midline mid-cingulate, right prefrontal cortex and left middle temporal gyrus. Anterior insula functional connectivity with the left subcallosal cingulate shows the opposite pattern, anterior insula-left subcallosal functional connectivity is decreased in I-AM patients compared with I-BM patients (Figure 14, Figure 15, Table 4).

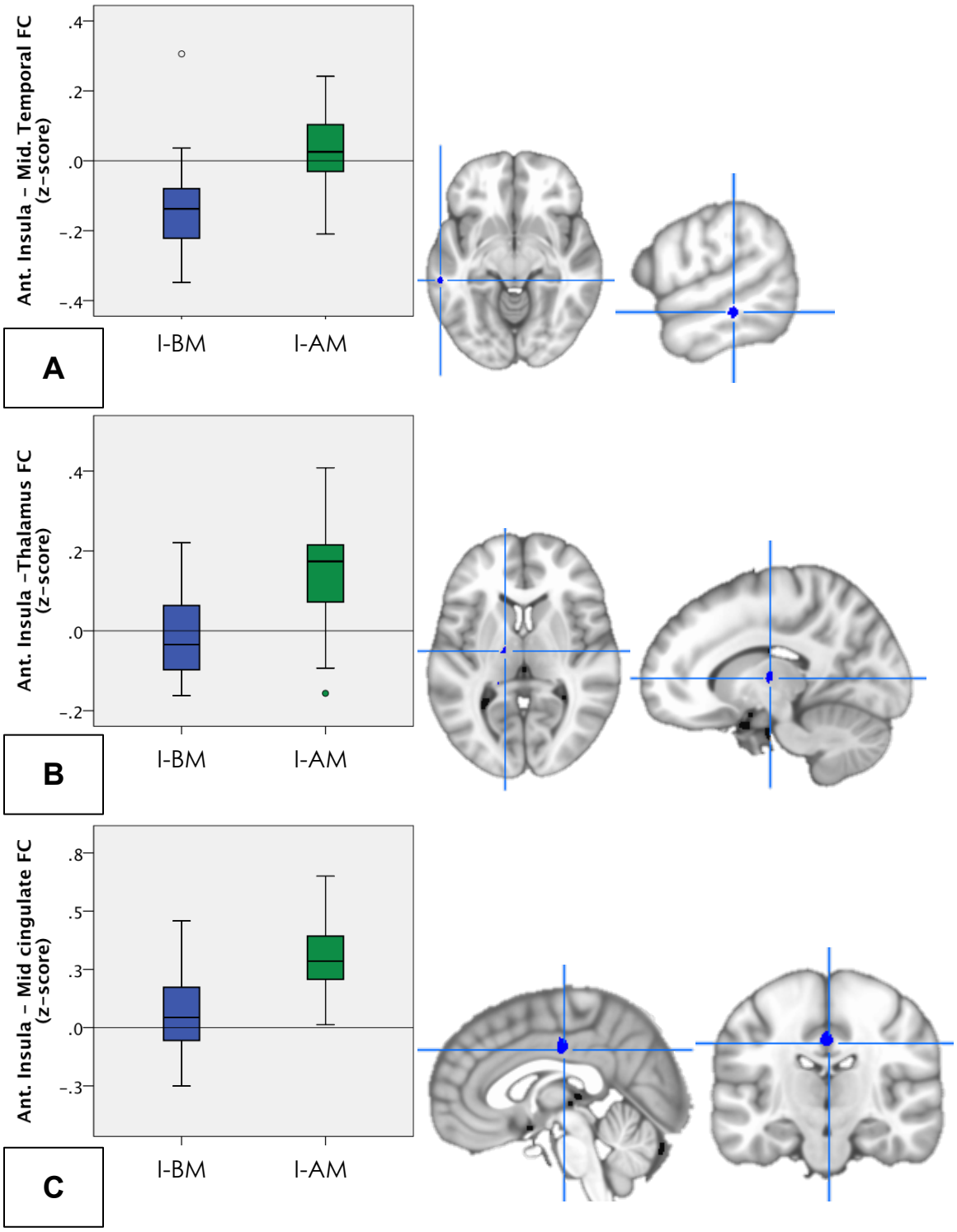

Figure 14 Right anterior insula FC differences between groups
Box Plots comparing anterior insula functional connectivity in anterior insula subgroups. A) Anterior-insula to middle temporal gyrus functional connectivity (FC) B) Anterior insula to left thalamus FC C) Anterior insula to mid-cingulate FC Patients with anterior insula metabolism below whole brain mean (I-BM), Patients with anterior insula metabolism above whole brain mean (I-AM)

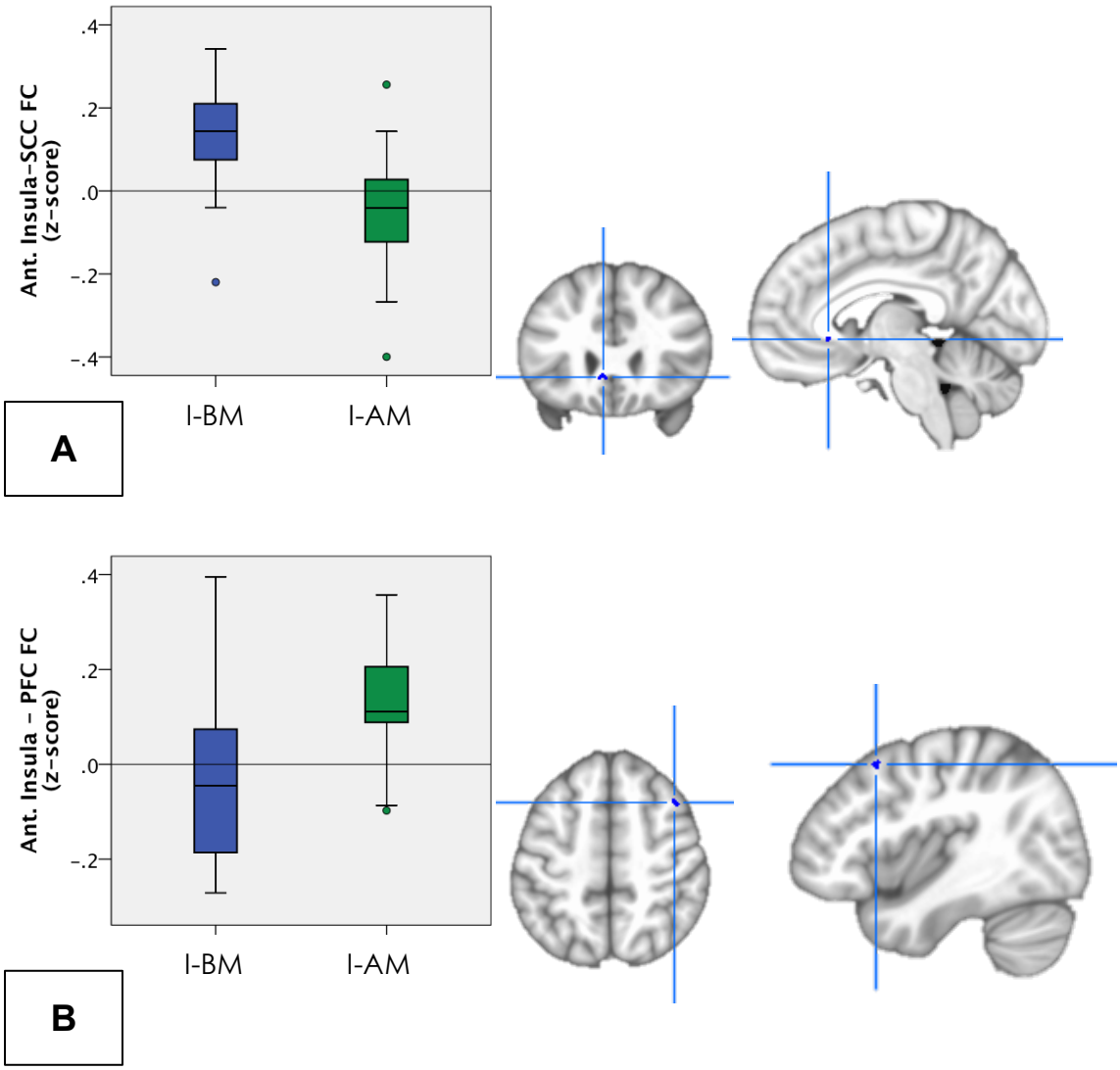

Figure 15: Right anterior insula FC differences between groups (cont'd)
Box Plots comparing anterior insula functional connectivity between anterior insula subgroups. A) Anterior insula to left subcallosal cingulate functional connectivity (FC) B) Anterior insula to prefrontal cortex FC
Patients with anterior insula metabolism below whole brain mean (I-BM), Patients with anterior insula metabolism above whole brain mean (I-AM)

Table 4: Ant. Insula FC T-test results

Region	**Side**	**Voxels**	**MNI coordinates** (center of mass) **x y z**
Mid-cingulate	midline	371	+0.3 -16.4 +37.5
Middle temporal gyrus	Left	80	+59.1 -32.8 -8.9 -
Prefrontal cortex	Right	68	-13.3 -12.6 +7.2
Thalamus	Left	67	+41.2 +17.8 +49.9
Subcallosal cingulate	Left	39	-6.2 +24.8 -3.6

Discussion

Anterior insula metabolism-based subtypes impact anterior insula functional connectivity. Functional connectivity of the anterior insula with the mid-cingulate, prefrontal cortex (PFC), thalamus, subcallosal cingulate (SCC), middle temporal gyrus varies between anterior insula subgroups. Mid-cingulate, PFC, middle temporal gyrus and thalamus show greater functional connectivity with the anterior insula in I-AM patients compared with I-BM patients. The left SCC is the only region that shows greater functional connectivity with the anterior insula in I-BM patients compared with I-AM patients. These differences in anterior insula functional connectivity may help explain why I-AM patients are responsive to sCIT and resistant to CBT, and I-BM patients are responsive to CBT and resistant to sCIT. Functional connectivity that varies between with anterior insula subgroups may either be a preserved relationship or adaptation that facilitates remission with a specific treatment, or may represent an abnormality that is better corrected by a specific treatment. These hypotheses may be better informed by studies looking at both changes in functional connectivity with treatment and comparisons with healthy controls. Specifically, such studies could help elucidate whether a pattern of functional connectivity is normal, normal but exaggerated, or abnormal, and whether differences in functional connectivity persist after successful (or unsuccessful) treatment.

Most of the differences in functional connectivity between groups show a lack of functional connectivity with the anterior insula in I-BM patients.

Psychotherapies, such as CBT may alter functional connectivity. Neuroimaging studies of Mindfulness training for depression treatment suggest that effective

psychotherapy alters region-to-region relationships. Mindfulness, and other psychotherapies 'train' the brain. The anterior insula is one region targeted by mindfulness training (Farb, Segal, & Anderson, 2013). Farb et al. show a limited functional connectivity between anterior insula and prefrontal cortex that is increased with mindfulness training. Our results show a decreased anterior insula-PFC functional connectivity in I-BM patients compared with I-AM patients. Activity in the anterior insula is essential for identifying the emotion significance of a stimulus and producing the associated affective state (Phillips et al., 2003a). Prefrontal cortex then interacts with anterior insula and other "ventral stream" regions, playing a role in emotional regulation. Potentially the prefrontal cortex's ability to regulate insula activity is disrupted in the I-BM patients, given the lack of functional connectivity between these two regions. Similar to mindfulness, CBT may retrain anterior insula-PFC functional connectivity. In patients where the functional connectivity relationship between insula and PFC is not absent, CBT may be less effective.

The neural model of biased responding to negative information in major depressive disorder (J. P. Hamilton et al., 2012) suggests that high pulvinar activity potentiates the amygdala, dorsal ACC and insula, and that activity in the dorsal ACC and amygdala fails to propagate up the ascending cortical-striatal-pallidal-thalamic circuit, which results in diminished responses to negative stimuli in the dorsal striatum and the dorsolateral prefrontal cortex. Anterior insula metabolic subgroups show differing functional connectivity between the anterior insula and thalamus. I-BM patients show low or negative anterior insula-thalamus functional connectivity compared with moderately positive

anterior insula-thalamus function connectivity in I-AM. If overactive thalamic activity is potentiating the salience network, then anterior insula subgroups are differently responding to such over activity. I-AM patients may show a stronger potentiation of thalamic over activity, leading to greater dysfunction in this cortical-striatal-pallidal-thalamic circuit.

Anterior insula functional connectivity with the mid-cingulate shows the strongest difference between anterior insula metabolic subgroups. Both the anterior insula and mid-cingulate are strong contributors to the salience network (K. S. Taylor et al., 2009). In this network, the anterior insula identifies salient information for deeper processing, particularly in the cingulate (Menon & Uddin, 2010). Anterior insula functional connectivity to the salience network has previously been shown to be interrupted in depressed patients (Veer et al., 2010). Anterior insula metabolism subgroups show different functional connectivity between the anterior insula and mid-cingulate. Because these regions normally act in concert, the decreased functional connectivity in the I-BM patients may be linked with a decreased ability to link salient information with action. The mid-cingulate is the cingulate's motor area, and plays a role in response selection (Vogt, 2005). Mid-cingulate may also play a role in cognitive tasks based on the reward value of particular behavioral outcomes (Bush, Luu, & Posner, 2000). Interruptions in functional connectivity of the anterior insula to the mid-cingulate may alter the flow of interoceptive information into evaluation of how rewarding a behavioral outcome may be.

Variability in anterior insula metabolism has direct implications for functional connectivity across multiple systems, including those involved in salience

monitoring and emotion regulation. Differences in anterior insula functional connectivity between these regions may underlie differential benefit from CBT and escitalopram treatments.

Chapter Summary

Chapter 3 addresses network differences between anterior insula subtypes. The neural network that underlies negative affective state in depression is different between the two anterior insula metabolism subgroups. Regions related to negative affective state in patients with anterior insula metabolism above whole brain mean matches well with regions indicated by the limbic-cortical dysregulation model. Patients with anterior insula metabolism below whole brain mean do not show a consistent relationship between brain activity and negative affect, however anterior insula metabolism below whole brain mean is abnormal compared with controls. Anterior insula metabolism subtypes also show differences in anterior insula functional connectivity between groups, with I-AM patients showing greater functional connectivity of the anterior insula with the mid-cingulate, thalamus, prefrontal cortex, and middle temporal lobe and I-BM patients showing greater functional connectivity of the anterior insula with the subcallosal cingulate. Anterior insula metabolism has implications for network dynamics, and network differences may underlie the anterior insula's treatment discrimination ability.

CHAPTER 4: BRAIN VARIANCE RELATED TO TWO-TREATMENT NON-RESPONSE[4]

Overview of Treatment Non-Response

Chapter 2 addressed a first-line biomarker, using a forced choice between two standard first-line treatments. Unfortunately, even multiple first-line treatments are not always effective. Less than 40% of depressed patients treated with first-line monotherapies achieve remission (American Psychiatric Association, 2000, 2010; Gaynes et al., 2012; Kennedy et al., 2009; National Collaborating Center Mental Health, 2009; Perlis, Patrick, Smoller, & Wang, 2009). After an initial treatment failure, subsequent steps generally involve switching between or combining first-line treatments. Common second-step treatment strategies include moving from psychotherapy to antidepressant medication (or vice versa), switching between antidepressant medications, or augmenting antidepressant medication treatment with psychotherapy or a second medication. However, such strategies result in additional remission rates of only 15-20% (Craighead & Dunlop, n.d.; Gaynes et al., 2012; Kocsis et al., 2009; Thase et al., 2007). Critically, the lack of response to initial treatments increases the vulnerability of non-remitting patients to ongoing suicidal ideation, social dysfunction, and treatment dropout (National Committee for Quality Assurance, 2007). While a first-line biomarker can help choose an optimal first treatment, some patients may require a different treatment option from standard care. Anterior insula

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[4] Parts of Chapter 4 are reprinted from Biological Psychiatry, epub ahead of print, CL McGrath, ME Kelley, BW Dunlop, PE Holtzheimer III, WE Craighead, HS Mayberg, Pretreatment Brain States Identify Likely Nonresponse to Standard Treatments for Depression, Copyright 2013, with permission from Elsevier

metabolism predicts remission to either CBT or sCIT in patients capable of achieving remission to one of those two treatments, but some patients will fail both CBT and sCIT. Chapter 4 examines potential metabolic predictors of patients who are unlikely to show meaningful improvement to either of these first-line treatments in order to characterize variance related to non-response to both CBT and sCIT (two-treatment non-response).

Experiment 3.1: Metabolic Predictors of Two-Treatment Non-response

We examined regional cerebral glucose metabolism that characterized non-response to both evidence-based psychotherapy (P) and a selective serotonin reuptake inhibitor (SSRI) (American Psychiatric Association, 2000). These P+SSRI treatment non-responders are defined as those patients who fail to respond over 6 months of treatment; the first 3 months randomized to either CBT or escitalopram (sCIT), the second 3 months receiving combined sCIT + CBT. Based on previous investigations of treatment failure in major depression (Dougherty et al., 2003; Konarski et al., 2009; Mayberg et al., 2005; Wu et al., 1999), we hypothesized that P+SSRI treatment non-responders would show increased pre-treatment subcallosal cingulate (SCC) metabolism as indexed by FDG-PET. Previous studies have shown hyperactivity in the SCC at baseline in patients who fail to respond to various treatments (Konarski et al., 2009), especially among those patients who have already failed at least one treatment (Dougherty et al., 2003; Mayberg et al., 2005; Wu et al., 1999). Many of the prior studies included patients on active treatment or patients who previously demonstrated treatment resistance. We explored the neural patterns associated

with non-response in depressed patients following randomized, controlled, stepwise treatment with two antidepressant interventions with different presumed mechanisms of action.

Methods

Subject Recruitment.

Recruitment of patients is detailed in Chapter 2.

FDG-PET Acquisition

FDG-PET acquisition is detailed in Chapter 2.

Treatment Protocol

Treatment protocol is as described in Chapter 2. Briefly, in Phase 1 patients were randomized to either CBT or sCIT. Patients who failed to remit to Phase 1 treatment were augmented with the second treatment in Phase 2.

Clinical Metrics

Clinical outcomes were defined using the HDRS with remission as the target endpoint. An HDRS score ≤7 at both weeks 10 and 12 of treatment defined Phase 1 remission. Similarly, Phase 2 remission was defined as an HDRS score ≤7 at both weeks 22 and 24 of treatment. Patients remitting at the end of Phase 1 or Phase 2 treatments were included in the 'remitter' group. P+SSRI non-response was defined by an HDRS change of <50% from baseline to the end of Phase 2 (Week 24). To avoid potential dilution of either the remission or P+SSRI non-response groups, dropouts and patients who achieved response but not remission (change in HDRS ≥50% but with an HDRS score >7) by the end of Phase 2 were not included in these main outcome groups, but were examined post-hoc. T-tests were performed to compare P+SSRI non-responders to remitters on clinical and

demographic variables as well as comorbid psychiatric disorders independent of the primary imaging analyses described below.

Imaging Acquisition

Imaging acquisition is as described in Chapter 2, Experiment 1.1.

Image Preprocessing

Image preprocessing is as described in Chapter 2, Experiment 1.1.

Image Analysis:

A priori region of interest analysis: subcallosal cingulate (SCC)

Based on the anatomical variability of the SCC in published reports of this region (variously incorporating Brodmann Areas 25,24,32) (Dougherty et al., 2003; Konarski et al., 2009; Mayberg et al., 2005; Wu et al., 1999), the entire subcallosal cingulate was surveyed using small volume correction methods (http://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html). The subcallosal cingulate volume was defined using FSL's Harvard-Oxford atlas (Desikan et al., 2006), SCC thresholded at 50% probability, centered on MNI coordinates -3, -17, 10. Within this bilateral subcallosal cingulate volume, the P+SSRI non-response and remitter groups were contrasted using a voxel-wise t-test. Results were considered statistically significant at a family-wise error (FWE) corrected $p < 0.05$ ($p < 0.005$ uncorrected, small volume cluster size 0.14 mL).

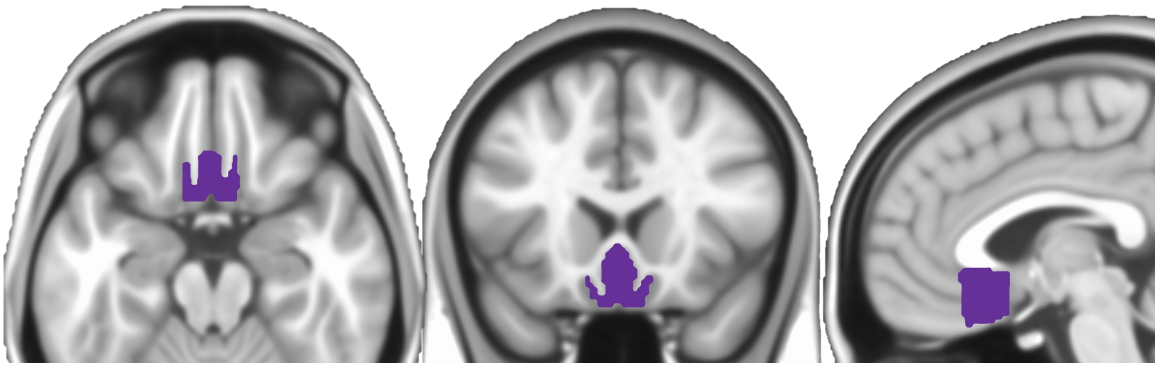

Figure 16: Atlas derived subcallosal cingulate (SCC) region of interest

Whole Brain Analysis

To probe other regions predictive of nonresponse to combined treatment, a whole-brain voxel-wise t-test was performed using the same P+SSRI non-response and remitter groups. Resulting clusters were considered statistically significant at a FWE corrected $p< 0.05$ ($p < 0.001$ uncorrected, cluster size 2.3 mL). Average metabolism was extracted in statistically significant regions of interest and *post-hoc* tests were performed.

Remitter by Phase, Remitter by Treatment Follow-up Analyses

The remitter group consisted of patients remitting to different treatments at different time points. To ensure that findings attributed to differences between P+SSRI non-responder and remitter groups were not due to systematic differences within the remitter group, two additional *post-hoc* t-tests were performed on all statistically significant regions. First, Phase 1 and Phase 2 remitters were compared to test for bias from different numbers of treatments (monotherapy vs. combined treatment). Second, to test for treatment specific effects, Phase 1 CBT monotherapy remitters were compared to sCIT monotherapy remitters.

Effect Size and Correlational Analyses

Effect sizes were calculated from the regions identified in the P+SSRI non-responder vs. remitter contrasts. To evaluate the relationship between regional metabolism and two-treatment outcome, percent change in HRDS from baseline to the Phase 2 endpoint was correlated with metabolism in each extracted region. Patients achieving remission during Phase 1, and those who did not enter or dropped out of Phase 2, were treated for a shorter period of time than Phase 2 completers and did not receive both treatments. In Phase 1 completers,

metabolism was separately correlated with the percent change in HDRS from baseline to week 12 (Phase 1 endpoint). These correlations allow for inclusion of patients with unclear outcomes in addition to those in the P+SSRI non-responder and remitter groups.

Comparisons with Healthy Controls

To further characterize the nature of identified patient group differences, mean metabolism in regions identified in the P+SSRI non-responder vs. remitter contrasts was extracted in the healthy control group (N=24,12 male/12 female; age mean±SD= 34.13± 7.74). A 3 group one-way ANOVA was performed, with post-hoc comparisons contrasting each patient group with controls.

Results:

Clinical Outcome:

Phase 1 and 2 outcomes are as described on in Chapter 2. Of the 27 patients, completing Phases 1 and 2, 12 remitted to the combined treatment, 6 achieved clinical response but not remission, and 9 were P+SSRI non-responders. Combined with the 24 remitters from Phase 1, the outcome groups analyzed included 36 remitters (35 with usable PET scans) and 9 P+SSRI non-responders (Figure 17).

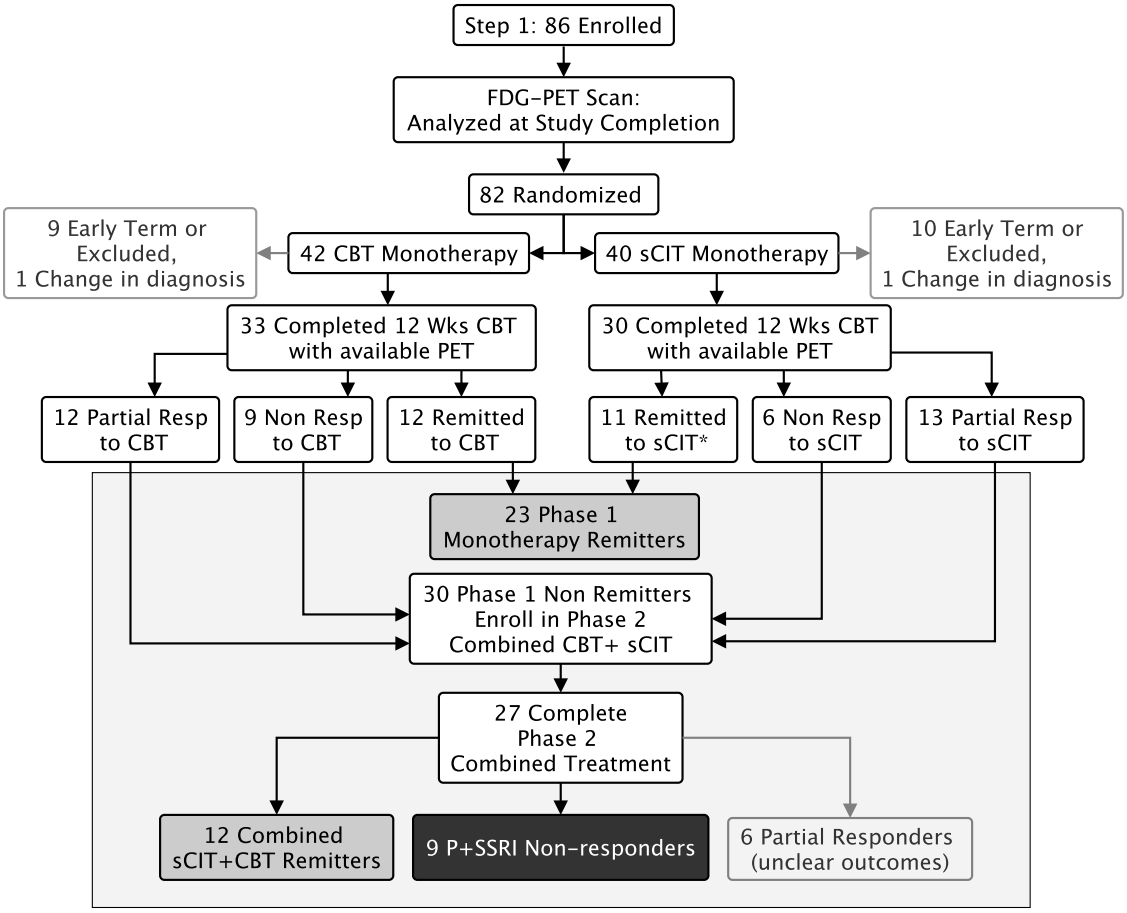

Figure 17: Combined Phase 1 and Phase 2 Outcomes

Remitter vs. P+SSRI Non-responder Comparisons of Clinical Variables

There were no demographic or behavioral differences between the remitter and P+SSRI non-responder groups (Table 5).

Subcallosal Cingulate Metabolism T-test Results:

Relative to the remitter group, significantly higher baseline left subcallosal cingulate metabolism was identified in the P+SSRI non-response group (FWE corrected $p \leq 0.05$) (Figure 18A, Table 2).

Whole Brain T-test of FDG-PET Results:

Only one region, the right superior temporal sulcus, met FWE corrected statistical significance ($p > .05$) in the whole brain t-test (Figure 18B, Table 6). Similar to the subcallosal cingulate, the right superior temporal sulcus showed relative hypermetabolism in the P+SSRI non-response group compared with the remitters.

Follow up T-tests between Remitter Groups

There were no differences in subcallosal cingulate or superior temporal sulcus metabolism between Phase 1 and Phase 2 remitters. There were also no metabolic differences in these regions between CBT and sCIT monotherapy remitters; indicating no compound treatment or treatment specific effects on these regions.

Effect Sizes and Full Sample Correlations with Outcome

Effect sizes are reported in Table 2. Right superior temporal sulcus showed the largest effect size (1.7 SD); the SCC effect size also exceeded 1 SD.

To verify that the regions defined by the P+SSRI non-responders were applicable to the complete sample and not just the extremes, correlations with percent change in HDRS were performed. We first tested the response in Phase 2

completers (n=27) to determine if the association of brain activity with response was consistent with the ANOVA results. The STS showed a strong correlation of metabolism with percent change in Phase 2 completers ($r= 0.655$, $p<0.0005$) while SCC metabolism showed a less strong correlation ($r= 0.364$, $p<0.06$) (Figure 19).

We also examined both regions for predictive potential in Phase 1 response; we limited these correlations to the 36 phase 1 completers who did not go on to phase 2. Although there was no significant correlation of percent change in HDRS with STS metabolism in this group ($r= -0.261$, $p<0.124$), the correlation of HDRS with SCC metabolism was significant ($r= 0.422$, $p<0.01$) (Figure 19).

Table 5: Demographic and behavioral comparisons between P+SSRI non-responders and remitters

Variable	P+SSRI non-responders (n = 9)	Remitters (n = 36*)	Test	p Value
Age, Years	43.8(8.0)	41.1(9.0)	t=-.830	.411
Duration of Current Episode, Weeks	156.6(250.7)	132.3(113.5)	t=.791	.791
Baseline HDRS	19.0(3.5)	18.5(3.1)	t=-.441	.661
Age of MDD Onset, Years	28.2(8.5)	27.8(12.1)	t=-.094	.926
Baseline HAMA	14.7(6.3)	14.0(2.9)	t=-.456	.650
Baseline BDI	21.6(3.0)	20.0(7.4)	t=-.611	.544
CTQ Total	40.3(10.3)	46.4(14.6)	t=.1.173	.247
Education, Years	15.0(1.8)	16.0(1.7)	t=1.357	.183
Gender, Female/Male	4/5	17/19	X^2=.200	.655
Treatment arm, CBT/sCIT	4/5	19/17	X^2=.200	.655
Current anxiety disorder No/Yes	7/2	26/10	X^2=.114	.736
Lifetime Substance Use Absent/Subthreshold/Threshold	4/3/2	21/10/5	X^2=.648	.723
Current MDD, None/Melancholic/Atypical	3/4/2	12/16/7	X^2=.022	.989
Previous Medication, No/Yes	1/8	10/26	X^2=1.083	.298
Previous Psychotherapy, No/Yes	5/4	17/19	X^2=.200	.655
Lifetime PTSD, No/Yes	9/0	33/3	X^2=.804	.370
Married or Cohabitating, No/Yes	5/3	14/21	X^2=1.337	.248
Employed Full Time, No/Yes	4/4	15/20	X^2=.135	.714
First Degree Family History, No/Yes	3/5	24/11	X^2=2.691	.101
Race, White/Black/Hispanic	8/0/0	26/5/4	X^2=2.602	.272
Lifetime Episodes, 1/2/3+	3/3/3	10/11/14	X^2=.145	.930

*Data are mean (SD) except as noted. Included only patients with available PET scans. HDRS, Hamilton Depression Rating Scale; HAMA, Hamilton Anxiety Scale ; BDI, Beck Depression Inventory; CBT, cognitive behavior therapy; sCIT, escitalopram; PTSD, Post traumatic stress disorder *Demographic and behavior data were available for all 36 remitters, PET scans were only available for 35 remitters*

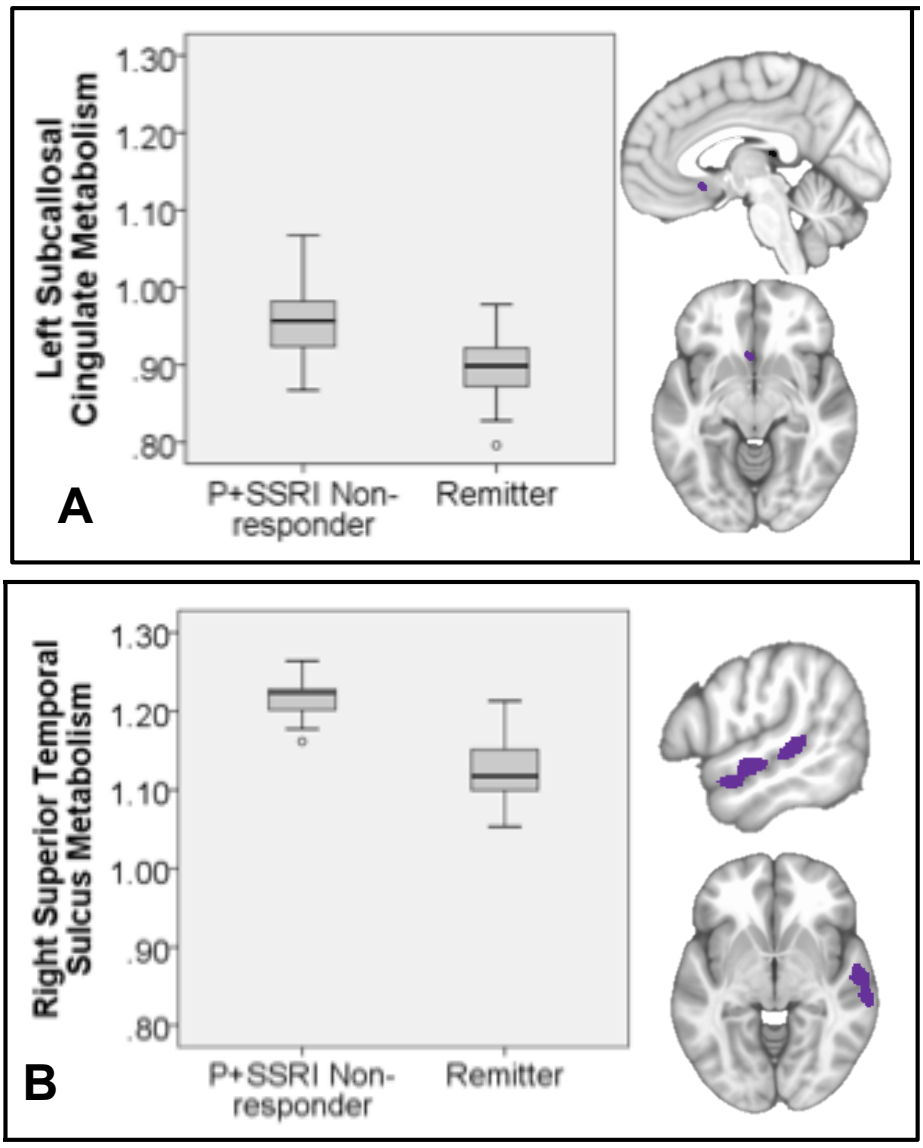

Figure 18: Subcallosal (SCC) region of interest and whole brain t-test results of P+SSRI non-responders compared with remitters.
Boxplots represent mean metabolism for each region of interest.

Table 6: Subcallosal Cingulate and Whole Brain T-Test Results

Anatomical Location	Cluster Size (voxels)	MNI coordinates x	y	z	T	Effect Size (N=44)
Subcallosal Cingulate T-Test						
Subcallosal Cingulate (L)	46	-1.5	-16.5	-9.0	2.963	1.15
Whole Brain T-Test						
Superior Temporal Sulcus (R)	1268	59.8	20.9	-3.0	3.530	1.76

MNI coordinates are center of mass. Cluster size reported at p 0.005 for small volume correction, p 0.001 for whole brain, p values FWE corrected at p< 0.05. L=left, R= right

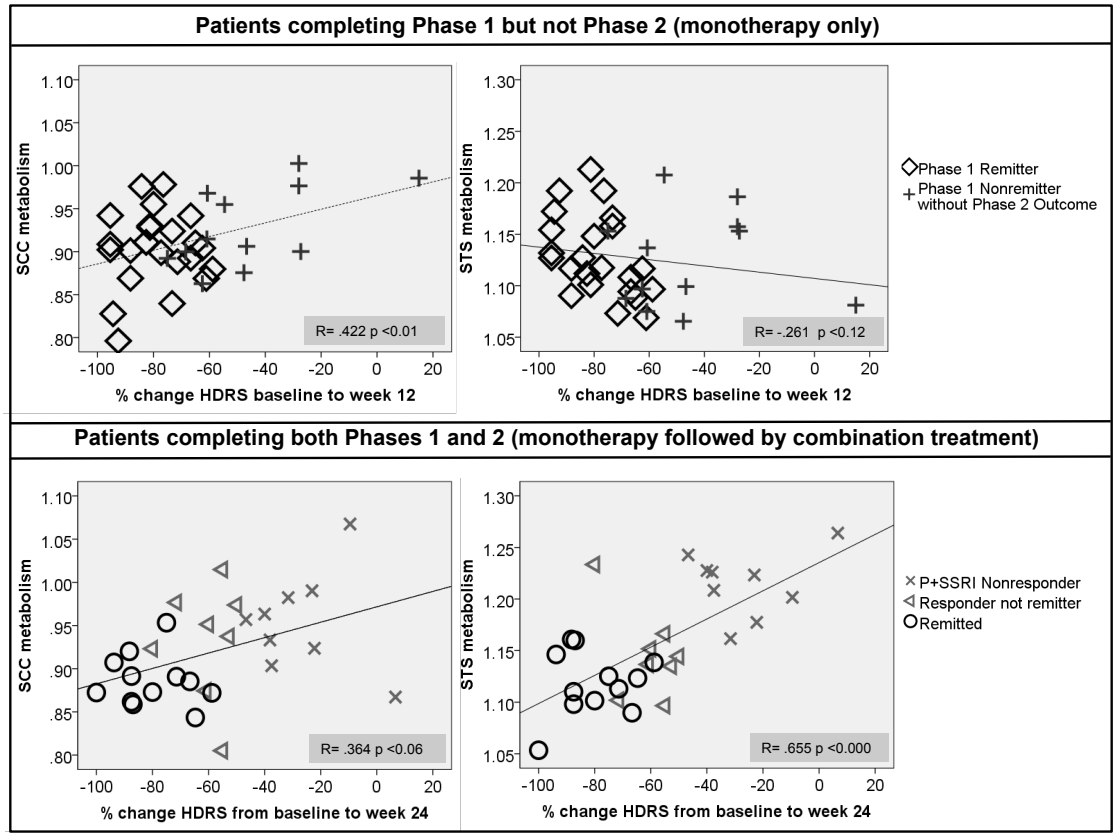

Figure 19: Subcallosal cingulate (SCC) and superior temporal sulcus (STS) metabolism correlated with outcome
Phase 1 Completers not entering/completing Phase 2 and Phase 2 Completers were correlated separately.

Comparisons with Healthy Controls

The one-way ANOVA of P+SSRI non-responder, remitter, and healthy control groups was significant for the SCC (F 4.767, p< 0.012) and STS (F 16.392 p <0.001) regions. *Post-hoc* t-tests showed higher SCC metabolism in P+SSRI non-responders compared with healthy controls (p< 0.022) but no difference between remitters and healthy controls (p< 0.854). STS metabolism was higher in P+SSRI non-responders compared to controls (p< 0.001) with remitters showing the opposite pattern; low STS metabolism in remitters compared with healthy controls (p<0.036). Although there was a significant difference in age between the control and patient groups, age did not correlate with SCC or STS metabolism (STS R= -0.087 p<0.388: SCC R=0.079 p< 0.434; N=101 [sample includes 77 patients with usable baseline FDG-PET scans and 24 controls]).

Discussion:

This study identified two candidate pretreatment FDG-PET biomarkers that distinguish P+SSRI non-responders from patients who remit to treatment with CBT or sCIT, delivered as monotherapy or in combination. These results extend Chapter 2's insula-based CBT/sCIT treatment-selection biomarker to identify brain states of patients who are potentially inappropriate for either intervention. As hypothesized, baseline subcallosal cingulate metabolism was higher in patients who went on to fail both treatments compared to those that remitted to either. Further, SCC activity in P+SSRI non-responders was uniquely increased compared with healthy controls. In the whole brain analysis, relative hypermetabolism was seen in the right superior temporal sulcus of P+SSRI non-responders.

The *a priori* hypothesis targeting the SCC was based on previous research identifying a relationship between treatment non-response and greater subcallosal cingulate activity, particularly in samples that have already failed at least one treatment (Dougherty et al., 2003; Greicius, Flores, Menon, & Glover, 2007; Mayberg et al., 2005; Wu et al., 1999). Baseline SCC hyperactivity has been reported in eventual non-responders to CBT or venlafaxine (Konarski et al., 2009) in a randomized 16-week monotherapy treatment study. SCC hyperactivity is also present in patients with multiple depression treatment failures, including ECT, compared with controls (Mayberg et al., 2005). This suggests over activity in the SCC is a core characteristic of eventual treatment nonresponse in depression. Functional connectivity fMRI studies further support over activity of the SCC, showing increased connectivity of the subcallosal cingulate to the resting-state default mode network in recurrently depressed patients with past treatment failures (Greicius et al., 2007). Lastly, the SCC is a surgical target for deep brain stimulation of treatment-resistant depression, with baseline SCC hyperactivity characterizing this extreme treatment resistant group (Mayberg et al., 2005). Chronic DBS in TRD is associated with a decrease in SCC activity with treatment, a similar change to that seen with successful response to SSRI, SNRI, rTMS, VNS, and ECT (Goldapple et al., 2004; Kennedy et al., 2001, 2011; Mayberg et al., 2000, 2005; Nobler et al., 2001; Pardo et al., 2008). The resting-state SCC hyperactivity seen here is unique to P+SSRI non-responders, compared with both remitting patients and healthy controls. SCC hyperactivity in P+SSRI non-responders may represent a non-responsive or ‘stuck’ sad mood state that

cannot be modulated by typical first-line interventions (Holtzheimer & Mayberg, 2011; Mayberg et al., 2005).

In addition to the SCC finding, right superior temporal sulcus (STS) showed significant metabolic differences between P+SSRI non-responders and remitters. The STS showed both a larger effect size and stronger correlation with two-treatment outcome suggesting it is a better discriminator of P+SSRI non-response than the hypothesized SCC region. STS and SCC metabolism were not significantly correlated ($r = .192$ $p > 0.095$, $N=77$) suggesting the two measures, while possibly related, are not redundant (Figure 20). Although a role for the STS in MDD is not strongly established, it has been identified in some fMRI connectivity and structural network studies of MDD, though without definitive interpretations (Liu et al., 2012; Sheline et al., 2009). More generally, the STS and other temporal lobe cortices are involved in evaluation of emotional valence (Peelen, Atkinson, & Vuilleumier, 2010), prospection (Schacter, Addis, & Buckner, 2007), and default mode network activity (Buckner, Andrews-Hanna, & Schacter, 2008). Alterations in the default mode network have been identified in MDD (J. P. Hamilton et al., 2013) as have changes in emotional self-evaluation (Fossati et al., 2003) potentially linking these STS findings to MDD more broadly. We speculate that STS hyperactivity may be related to the over-engagement of the default mode at rest in treatment-resistant MDD, contributing specifically to alterations in how the resting brain contextualizes emotion.

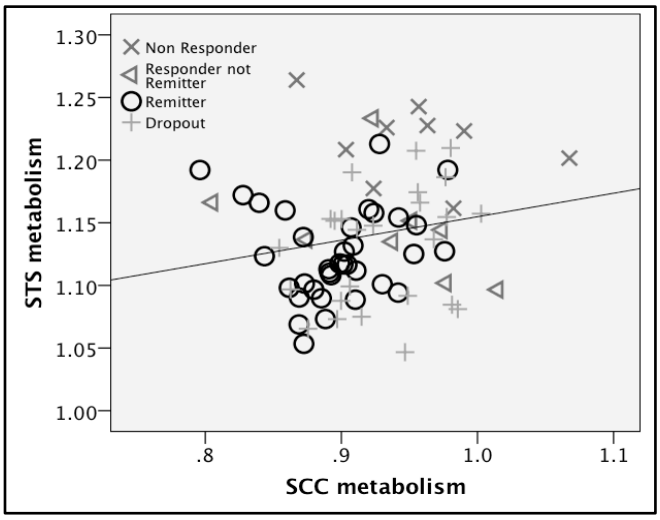

Figure 20: Correlation of STS and SCC metabolism in all patients

The results presented here contribute to the growing literature on predictors of response to treatments in patients with MDD. Fully integrating the current results with those of previous studies is challenging due to differences in patient samples and research methodology. One notable absence from the whole brain analysis was the rostral cingulate. Rostral cingulate activity has been repeatedly demonstrated to predict depression treatment outcome (Pizzagalli, 2011) primarily in studies of a single medication. We failed to identify a rostral cingulate discriminator in the initial whole brain analysis to define CBT-sCIT outcome differences. A post hoc analysis within each treatment did, however, identify a non-significant correlation between rostral cingulate metabolism and treatment outcome in escitalopram treated patients, but not CBT treated patients (Appendix A). This escitalopram specific trend is consistent with published studies demonstrating this same association of activity in Brodmann area 24a (rostral anterior cingulate) with hypermetabolism associated with response and hypometabolism associated with nonresponse to a single pharmacological treatment (Davidson, Irwin, Anderle, & Kalin, 2003; Kennedy et al., 2001; Mayberg et al., 1997). None of these published studies used remission as an outcome variable or examined patterns that specifically discriminated across different classes of treatments. However, to further explore this potential relationship, a post-hoc comparison of P+SSRI non-responders and remitters was performed using a rostral anterior cingulate region that included the entire perigenual anterior cingulate cortex. There were no differences in rostral cingulate metabolism between patients remitting to either treatment compared with those failing both treatments

Some limitations may affect the interpretation of these findings. The P+SSRI non-response group was not intentionally powered and is small. Patients achieving response but not remission at some point during the study (N=6) were excluded from t-tests identifying potential biomarker candidates further decreasing the non-responder sample size (N=9). To ensure detection of a difference that would represent the biology of unambiguous two-treatment non-responders, we intentionally avoided including patients responding but not remitting. Additionally, we did not group responders with remitters because of the known relationship between residual symptoms and greater likelihood of clinical relapse (Paykel et al., 1995). Although this decision provides for the most unambiguous biological signal detection, a next-step treatment choice would still need to be made for these patients. While SCC and STS metabolism show consistency across all levels of response, our data do not address this issue of choosing a next-step treatment.

A second limitation concerns the age difference between patients and healthy controls. While the age difference may be a factor in the differences between the patient group and controls, the lack of correlation between age and either SCC or STS metabolism, indicates that age is unlikely to be driving the identified differences. Age was consistent between P+SSRI non-responders and remitters, with SCC results showing a difference between P+SSRI non-responders and healthy controls and no difference between remitters and healthy controls. More importantly, analysis of the control subject data provides some context for understanding the nature of the regional hypermetabolism identified here, but

the comparison does not directly influence the interpretation of the biomarker itself for discriminating the two patient groups.

Although the SCC and STS show promise as biomarkers of P+SSRI non-response, replication in additional studies will be necessary before these patterns could be considered reliable for clinical use. Notably, the Phase 2 P+SSRI non-responders were a subset of those patients in the Phase 1 analysis (McGrath et al., 2013) with hyperactivity of the right anterior insula which predicted response to sCIT and failure to CBT (7 of 9). Taken together, a patient presenting with hypermetabolism of the anterior insula in combination with increased metabolism in the SCC and STS may benefit most from starting treatment with a non-SSRI antidepressant medication or an alternative therapy. This speculation will require explicit testing of alternative antidepressant medications and non-pharmacological treatments now reserved for more treatment-resistant patients. Future directions for this line of research will be to develop treatment algorithms based on imaging biomarkers, and clarification of whether these baseline findings represent trait markers or state markers that change with successful treatment. Replication of these imaging biomarkers in other cohorts will be required to achieve these goals.

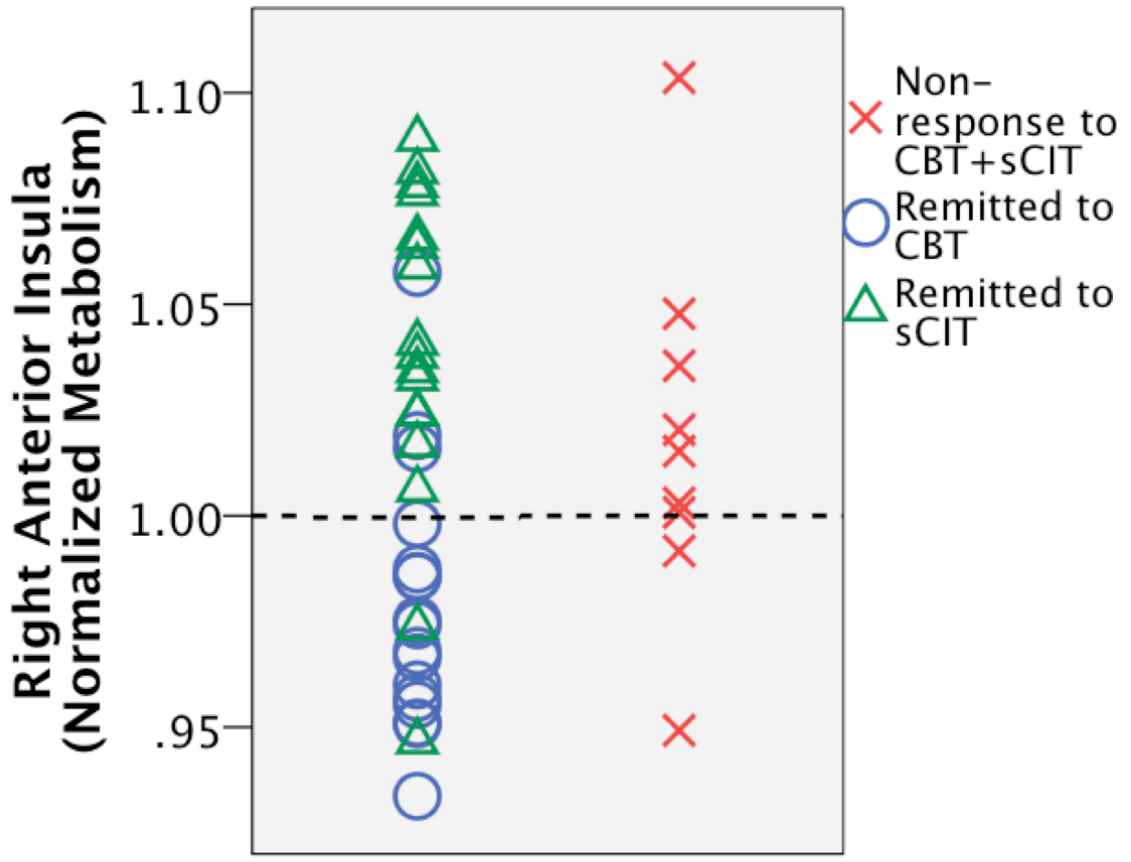

Figure 21: Right anterior insula metabolism by two phase outcome
Anterior insula metabolism graphed by outcome to Phases 1 and 2 of treatment. Red X's are subjects who fail to respond to CBT, sCIT or their combination, Green circles are patients who remit to CBT in either Phase 1 or Phase 2 of treatment. Blue triangles are patients who remit to sCIT in either Phase 1 or Phase 2 of treatment. The dotted line at one divides anterior insula subtypes into patients with anterior insula metabolism above and below whole brain mean.

Integration of SCC Marker with Anterior Insula Function

Seven of nine P+SSRI non-responders have anterior insula metabolism greater than whole brain mean (Figure 21). These data indicate that patients with anterior insula metabolism less than whole brain mean are unlikely to be non-responsive to the combination of CBT plus SSRI treatment, however replication is needed to support this interpretation. Interestingly, Chapter 3 Experiment 2.2 shows decreased functional connectivity with between the anterior insula and left subcallosal cingulate in the I-AM patients compared with the I-BM patients This difference in functional connectivity may underlie the different prevalence of two-treatment non-responders between anterior insula subgroups.

Experiment 3.2: Variance in Network Dynamics Related to Treatment Non-Response

The approach in Experiment 3.2 parallels Experiment 2.2's approach. Both experiments explore the network dynamics related to variability in a metabolism based predictor of treatment outcome. Here, we explore differences in rs-fMRI connectivity related to variance in SCC metabolism. SCC metabolism was elevated in two-treatment non-responders compared with both remitters and healthy controls. SCC variability across patients may not be limited to metabolism, but may extend to functional connectivity. Experiment 3.2 will measure the relationship between SCC metabolism and SCC functional connectivity.

Methods

Subject Recruitment.

MDD patients were recruited as in Chapter 2, Experiment 1.1. Of the 77 patients with available PET data, only 45 have corresponding resting-state fMRI data.

Patient Subgroups Groups

Patient subgroups were compared in follow-up analyses only. Anterior insula subgroups are as defined in Chapter 2. P+SSRI non-responder and remitter groups are as described in Experiment 3.1.

FDG-PET Acquisition

FDG-PET acquisition is as detailed in Chapter 2, Experiment 1.1.

FDG-PET Preprocessing

FDP-PET Preprocessing is as detailed in Chapter 2, Experiment 1.1.

fMRI Acquisition

fMRI data was acquired as in Chapter 3, page 80.

fMRI Preprocessing

fMRI preprocessing is as described in Chapter 3, page 88.

fMRI Seed Selection

Seed selection for seed-based functional connectivity was data-driven, based on Experiment 3.1. In Experiment 3.1, both SCC and STS regions were identified as predictors of two-treatment non-response. The SCC was selected as a seed for functional connectivity because 1) the SCC showed a relationship with treatment outcome across Phases 1 and 2 of treatment. 2) the SCC's role in depression is strongly supported in the literature.

The left SCC region indicated in the whole-brain P+SSRI non-responder vs. remitters t-test was transformed into fMRI space using AFNI. SCC seed-based functional connectivity maps were calculated for each patient.

fMRI analysis

For reference, whole-brain functional connectivity of the left SCC in the full MDD sample was calculated and thresholded using a one-sample t-test ($p>0.05$, false discover rate [FDR] corrected). Left SCC metabolism was regressed on left SCC functional connectivity using AFNI software. Activity in statistically significant regions (p 0.001, uncorrected) was extracted from the peak voxel. SCC functional connectivity with extracted regions was compared between P+SSRI non-responders and remitters, and between anterior insula subgroups using t-tests.

Results

Whole-brain SCC Functional Connectivity Map

The SCC shows functional connectivity in all MDD patients with bilateral orbital frontal cortex, medial prefrontal cortex, temporal cortex, posterior cingulate,

anterior cingulate, anterior insula, parietal cortex, amygdala and hippocampus (Figure 22).

Relationship of SCC Metabolism with SCC FC

Left SCC Metabolism correlates with left SCC functional connectivity with right rostral anterior cingulate (rACC), right medial orbitofrontal cortex (mOFC), right posterior hippocampus/parahippocampus (HC) and right prefrontal cortex (BA8). SCC-rACC functional connectivity correlates positively with SCC metabolism. SCC-mOFC, SCC-HC and SCC-BA8 functional connectivity correlate negatively with SCC metabolism (

Figure 23, Table 7).

SCC FC Regions Compared Between Groups

Results of the independent samples t-tests comparing SCC functional connectivity results between P+SSRI non-responders (N=7) and remitters (N=24) were significant for SCC-HC and SCC-rACC functional connectivity (Table 8). Independent t-tests comparing anterior insula subgroups also showed a difference in SCC-HC functional connectivity (Table 8).

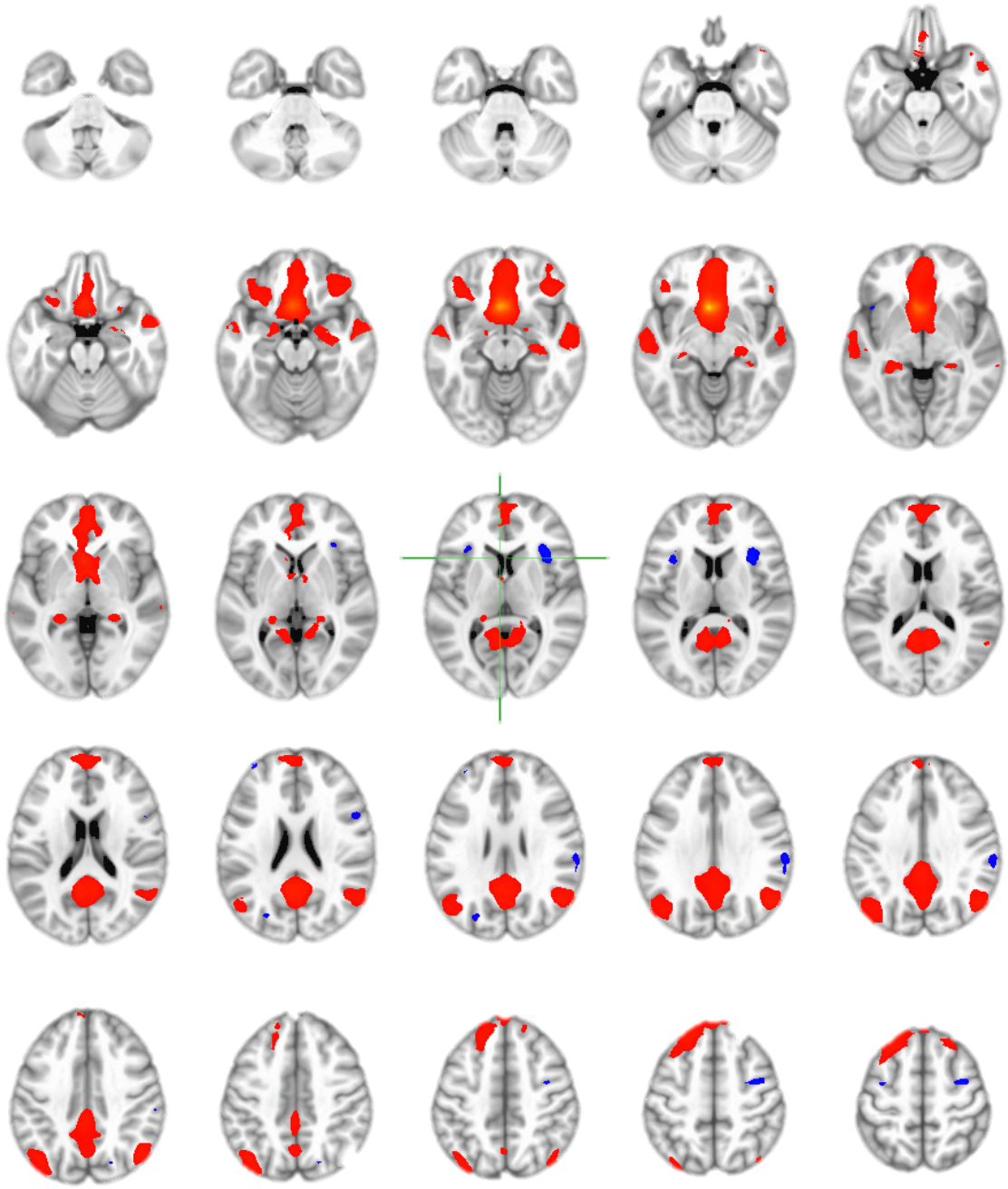

Figure 22: SCC Functional Connectivity Map
All MDD Patients SCC-seed based functional connectivity versus zero.

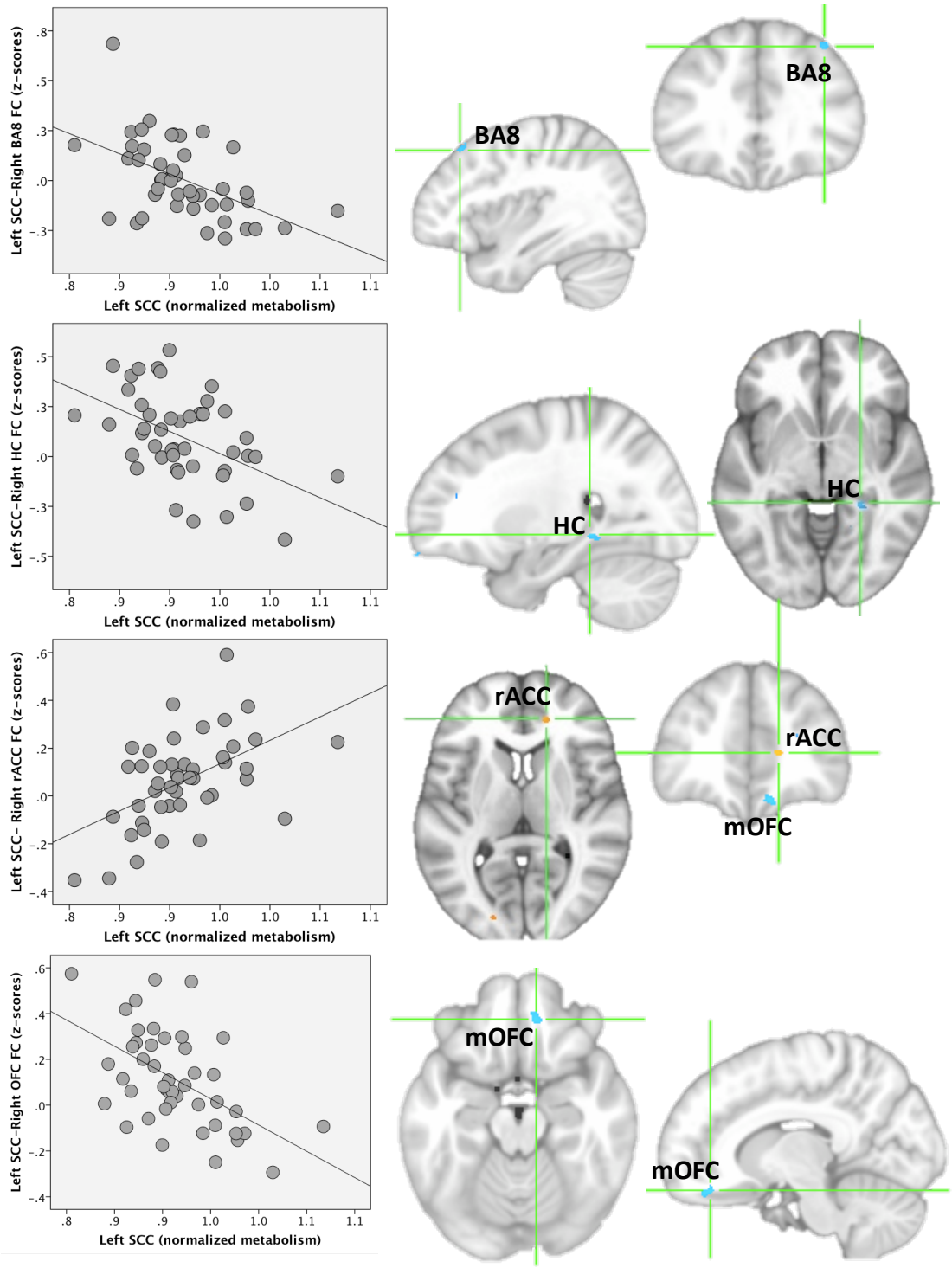

Figure 23: SCC metabolism correlates with SCC FC
A) Regions showing correlations between left SCC metabolism and left SCC functional connectivity. B) Scatter plots of left SCC metabolism correlated with left SCC functional connectivity (peak) with rostral anterior cingulate (rACC), medial orbitofrontal cortex (mOFC), posterior hippocampus/parahippocampus (HC) and prefrontal cortex (BA8).

Table 7: Correlation of Right SCC FC and Metabolism in Extracted Regions

Region	Side	Voxels	MNI coordinates	R (p 0.001)
Right mOFC	R	175	+11.0 +41.0 -18.0	-.536
Right HC	R	99	+25.0 -38.0 -5.0	-.494
Right BA8	R	95	+37.0 +32.0 +48.0	-.511
Right rACC	R	39	+16.0 +42.0 +7.0	.512

Table 8: Results of Subgroup Comparisons in SCC FC Regions

	P+SSRI NR v REM		I-AM v I-BM	
Region	***t (df=30)***	***Sig.***	***t (df=43)***	***Sig.***
Right mOFC	.752	.458	.552	.584
Right HC	3.681	.001**	-2.014	.050*
Right BA8	.910	.370	-1.851	.071
Right rACC	-2.140	0.041*	.572	.570

I-AM = patients with anterior insula metabolism above whole brain mean, I-BM = patients with anterior insula metabolism below whole brain mean

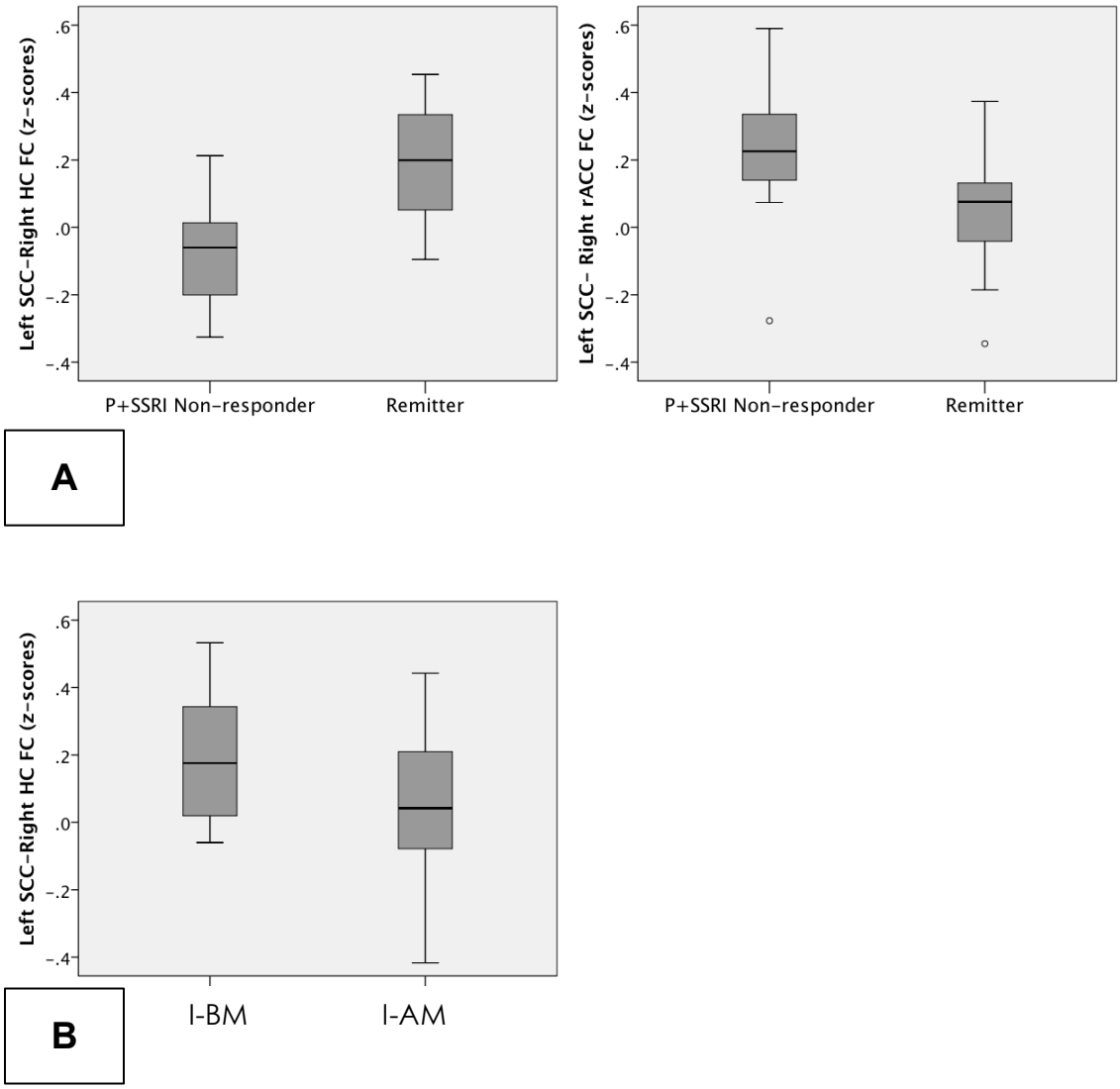

Figure 24: SCC-HC and SCC-rACC group differences
A) Boxplots of SCC-HC and SCC-rACC FC differences between P+SSRI non-responder and remitter groups. B) Boxplots of SCC-HC differences between anterior insula subgroups, anterior insula metabolism above whole brain mean (I-AM), anterior insula metabolism below whole brain mean (I-BM).

Discussion

Variance in left subcallosal cingulate metabolism is echoed by variance in left SCC functional connectivity. Functional connectivity between the left SCC and rACC increases as metabolism increases. SCC functional connectivity with right mOFC, right PFC and right HC increases as metabolism decreases. Tract-tracing studies show structural connections between SCC and each of these areas (for review see Hamani et al., 2011).

Variability in SCC connectivity and metabolism may be related to volume loss in the subcallosal cingulate. Many studies show reduced SCC volume, primarily loss of glial cells without loss of neurons, (Botteron, Raichle, Drevets, Heath, & Todd, 2002; Drevets et al., 1997; Hirayasu et al., 1999; D Ongür, Drevets, & Price, 1998) however findings are variable. Explanations for variance in findings range from the suggestion that patients with severe depression have greater volume reduction (Brambilla et al., 2002) to genetic based differences in cingulate volume (family history of depression, variance in volume with the serotonin transport). Functional variability in this region in depressed patients is mirrored by and may be related to structural variability in this region.

As discussed in Experiment 3.1, hyperactive SCC is linked with non-response to treatment. In this cohort, left SCC metabolism is higher in P+SSRI non-responders compared with remitters to either treatment. P+SSRI non-responders also show decreased SCC-HC connectivity and increased SCC-rACC connectivity compared with remitters to either treatment. Both P+SSRI non-responders and remitters show SCC functional connectivity with the rACC, but P+SSRI non-responders show a stronger connectivity between these regions. The SCC and

rACC share connections with amygdala, hypothalamus and brainstem regions involved in visceral monitoring. Increased functional connectivity between these regions may reflect a heighted state of vigilance in these patients.

SCC-HC functional connectivity is greater in remitters than P+SSRI non-responders. The hippocampus is a focus of depression research. Animal models have a rich literature on the role of stress and antidepressants in hippocampal plasticity (Duman, Malberg, & Thome, 1999; Warner-Schmidt & Duman, 2006). Patients show reductions in hippocampal volume that may be linked with recurrent depression and/or treatment outcomes (Frodl et al., 2008; Fu et al., 2012; MacQueen & Frodl, 2011; Sheline, Wang, Gado, Csernansky, & Vannier, 1996). Specifically, lower hippocampal volumes have been linked with treatment non-response. Such variance in hippocampal volume may impact the functional relationship between SCC and HC. Hippocampal dynamics related to the SCC may play a role in treatment non-response.

Further, SCC-HC functional connectivity also varies between anterior insula subtypes, with patients with anterior insula metabolism greater than whole brain mean showing a decreased connectivity between these regions. This result is consistent with a larger prevalence of P+SSRI non-responding patients with anterior insula metabolism greater than whole brain mean. Patients with anterior insula metabolism less than whole brain mean show preserved SCC-HC connectivity similar to the remitter group, while the patients with anterior insula metabolism greater than whole brain mean show decreased SCC-HC functional connectivity similar to the P+SSRI non-responders. The patients with anterior insula metabolism greater than whole brain mean show greater variability, which

is likely driven by the larger number of P+SSRI non-responders in this anterior insula subgroup.

Chapter Summary

Predictors of two-treatment non-response help explain heterogeneity in depression. SCC and STS hyperactivity is seen in two-treatment non-responders compared with remitters to either treatment. I-AM patients make up the majority of two treatment non-responders, suggesting this anterior insula subgroup may be more vulnerable to treatment non-response. SCC metabolism impacts network dynamics via functional connectivity of the SCC with rostral anterior cingulate, medial orbitofrontal cortex, parahippocampus/hippocampus and prefrontal cortex. SCC-hippocampus functional connectivity shows the most variance, with differences between both two-treatment non-responders and remitters, and anterior insula subgroups.

CHAPTER 5: INCORPORATING VARIABILITY INTO DEPRESSION MODELS

Summary and Significance of Findings

This dissertation evaluated neurobiological variability driven by differences in treatment outcome-based patient subgroups. By directly integrating neurobiological variability into current models, we can improve our understanding of network dynamics in major depression as well as move towards a more clinically relevant network conceptualization.

Chapter 2 defined two brain-based subtypes related to unambiguous treatment outcomes to cognitive behavior therapy (CBT) or escitalopram (sCIT). Experiment 1.1 identified anterior insula metabolism as a potential discriminator of CBT/sCIT remission. Experiments 1.2 and 1.3 supported anterior insula metabolism as a discriminator of treatment specific outcome in internal (Experiment 1.2) and external (Experiment 1.3) follow-up studies. Together, Chapter 2 findings suggest that right anterior insula metabolism above whole brain mean, and right anterior insula metabolism below whole brain mean represent two different brain-based depression subtypes that are linked with differential treatment outcomes. This candidate brain-based biomarker has the potential to directly impact clinical decisions regarding how to treat patients. While anterior insula metabolism would need to be prospectively tested before clinical use, metabolism in the anterior insula has the potential to determine whether patients should be treated with CBT or sCIT.

Chapter 3 investigated the relationship between anterior insula metabolism and network dynamics in depression. Experiment 2.1 showed differences between anterior insula subgroups in the neural network that underlies state negative mood measures in depressed patients. In I-AM patients, a latent variable including amygdala, hippocampus, parietal cortex, motor cortex, premotor cortex, dorsolateral prefrontal cortex and thalamus was associated with negative affective. I-BM patients did not show a consistent relationship between brain activity and negative affect, but patients endorsing negative across both anterior insula subtypes showed increased subcallosal cingulate activity. Experiment 2.2 tested the impact of anterior insula metabolic subtypes on anterior insula functional connectivity. Anterior insula functional connectivity with prefrontal cortex, thalamus, mid-cingulate, subcallosal cingulate, and middle temporal gyrus varied as a function of anterior insula metabolism. Together, Experiments 2.1 and 2.2 show that differences in anterior insula metabolism impact network dynamics. These differences in network dynamics are potentially related to how treatments induce remission. Analyzing functional connectivity differences based on a metabolically defined treatment biomarker is a new approach to understanding variance in depression neurobiology. The pairing of metabolic and rs-fMRI functional connectivity allow for a better understanding of what regional interactions may relate underlie responsiveness to specific treatments.

Chapter 4 expanded the treatment specific subtype analysis related to anterior insula metabolism to include variability related to two-treatment non-response. Patients failing to respond to both CBT and sCIT show hypermetabolism in the SCC and STS compared with remitters to either treatment or their combination.

Two-treatment non-responders primarily show I-AM suggesting that patients with relatively hypermetabolic insula activity may be more vulnerable to two-treatment non-response. Clinically, Chapter 4 works toward improving first-step biomarkers, like anterior insula metabolism, by identifying patients who may need an alternative first-line treatment strategy. Defining neural activity patterns predictive of failure to both a standard antidepressant medication and an evidence-based course of psychotherapy could help "fast-track" such patients to alternative treatments, partially circumventing the protracted trial-and-error process of current clinical care.

No published neural circuitry models directly address possible biological subgroups. Previous studies have looked at variability between different groups of patients in the context of a network model (Seminowicz et al., 2004), but variability has not been directly incorporated into neural circuitry models. Linking brain activity with treatment specific-subgroups and two-treatment non-response variability can help accommodate the "consistent inconsistencies" seen throughout depression research. Both treatment specific outcome-based subgroups (Chapters 2, 3) and variance related to non-response to two standard treatments (Chapter 4) contribute clinically meaningful variability at the brain level. By incorporating brain regions that differ between clinically relevant groups and their impact on network dynamics, we inform depression neural circuitry in a clinically useful way.

Incorporating Variance into Neurobiological Models

As detailed in the introduction, depression neural circuitry models are framed using different approaches. Here, we incorporate treatment outcome related variance into three published models of depression, the limbic-cortical dysregulation model (Mayberg, 1997, 2003; Mayberg et al., 1999b), the neural model of biased responding to negative information (J. P. Hamilton et al., 2012) and Phillips et al.'s neurobiology of emotion perception model (Phillips et al., 2003a, 2003b).

Limbic-cortical Dysregulation Model

Results from Chapters 2 through 4 are most easily integrated with the limbic-cortical dysregulation model. The limbic-cortical dysregulation model compartmentalizes brain regions implicated in depression by their function and functional interactions. Here we have refined and expanded the limbic-cortical dysregulation model to directly incorporate clinically relevant variance (Figure 25). This refinement preserves the models framework, while adding clinical utility.

The limbic-cortical dysregulation model depicts regions with known anatomical interconnections that also show synchronized PET changes across behavioral states, baseline post-treatment changes, and transient induced sadness. Dysfunction in this network can explain different combinations of clinical symptoms seen in depressed patients across mood, motor, cognitive, and vegetative domains. Regions are grouped into 4 main compartments, based on anatomy. The frontal-limbic segregation additionally identifies brain regions where an inverse relationship is seen across both sadness and depressive illness. This inverse relationship consists of decreases in dorsal neocortical regions and

relative increases in ventral limbic and paralimbic areas during sadness/depressive illness. The model also proposes that illness remission is related to appropriate modulation of dysfunctional limbic-cortical interactions . Our expanded model identifies functional compartments that predict treatment outcome and their functional interactions (although these regions do not necessarily interact with treatments).

Both treatment specific and two-treatment non-response predictors are similarly compartmentalized in the “interoception” functional compartment. Functional connectivity between predictive nodes of the “interception” and regions in the “cognition” compartment varies. In the context of the limbic-cortical dysregulation model, treatment-linked variance in activity between these ventral limbic and dorsal cortical regions may represent different targets for modulating dysfunctional limbic-cortical interactions. Further support for this interpretation is provided by the differential relationships between negative affect and brain activity between anterior insula subtypes, with patients with anterior insula metabolism greater than whole brain mean showing the more classic ventral limbic over activity, and dorsal cortical under activity associated with negative mood. Variance in these regions may make an individual patient more or less susceptible to modulation of dysfunctional limbic-cortical interactions with different treatments. This variation in activity may represent either a preserved function that facilitates functional recovery with a specific treatment, or may be an abnormality that a specific treatment can better target and correct.

Unique to the two-treatment non-responders, interactions between the “interoception” and “self awareness” compartments vary as a function of

predictors of two-treatment non-response. Variance in functional relationships between regions responsible for “self awareness” and “interoception” may be an one component of what differentiates treatment resistant patients from treatment responsive patients.

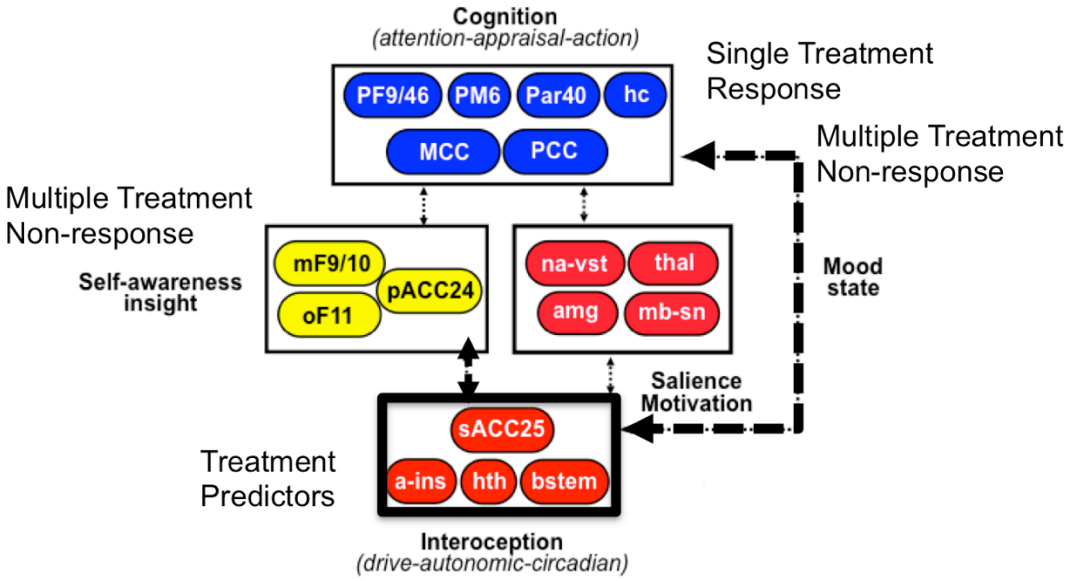

Figure 25: Adapted Limbic-cortical dysregulation model
Limbic cortical dysregulation model adapted and expanded from Mayberg 2009 to incorporate variance driven by outcome to treatment. Bolded black lines have been added around nodes and overlaid on interactions between regions that vary with treatments. Abbreviations: mF, medial prefrontal; dF, prefrontal; pm, premotor; par, parietal; aCg, dorsal anterior cingulate; pCg, posterior cingulate; rCg, rostral cingulate; thal, thalamus; bstem, brainstem; mOF, medial orbital frontal; Cg25, subgenual cingulate; Hth, hypothamus; Hc, hippocampus; a-ins, anterior insula; amyg, amygdala; p-ins, posterior insula, STS, superior temporal sulcus, motor, motor cortex, inf-temp, inferior temporal lobe. Numbers are Brodmann designations.

Negative Bias Model of Depression

Hamilton et al. describe a "neural model of biased responding to negative information" where over activity in the pulvinar nucleus of the thalamus primes anatomically connected regions important to the salience network, particularly the amygdala, dorsal anterior cingulate, and insula for a potentiated response to negative stimuli. Compounding this, nigrostriatal relays fail to propagate information to the dorsal striatum (specifically the caudate) and dorsolateral prefrontal cortex, limiting the ability of these regions to reduce the impact of negative stimuli. Hamilton's model assumes a primary problem in thalamus (a thalamic 'driver'), with maladaptation to that primary problem. This model assumes the same maladaptation across all patients. By incorporating our results with Hamilton's model, we identify multiple nodes in the neural model of biased responding to negative information that vary pre-treatment and are linked with treatment outcome. Our data is not consistent with the Hamilton model's assumption that all patients share the same basic maladaptation to a primary problem.

First, the Hamilton model does not include regions indicated by Chapter 4 as related to multiple-treatment failure, so it may apply less to that subgroup of patients. Excluding variability related to two-treatment non-response, we still have difficulty reconciling results from Chapters 2 and 3 with the Hamilton model.

A direct relationship between anterior insula and thalamus is not depicted in the Hamilton model, but variation in functional connectivity between the anterior insula and thalamus may be important to treatment specific outcomes. If the

thalamus is a primary driver, anterior insula metabolic subgroups have a different response to this primary stimulus. Anterior insula subgroups show differing anterior insula-thalamus functional connectivity. I-BM patients show a low or negative correlation between insula and thalamus compared with a moderately positive correlation between these regions in I-AM. Potentially, one subgroup may be showing a direct interaction between these regions that could be incorporated to refine this model.

The Hamilton model attempts to accommodate variability between patients using the relationship between the DLFPC and striatum. However, there are many more nodes that show variability that are not accommodated by the model. Given the data here, it is more plausible that this model may be most relevant to a specific subgroup, rather to depression more generally. In support of this premise, the increased connectivity between thalamus and anterior insula in patients with comparatively higher anterior insula metabolism fits with the concept that the thalamus primes the salience network. However, incongruent with salience network "priming", is the relationship between anterior insula and amygdala metabolism. Both amygdala and insula are important nodes in the Hamilton model, but both vary in relation to outcome to treatment. Anterior insula and amygdala metabolism are negatively correlated, meaning that in I-AM patients, the amygdala activity is lower than in I-BM patients. Unless thalamic priming has variable impact on different regions in the salience network, it seems that the salience network as described by Hamilton is a mixture of high and low states in different patients.

Adapted Neurobiology of Emotion Perception model

Phillips et al (2003a, 2003b) provide a conceptual framework of emotional regulation, which they extend to depression. Phillip's emotional regulation model is centered on three stages, identification and appraisal of a stimulus, producing an affective state in response to a stimulus, and regulating the produced affective state (Phillips et al., 2003a). The ventral system (amygdala, insula, ventrolateral prefrontal cortex, orbitofrontal cortex, ventral anterior cingulate, thalamus, ventral striatum and brainstem nuclei) identifies emotional significance, produces affective state, and regulates autonomic responses. The dorsal system (dorsolateral prefrontal cortex, dorsomedial prefrontal cortex, dorsal cingulate and hippocampus) is important for executive function, including selective attention, planning and effortful regulation of the resulting affective states. Potentially a reciprocal relationship occurs between these two systems. According to this model, volume reductions in the ventral system combined with increased activity in ventral system regions may result in a restricted emotional range, biased by the amygdala towards the perception of negative emotions. Amygdala and insula, two ventral stream nodes show variability in patient data. Patients with anterior insula metabolism greater than whole brain mean generally show low amygdala activity (and vice versa) suggesting that both nodes cannot be simultaneously overactive. Further, given the variance in amygdala activity, it is unlikely that the amygdala is similarly biasing the perception of negative emotions in all patients. Possibly this model is most applicable to patients with relatively low anterior insula and high amygdala metabolism. This interpretation is consistent with anterior insula subgroups engaging different neural systems

related to negative affect. As a caveat, this model does not link to motor and vegetative systems, so variance in these regions may be linked to domains outside of emotion and cognition.

In the dorsal system, structural and functional impairments associated with executive function and effortful regulation of emotional behavior may exacerbate this issue of ventral stream biasing, resulting in depressed mood and anhedonia. This element of the model is consistent with our data. I-BM patients (relatively low anterior insula and high amygdala metabolism) show a decreased functional connectivity between the anterior insula and dorsolateral prefrontal cortex. Further, hypermetabolism in the SCC predicts two-treatment non-response, and is related to a lack of functional connectivity between the hippocampus and SCC, although this relationship is seen in primarily in I-AM patients.

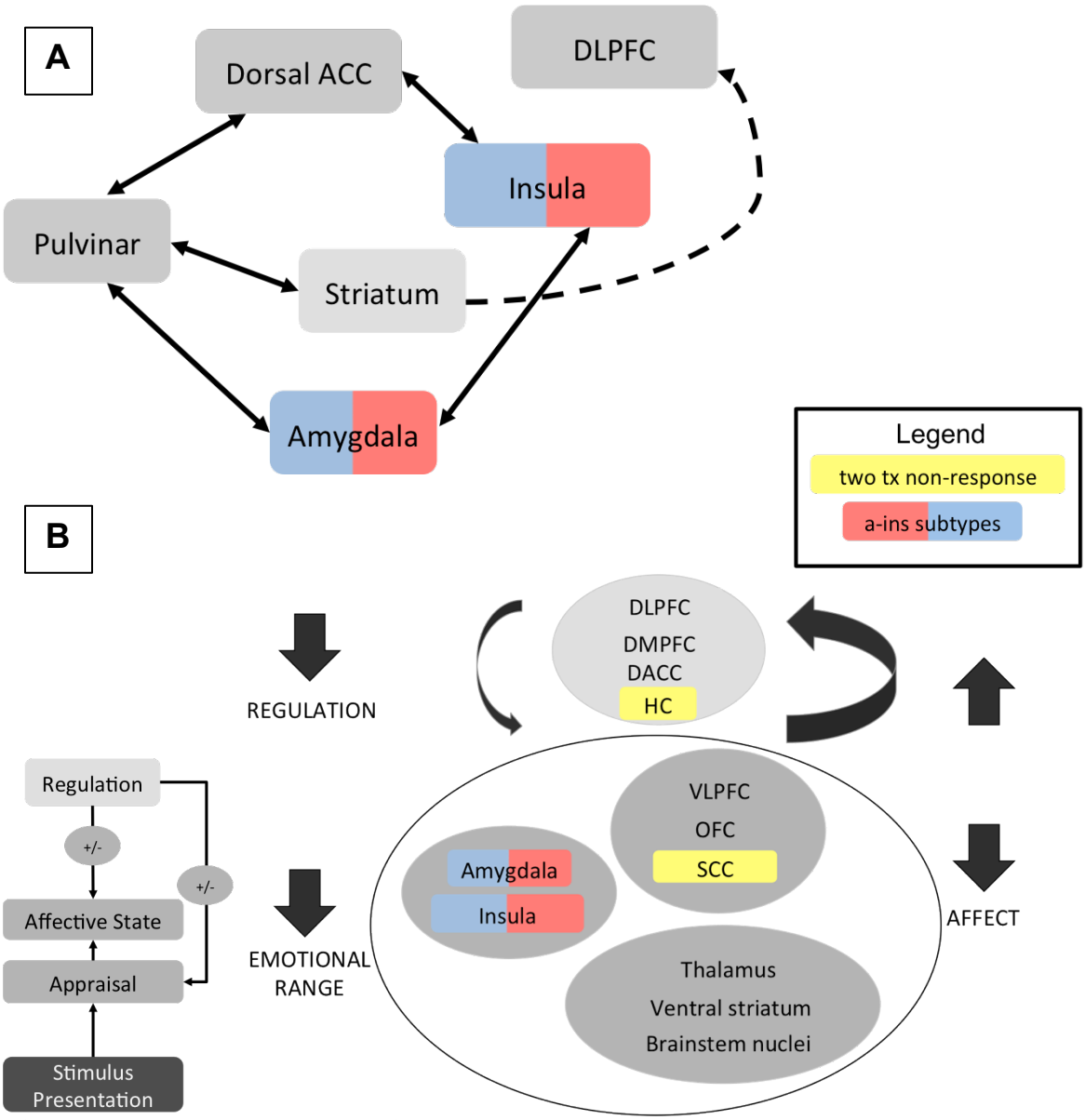

Figure 26: Adapted Depression Models
A) *Neural model of biased responding to negative information adapted from Hamilton et al. 2013. B) Neurobiology of Emotion Perception model Adapted from Phillips et al. 2003*

Future Directions

A next step in this line of research is to test the utility and scope of anterior insula metabolism as a clinical biomarker. First, how well anterior insula metabolism predicts remission to CBT and sCIT needs to be tested. In this initial study, evidence was slightly stronger for anterior insula metabolism as a predictor of CBT remission than escitalopram remission. Both anterior insula metabolism subtypes should be tested prospectively to see if they can reliably and robustly predict differential outcome to CBT and sCIT. If this candidate biomarker is replicated, its use in the clinic will need to be tested more broadly across different centers and other treatments. For instance, anterior insula metabolism below whole brain mean may or may not generalize as a predictor of remission to non-CBT types of psychotherapies. Similarly, anterior insula metabolism above whole brain mean may or may not generalize as a predictor of remission to any SSRI, or other antidepressant medications with different mechanisms of action. Further, the two-treatment non-responders identified in this study primarily showed I-AM. Whether I-BM is protective against treatment resistance will need to be tested. In addition, studies investigating whether anterior insula activity changes with worsening of depression symptoms that accompany relapse or recurrence are of interest.

Moving forward, studies of change with treatment can also be informative. Anterior insula metabolism was measured pretreatment. Previous studies have shown changes in the anterior insula with treatment, but have not looked at the interaction of treatment and pre-treatment subtypes. Whether anterior insula metabolism subgroups are differently targeted by the different treatments is

unknown. Mindfulness has been show to modify anterior insula functional connectivity. This should be directly tested using CBT in anterior insula subtypes. Further, some previous studies of change with treatment show “corrections” of abnormalities while others show “adaptive” changes in regions that were not abnormal to start. Change studies may be one approach towards understanding compensatory changes versus “lesions”.

Changes in SCC activity with treatment should also be assessed. Previous studies have shown decreases in SCC activity with successful treatment, but questions remain about the interaction of change with treatment and pretreatment activity. In Experiment 2.1, some patients experienced intrusive negative affect during the resting state, while other patients did not. Further, brain activity in anterior insula subgroups was differently related to negative affect. This data suggests that the ability to prevent intrusive negative affect may represent a positive adaptation in depression. If so, anterior insula subgroups appear to achieve such mood regulation with different strategies, and some patients in each group are successful and some are not. How anterior insula subgroups differently regulate mood state and why some patients are successful while others fail should be pursued.

Future studies can also investigate how other biological measures relate to anterior insula subgroups as well as how such measures could A) be contributing to anterior insula metabolic variability and B) be used as potential bedside surrogates for anterior insula metabolism. A number of candidate biological measures, including but not limited to genetics, stress responses, heart rate variability, inflammation and pain processing should be investigated.

Potential Relationships of the Anterior Insula to Biological Measures

Hypothalamic-pituitary-adrenal (HPA) Axis

Alterations in hypothalamic-pituitary-adrenal (HPA) axis activity have been reported in depressed patients, but are not seen in all patients (Pariante & Lightman, 2008). Regulation of both adrenocorticotropic hormone (ACTH) and cortisol has been associated with right insula activity during induced sadness, where increased cerebral blood flow in the right insula was associated with increased ACTH and cortisol measures (Ottowitz et al., 2004). Anterior insula metabolic subgroups may show differences in HPA axis activity, either at rest or when provoked. The Ottowitz et al. study suggests that I-AM patients may show increases in cortisol, particularly in patients endorsing negative mood, as anterior insula metabolism is correlated with negative affect in this anterior insula subgroup.

Inflammatory Markers

Inflammation has been linked with treatment non-response in major depression. A relationship between anterior insula metabolism and inflammation should be investigated. In a preliminary analysis of inflammatory measures in this patient cohort, interleukin-6 (IL-6), a pro-inflammatory cytokine, did not correlate with response to treatment. However, in patients who received a first treatment matching to their anterior insula subtype, patients who did not achieve remission showed greater levels of inflammation (See Appendix B). Increases in inflammation may explain why some patients failed initial treatment even though they received treatment matching their insula status.

Autonomic Functions

Activity in the anterior insula has been related to autonomic function, particularly cardiovascular function (Critchley et al., 2005; Ruggiero, Mraovitch, Granata, Anwar, & Reis, 1987). The anterior insula shows an asymmetric relationship with autonomic function where the right anterior insula has been linked to regulation of sympathetic activity, and the left anterior insula has been linked with regulation of parasympathetic activity (Craig, 2005). Anterior insula subtypes may show a differential balance between left and right anterior insula activation of sympathetic and parasympathetic systems.

Heart rate variability is one candidate for a surrogate measure of anterior insula metabolism as a predictor of treatment outcome. Low heart rate variability has been reported in depressed patients (Musselman, Evans, & Nemeroff, 1998) and in healthy controls, changes in heart rate variability correlate with functional connectivity between the anterior insula and amygdala (Chang et al., 2013), directly linking heart rate variability measures with anterior insula activity.

Studies suggest that decreases in heart rate variability in depression may be linked with decreased parasympathetic innervation, leaving an unopposed stimulation by the sympathetic nervous system (Gorman & Sloan, 2000). Differences in the balance of sympathetic and parasympathetic innervation may explain why the anterior insula predictor is unilateral. Pupillary function is a second measure of autonomic activity that may be related to anterior insula function. Similar to heart rate, pupillary function is regulated by a combination of sympathetic and parasympathetic inputs. Changes in pupil size in the context of expressions of sadness are associated with activity changes in amygdala,

insula, superior temporal sulcus and brainstem (Harrison, Singer, Rotshtein, Dolan, & Critchley, 2006). Further, pupillary response to emotional information has been previously indicated as a potential predictor of remission to cognitive therapy where low sustained pupil responses to negative information may indicate which high-severity patients will respond to cognitive therapy (Siegle, Steinhauer, Friedman, Thompson, & Thase, 2011).

Genetics

Multiple single nucleotide polymorphisms (SNPs) confer risk for major depressive disorder. Potentially, differences in anterior insula metabolism may have an underlying genetic component that should be investigated. Given the potential relationships between insula activity and HPA axis, autonomic processing and/or inflammation, SNPs linked with HPA axis function, autonomic processing and/or the immune system are good candidates for studies of genetic contributors to anterior insula metabolic subgroups. For example, genes that contribute to HPA axis regulation, corticotrophin-releasing hormone receptor 1 and FKBP5, have previously been linked with treatment response (Binder et al., 2004; Licinio et al., 2004) and may also relate to anterior insula metabolism.

Pain Processing

Alteration of pain threshold is another candidate biological measure that could help explain differences in anterior insula metabolism between patients. Pain thresholds have been show to be variably altered in depression (Bär et al., 2005), and pain processing is linked with activity in the insula (Zaki, Ochsner, Hanelin, Wager, & Mackey, 2007). Possibly changes to pain thresholds may vary in depression patients as a function of insula activity. Further, previous studies

show altered pain processing in the insula in depressed patients (Mutschler, Ball, Wankerl, & Strigo, 2012; Strigo, Simmons, Matthews, Craig, & Paulus, 2008). Pain processing may be different between anterior insula subgroups at both the brain and body levels.

Multiple biological measures have potential to help explain variance in anterior insula metabolism, to help explain variance in addition to the anterior insula or to be developed as non-imaging behavioral or psychophysiological surrogates for insula metabolic activity. By integrating non-brain measures of biological systems related to depression with future studies of variation related to anterior insula metabolism, we can potentially better refine treatment outcome related depressive subgroups.

Final Words

Our understanding of depression neurobiology will remain incomplete without accommodating the well-known heterogeneity seen in clinical and biological manifestations of depression. Previous approaches to characterizing patient heterogeneity generally focused on symptom variability rather than treatment outcome as a primary biological feature. Our approach, subgrouping patients based on treatment outcomes is a valid new strategy that appears to biologically cluster patients into clinically relevant brain-based subtypes. Long term, by further characterizing these treatment-specific neural networks, we can both improve patient care and better understand depression pathophysiology.

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APPENDICES

APPENDIX A: POST-HOC TESTING OF THE ROSTRAL ANTERIOR CINGULATE AS A PREDICTOR OF TREATMENT OUTCOME

The rostral anterior cingulate is among the best replicated predictors of outcome to a single treatment (Fu et al., 2012; Pizzagalli, 2011), but was not identified in the primary treatment by outcome ANOVA interaction (Experiment 1.1) nor in the whole-brain t-test comparing P+SSRI non-responders and remitters (Experiment 3.1). To determine the treatment outcome related variability in the rostral cingulate, a post-hoc examination was performed.

Methods

A region of interest of the rostral anterior cingulate was centered on MNI coordinates x=0, y=38, z=5, extending from the edge of the subcallosal region in the primary analysis up through the anterior cingulate to the middle cingulate (Bush et al., 2000; Dost Ongür, Ferry, & Price, 2003) (Figure 27). Within this bilateral rostral anterior cingulate volume, post-hoc analyses related to treatment outcome were performed to supplement Experiments 1.1 and 3.1

Experiment 1.1

Correlation of percent change in HDRS score was correlated with baseline metabolism separately in CBT and sCIT treated groups. Analysis was restricted to the rostral anterior cingulate region of interest. Given the emphasis on this region in previous studies, a liberal threshold was used in this *post-hoc* analysis (uncorrected p-value of 0.05).

Experiment 3.1

The P+SSRI non-responder and remitter groups were contrasted using a voxel-wise t-test. The same liberal threshold was used in this *post-hoc* analysis (uncorrected p-value of 0.05).

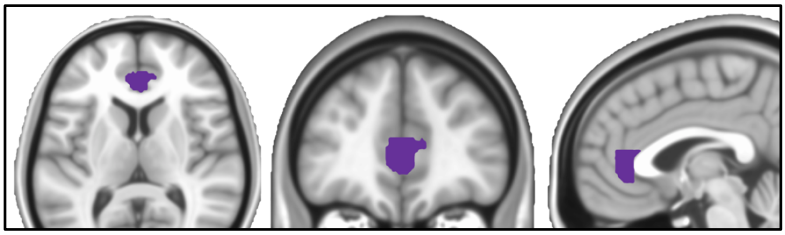

Figure 27: Atlas-based Rostral Cingulate Region of Interest

Results

Experiment 1.1

Rostral cingulate metabolism was correlated with treatment outcome, but solely in the sCIT group. In sCIT treated patients, metabolism in responders was greater than non-responders in a very small region (Figure 28, 2 voxels, MNI coordinates x= -1 y= 39 z= -6).

Experiment 3.1

No voxels showed a significant difference between P+SSRI non-responders and remitters.

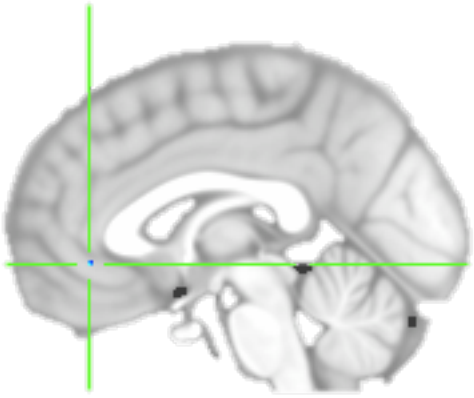

Figure 28: rACC region correlated with outcome in sCIT treated patients

APPENDIX B: INFLAMMATORY INFLUENCES ON BRAIN VARIABILITY

Anterior insula metabolism subtypes show good potential for clinical utility, but they are not accurate for all patients. In addition to the variance related to treatment-specific predictors, non-specific predictors may be informative. Many previous studies link pretreatment brain activity with response variables in studies of a single treatment (Fu et al., 2012; Milak et al., 2009; Pizzagalli, 2011). While less clinically applicable, non-specific predictors can inform depression neurobiology and may share a relationship with other biological measures. While there was no significant main effect of remission across escitalopram and CBT treated patients, taking a more graded approach may be more effective and provides an opportunity to measure overlapping variance in brain metabolism related to other biological measures, specifically inflammation.

Inflammation may be one source of variability in non-specific treatment outcomes. Some MDD patients show increased inflammatory markers compared with healthy controls (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Irwin & Miller, 2007; Miller, Haroon, Raison, & Felger, 2013), adding another source of biological variability in MDD. Inflammation has been indicated as a potential causal factor in MDD, with up to 50% of patients treated with interferon alpha, a Hepatitis C treatment that causes an inflammatory response develop major depression (Raison, Capuron, & Miller, 2006). Increased inflammation contributes to treatment non-response to traditional treatment approaches (Raison et al., 2013).

Here, we will correlate percentage change in the Hamilton Depression Rating Scale across both treatments with whole brain metabolism and test the impact of increased inflammation on brain activity.

Methods

Subject Recruitment.

Recruitment of patients is detailed in Chapter 2.

Treatment Outcomes

Treatment outcomes are overviewed in Chapter 2. The outcome measure of interest for this analysis is percent change in Hamilton Depression Rating Scale (HDRS). For each patient, the percent change in HDRS is calculated from baseline to week 12 of treatment.

FDG-PET Acquisition

FDG-PET acquisition is detailed in Chapter 2.

FDG-PET Preprocessing

FDP-PET Preprocessing is detailed in Chapter 2.

FDG-PET Analysis

Baseline FDG-PET scans were correlated with percent change in HDRS, from baseline to week 12 in all Phase 1 completers. Follow-up analysis repeated the correlation of FDG-PET with HDRS separately in CBT treated and sCIT treated patients.

Immune measures

Immune measures were collected and analyzed by colleagues in the Miller lab at Emory University. At baseline, blood was drawn from each patient. Plasma was analyzed for concentrations of IL-6. Plasma concentrations of IL-6 were determined in duplicate using sandwich ELISA according to manufacturer‘s

protocol (R & D Systems, Minneapolis, MN, USA, http://www.mdsystems.com/pdf/hs600b.pdf.). The mean inter- and intra-assay coefficients of variation for control samples in this assay are reliably 10% or less (Pace et al., 2009).

Analysis of Immune Measures

Because distributions of the IL-6 measures were skewed, we took the natural log of IL-6 measures to normalize the distribution. We then correlated IL-6 measures with whole brain FDG-PET activity, baseline demographic data and anterior insula activity.

Results

Treatment Groups

The full MDD group consisted of sixty-three Phase 1 completers with usable PET scans. Of the 63 completers[5], 33 were treated with CBT, and 30 were treated with escitalopram. Percentage change ranged from -95.45 to 26.67, with the mean percent change across both treatments -52.62, -50.3 (SD 20.03) for CBT treated and -55.18 (SD 30.16) for escitalopram treated.

Metabolic Correlates of Phase 1 Outcomes, All Completers

Inferior parietal lobule (BA40) metabolism positively correlated with percent change in HDRS in patients treated with either CBT or sCIT (R=0.523, p>0.000011, Figure 29). This region of interest was identified as inferior parietal lobule (Caspers et al., 2008) using SPM Anatomy toolbox (Eickhoff et al., 2005) (http://www.fz-juelich.de/ime/spm_anatomy_toolbox, see Figure 30) implemented in Matlab. Follow-up analyses indicate that both anterior insula

[5] *With valid PET scans, 66 total patients completer Phase 1 12 weeks of treatment*

subgroups show a similar correlation with percent change HDRS and BA40 metabolism (I-BM R= 0.583, p>0.003, I-AM R=0.530, p>0.001) with no difference in mean metabolism between groups (T=-1.215, p>0.228, I-BM, mean 1.0834, I-AM, mean 1.1038).

Metabolic Correlates of IL-6 Measures

Correlating whole brain metabolism with ln (IL-6) reveals one significant finding. Activity in the inferior parietal lobule (BA40) positively correlates with IL-6 measures (MNI coordinates at peak, x -55.5 y 18 z 37.5, R=0.470, p<0.000). Correlations with demographic measures also presented significant results. IL-6 levels correlated with increased severity of symptoms at baseline, R=0.416 p>0.000. IL-6 levels did not correlate directly with percent change in HDRS (R=0.165, N=60, p>0.206).

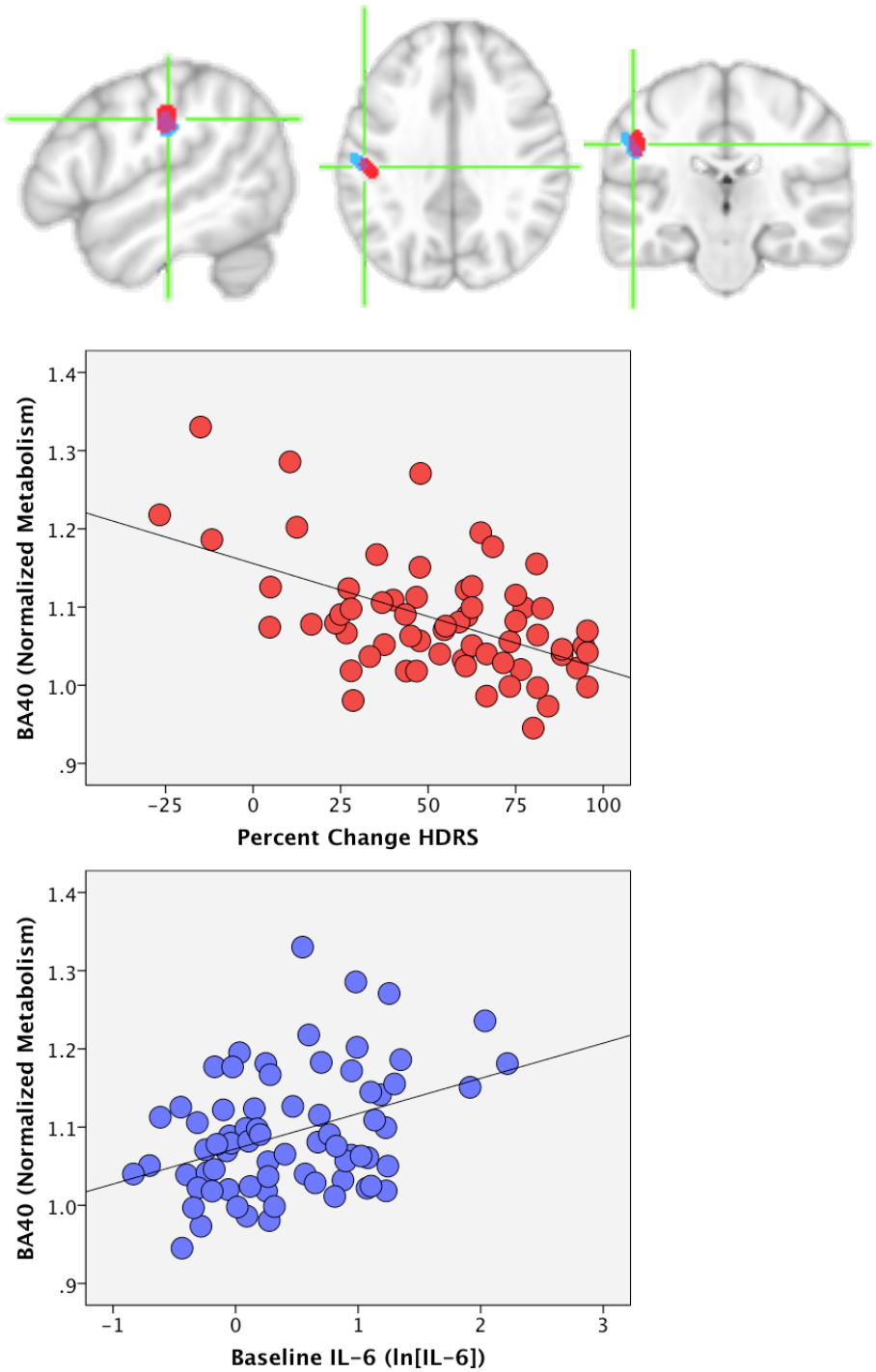

Figure 29: BA40 activity correlated with HDRS and IL-6
A) BA40 region correlated with both percent change in HDRS and IL-6. Red region is correlated with percent change in HDRS, blue region is correlated with IL-6 log scores, Purple region is overlapping area that correlates with both (thersholded at p 0.001) B) Scatter plot of Percent Change in HDRS and BA20 metabolism C) Scatter plot of IL-6 and BA20 metabolism

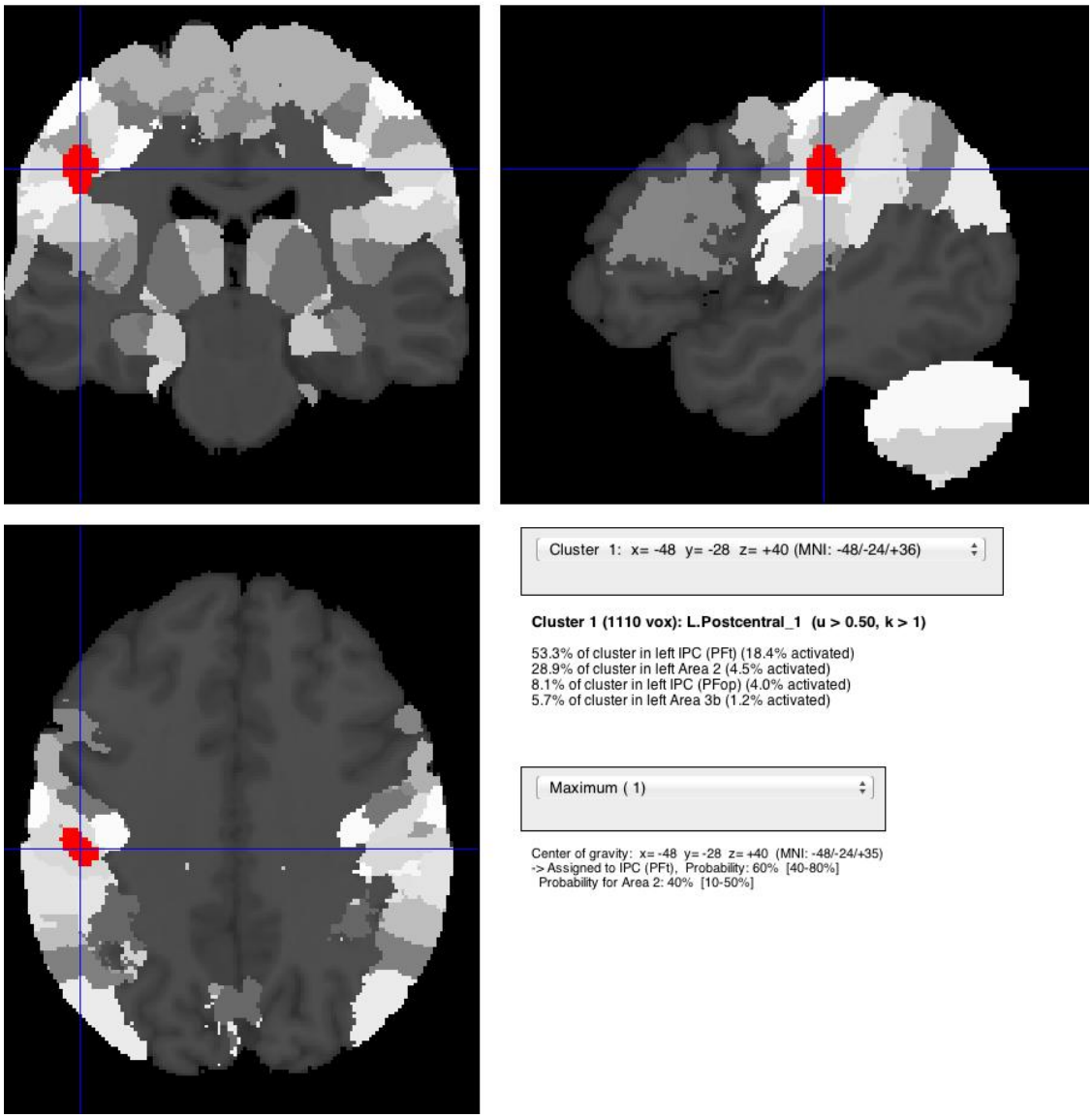

Figure 30: Cytoarchitecture-based MNI atlas region identification

Discussion

Variability in BA40 metabolism in depressed patients is related to both treatment outcome and inflammatory markers. The current experiment presents another avenue to explore what factors might contribute to neurobiological heterogeneity in MDD. Variance in BA40 metabolism relates to treatment outcome in patients treated with either CBT or escitalopram. This non-specific predictor of treatment outcome is applicable across two treatments with different presumed treatment mechanisms. Whether treated with anti-depressant medication or psychotherapy, increased BA40 metabolism prior to initiation of treatment is an indicator of poor outcome. Inferior parietal lobule is a region of association cortex that plays a role in multi-modal integration and attention (Rushworth, Johansen-Berg, Göbel, & Devlin, 2003). Inferior parietal lobule projections to the retrosplenial and cingulate cortices may integrate supramodal sensory data and limbic information (Mesulam, Van Hoesen, Pandya, & Geschwind, 1977). This interpretation of parietal lobule connectivity is consistent with previous evidence of BA40's role in major depression. BA40 is a node in the limbic-cortical dysfunction model of depression and shows decreased activity in depression. Multiple studies report treatment induced changes in inferior parietal cortex in depressed patients (Goldapple et al., 2004; Mayberg et al., 1999b, 2000).

As inflammation increases, BA40 metabolism increases. The region correlating with IL-6 measures overlaps with the BA40 region that correlates with outcome to after either CBT or escitalopram (Experiment 3.1). Inflammation may partly drive variability BA40. Previous literature links inflammation with treatment non-response. Here, inflammation impacts non-specific predictors of outcome.

Levels of inflammation may play a role in the accuracy of the insula as a biomarker for initial treatment, where higher levels of inflammation may interfere with remission to predicted treatment. To test this hypothesis, we compared IL-6 levels and BA40 metabolism between patients achieving remission, and patients not achieving in remission in all Phase 1 patients who received the treatment predicted by their insula. In these patients who received the treatment indicated by their insula, IL-6 measures are increased in non-remitting patients who received insula-matched treatment, but only at trend significance levels (t 1.833, $p<0.071$) (Figure 31). BA20 metabolism shows a stronger difference. BA40 Metabolism is increased in non-remitting patients (N=16) compared patients remitting after 12 weeks of monotherapy treatment with either CBT or escitalopram (t 2.925, p 0.006) (Figure 31). IL-6 and BA20 metabolism measures may contribute to non-remission in patients who get the 'right' 1st treatment, but a relationship with two-treatment failure is less straightforward.

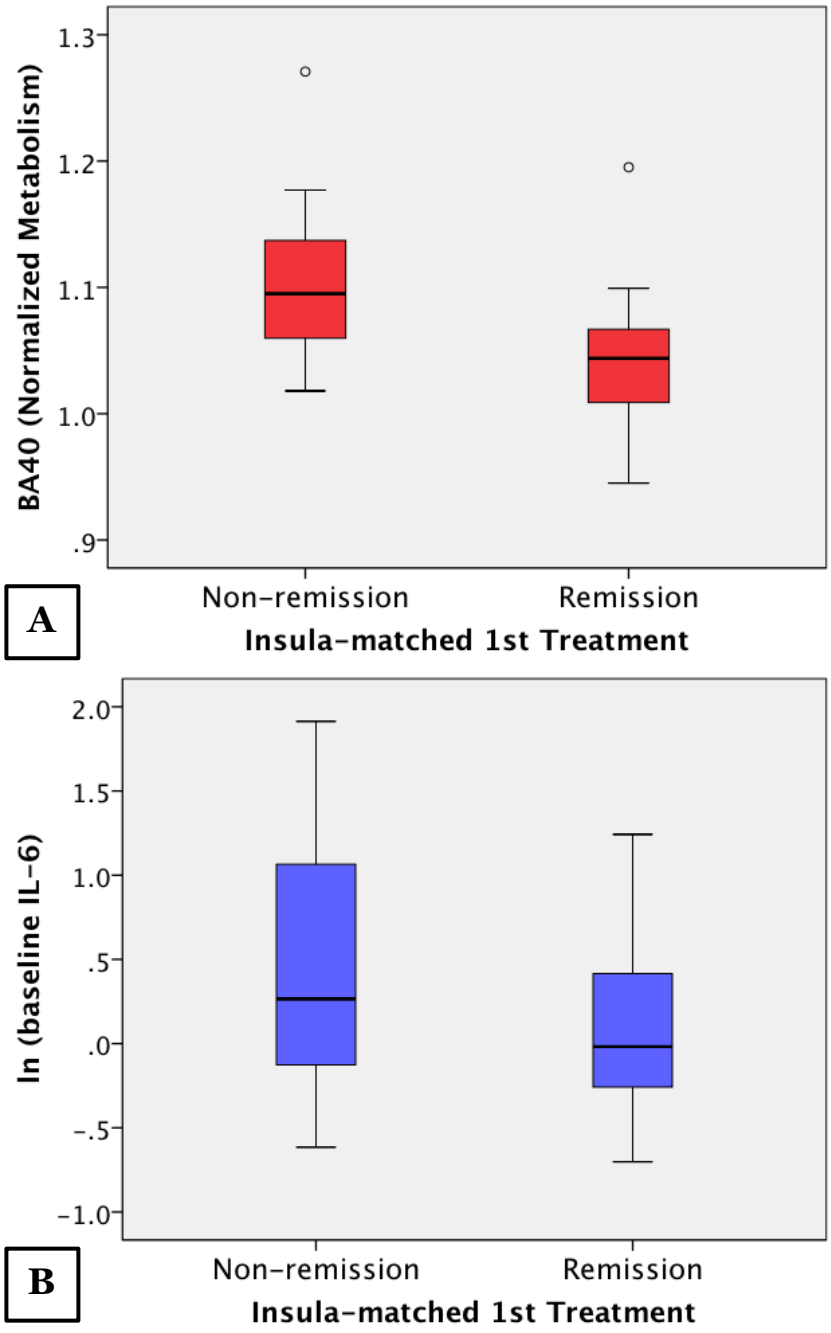

Figure 31: Differences in remitters and non-remitters treated with monotherapy matched with anterior insula predicted treatment.
A) BA40 is significantly increased in non-remitting patients. B) IL-6 levels are increased in non-remitting patients at the trend level.

APPENDIX C: NEUROIMAGING METHODS AND ANALYSIS STRATEGIES

Resting State

The rationale for choosing resting state over a task design is multipart. Resting state networks have been reliably demonstrated as stable in healthy controls and have shown disruptions in disorders including depression as well as Alzheimer's disorder, schizophrenia, ADHD and autism. Major depression is a continuous state, meaning that it doesn't require a provocation to show symptoms. The dysfunction of the disorder can be captured at rest because that dysfunction occurs during the resting state. Resting is requires less engagement by patients. Subjects experiencing greater severity may have greater difficulty performing a given task, making interpretation potentially problematic.

Positron Emission Tomography

Positron Emission Tomography or PET imaging is a biological imaging technique developed in the early 1980's that quantifies the release of positrons from a radioactively labeled biological probe in order to assay biological systems. FDG-PET measures glucose metabolism using radioactively labeled Fluorine (^{18}F) incorporated into fluro-deoxyglucose (FDG), which is a glucose analog. FDG is injected into the bloodstream, passes through the blood-brain barrier and is taken up by cells requiring glucose. FDG is phosphorylated, which causes a size increased preventing FDG from leaving the cell, meaning that on average the cell FDG enters first is where it stays localized during the scan.

PET scan measurement of glucose metabolism was selected based on its high reliability and availability combined with its established use for studies of

baseline scan patterns in depression and effects of various antidepressant treatments (Bartlett et al., 1991; Brody et al., 1999; Drevets et al., 2002; Goldapple et al., 2004; Kennedy et al., 2007; Kimbrell et al., 2002; Konarski et al., 2009; Little et al., 2005; Mayberg et al., 1997; Milak et al., 2009; Saxena et al., 2003).

Partial Least Squares Analysis

Partial least squares (PLS) analysis is a multivariate technique that has many applications. PLS techniques are tailored to accommodate large datasets, like neuroimaging data. Applied to neuroimaging, PLS can identify the underlying covariance structure in neuroimaging data. PLS has four types, behavior, task, seed and multi-table. Our approach takes advantage of behavioral PLS. In Chapter 3, behavioral PLS allows us to analyze associations between a behavior (negative affect) and brain activity (metabolism).

Behavior and brain activity are entered into two separate matrixes. Matrixes are centered and normalized within each condition. The matrix of correlations for each condition is then computed and then condition-wise matrixes of correlations are stacked, forming the combined matrix of correlations. This combined matrix of correlations is the input for singular value decomposition (SVD), the main analytic tool in PLS analysis. Output from the SVD is used to create brain saliences, with are voxel-dependent differences in the brain behavior correlation. Brain saliences are used to compute latent variables.

The goal of a PLS analysis is to extract information common to two datasets (e.g., negative affect, metabolism) that is generalizable. This is done through a combination of computational approaches, namely permutation tests (to obtain p

values) and bootstrapping (to determine stability). Essentially, permutation tests determine if a signal is strong enough to detect, and bootstrapping determines that signal's reliability (McIntosh & Lobaugh, 2004).

Functional Magnetic Resonance Imaging

Resting state functional magnetic resonance imaging (rs-fMRI) is a task-free non-invasive functional neuroimaging approach. The signal measured by rs-fMRI is blood oxygen level dependent (BOLD). BOLD is an indirect measure of blood flow. As activity in a region increases, blood flow increases, and so does the BOLD signal. In resting state data, measures of spatio-temporal correlations between spatially distinct regions (functional connectivity) are measured. These temporally correlated fluctuations in regional activity can be defined using model-driven (seed-based functional connectivity) and data-driven (e.g., Independent Components Analysis) methods.