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The Functional and Structural Consequences of Aberrant Microglial Activity in Major Depressive Disorder

Jasmine D. Cakmak, *The University of Western Ontario*

Supervisor: Palaniyappan, Lena, *The University of Western Ontario*
Co-Supervisor: Vasudev, Akshya, *The University of Western Ontario*
Co-Supervisor: Anazodo, Udunna, *The University of Western Ontario*
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Abstract

Major depressive disorder (MDD) is a highly debilitating neuropsychiatric illness which has been linked with increases in both peripheral and central inflammation, as well as with changes in connectivity. Although countless studies have investigated these two topics, the relationship between neuroinflammation and functional/structural connectivity has not been explored. Using [^{18}F]FEPPA PET imaging, we measured translocator protein-related (TSPO) microglial activity in the subgenual anterior cingulate cortex (sgACC) and insula and confirmed significantly increased [^{18}F]FEPPA uptake in depressed patients (N=12) compared to healthy controls (N=23). Using a seed-based ROI analysis of fMRI data, we found that patients show overall decreased connectivity between the sgACC and the insula. To test the relationship between inflammation and brain connectivity, we performed regressions which found functional (sgACC-insula) and structural (cingulum bundle) connectivity to be significant factors in explaining the microglial activity in the left sgACC. Our study suggests that neuroinflammation relates to network function in MDD.

Keywords

Major Depressive Disorder, Depression, Late-Life Depression, Neuroinflammation, [^{18}F]FEPPA, Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), Microglia, PET/MR, Neuroinflammation; Functional connectivity; Treatment-resistance; Diffusion Tensor Imaging (DTI); White Matter Integrity

Summary for Lay Audience

Major depressive disorder is a brain illness which affects many people worldwide. The main symptoms are depressed mood and loss of pleasure, with additional symptoms such as lack of concentration and fatigue. Previous studies have shown that there are a variety of reasons why depression could occur, but the focus of the current thesis is the inflammation hypothesis. In the brain, when the inflammatory response is activated, microglia, the resident immune cells in the brain, will become activated. Microglia influence the body's inflammatory response, and their activation can be measured through positron emission tomography (PET) imaging of a protein on their surface. It has been shown in the past that certain areas of the brain have greater microglial activity in depression.

The functioning of different brain areas also plays a role in the development of depression. The simultaneous activation of areas such as the anterior cingulate cortex and the insula can be disrupted due to depression; this simultaneous activation represents an activation-related connectivity between the regions. Those two areas are involved in mood and self-examination. We can measure levels of activation of specific brain areas with magnetic resonance imaging (MRI). What we do not know is how disrupted brain connectivity is related to microglial activity in these areas.

The first goal of this thesis was to measure brain inflammation through microglial activity, particularly in areas noted by other studies. The second goal was to measure brain connectivity between these areas in the brain. The third goal was to see if brain connectivity and microglial activity are related in any way. We expected increased inflammation and decreased brain activity in depressed individuals. We also expected that they would be related to one another.

We found that, as we predicted, in these brain areas inflammation was increased and brain activity was decreased in depression. We next found that brain connectivity is related to inflammation. We believe that the continuous study of the neurological basis for depression will help physicians and pharmaceutical scientists develop new and more specific treatments to better the lives of the many of those who suffer from depression worldwide.

Co-Authorship Statement

This thesis was adapted from "The Functional and Structural Consequences of Aberrant Microglial Activity in Major Depressive Disorder," a manuscript submitted to the Journal of Psychiatry and Neuroscience and co-authored by Jasmine D. Cakmak, Linshan Liu, Stefan Poirier, Betsy Schaefer, Dr. Amer M. Burhan, Dr. Priyadharshini Sabesan, Dr. Raju Poolacherla, Dr. Elizabeth Finger, Dr. Justin W. Hicks, Dr. Jean Théberge, Dr. Keith St. Lawrence, Dr. Lena Palaniyappan, and Dr. Udunna C. Anazodo. Jasmine D. Cakmak performed PET and fMRI data preprocessing, statistical analyses, and drafted the manuscript. Drs. Udunna Anazodo and Lena Palaniyappan collected data. Dr. Udunna Anazodo and Linshan Liu assisted in PET analysis through writing of Matlab scripts and PET image reconstruction. Stefan E. Poirier completed the DTI data processing and value extraction. Betsy Schaefer recruited participants for the study. Dr. Poolacherla assisted in data acquisition. Dr. Sabesan participated in recruitment and clinical data acquisition. Dr. Justin W. Hicks synthesized [^{18}F]FEPPA for the study. Dr. Burhan aided in recruitment and clinical data acquisition. Dr. Théberge and Dr. Anazodo assisted in data acquisition. Dr. Palaniyappan and Dr. Finger assisted with recruitment. Funding was secured by Dr. St. Lawrence, Dr. Théberge, Dr. Palaniyappan (Lawson Strategic Research Fund), Dr. Finger, and Dr. Anazodo (as a co-investigator for the FTD study for controls). Dr. Lena Palaniyappan, Dr. Jean Théberge, Dr. Anazodo, Dr. St. Lawrence, Dr. Finger, and Dr. Burhan conceptualized the experiment. Dr. Udunna Anazodo and Dr. Lena Palaniyappan supervised the data analyses and the final draft. All co-authors participated in review and editing of the manuscript.

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List of Symbols and Abbreviations

[^{18}F]FEPPA	[^{18}F]N-2-(fluoroethoxyl)benzyl-N-(4phenoxypyridin-3-yl)acetamide
3-HK	3-hydroxykynurenine
ACC	Anterior Cingulate Cortex
ACTH	Adrenocorticotropic Hormone
AD	Axial Diffusivity
BBB	Blood Brain Barrier
BDI	Beck Depression Inventory
CGI-S	Clinical Global Impressions – Severity Scale
COX-2	Cyclooxygenase-2
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
Cldn5	Claudin-5
CX3CR1	C-X3-C Motif Chemokine Receptor 1
dACC	Dorsal Anterior Cingulate Cortex
DLPFC	Dorsolateral Prefrontal Cortex

DMPFC	Dorsomedial Prefrontal Cortex
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
ECT	Electroconvulsive Therapy
EEG	Electroencephalography
FA	Fractional Anisotropy
FDG	Fluorodeoxyglucose
fMRI	Functional Magnetic Resonance Imaging
FWHM	Full Width Half Maximum
GDS	Geriatric Depression Scale
HAM-D	Hamilton Rating Scale for Depression
HPA	Hypothalamus-Pituitary-Adrenal
IDO	Indoleamine 2,3 Dioxygenase
IFN-α	Interferon-Alpha
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-8	Interleukin-8
KAT	Kynurenic Acid
KMO	Kynurenine-3-monooxygenase
KYN	Kynurenine
KYNA	Kynurenine Aminotransferase
LLD	Late-Life Depression
LPS	Lipopolysaccharide
MCP1	Monocyte Chemoattractant Protein 1
MCP4	Monocyte Chemoattractant Protein 4
MD	Mean Diffusivity
MDD	Major Depressive Disorder
MDE	Major Depressive Episode
MNI	Montreal Neurological Institute
MoCA	Montreal Cognitive Assessment
MPFC	Medial Prefrontal Cortex
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
Nac	Nucleus Accumbens
NSAID	Non-Steroidal Anti-Inflammatory Drug
PET	Positron Emission Tomography
PSF	Point Spread Function
PUFA	Polyunsaturated Fatty Acid
QUIN	Quinolinic Acid
RD	Radial Diffusivity
RF	Radio Frequency
ROI	Region of Interest
rSUV	Relative Standardized Uptake Value
rTMS	Repetitive Transcranial Magnetic Stimulation
sgACC	Subgenual Anterior Cingulate Cortex

SNRI	Selective Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitors
sTNF-R2	Soluble Tumour Necrosis Factor Receptor 2
SUV	Standardized Uptake Value
TBSS	Tract-Based Spatial Statistics
TNF	Tumour Necrosis Factor
TRD	Treatment-Resistant Depression
TRLLD	Treatment-Resistant Late-Life Depression
TRP	Tryptophan
TSPO	Translocator Protein
VMPFC	Ventral Medial Prefrontal Cortex
WM	White Matter

List of Appendices

Chapter 1

1 Depression, Its Burden, and the Knowledge Gap

This thesis contains research pertaining to the etiology of major depressive disorder (MDD), a highly debilitating mental illness. Mental illnesses negatively impact an individual's mood, thoughts, or behaviour, leading to distress and impairment in daily activities. Depression is the most common of all mental illnesses and the leading cause of disability worldwide, having a lifetime prevalence of 20.6% in the US and 11.2% in Canada, and while manageable when treated, can have serious implications for quality of life when left unmanaged (1,2). There exists no one-size-fits-all cure for MDD; it is hypothesized that many different physiological pathways can contribute to its etiopathology. Modern treatment options rely on symptom management and include a combination of medication (selective serotonin reuptake inhibitors etc.) and psychotherapies. Despite the various options that are available, many patients develop a treatment-resistant state. In part, resistance may relate to our failure to address the mechanistic processes that underlie the illness. This thesis is an attempt to investigate one such pathway – neuroinflammation – in MDD. Several lines of clinical research on neurobiological pathways of depression, reviewed below, point toward depression being a systemic disorder with an inflammatory basis. The aim of this work is to fill the knowledge gap linking the notion of neuroinflammation to depressive symptoms and behaviours mediated by dysfunctional brain networks.

1.1 Clinical Symptoms of Depression

1.1.1 Major Depressive Disorder (MDD)

MDD is a psychiatric illness which tends to run a remitting and relapsing course. It is common to experience multiple episodes; about 75% of individuals who have experienced a single episode of MDD will experience two or more at different points throughout life. With each subsequent episode, the risk of having another one increases

exponentially, while the time between each episode decreases (3,4). For an individual who has had at least one episode, the average number of MDD episodes is four, and each episode has an average length of twenty weeks (5,6). To be diagnosed with MDD, as defined by the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5), one must have at least five of the following symptoms, with one of them being either depressed mood or loss of pleasure: depressed most of the day; diminished pleasure in activities all day or most of the day, every day; weight loss or weight gain without dieting or increase/decrease in appetite nearly every day; insomnia or hypersomnia almost every day; psychomotor agitation or retardation; fatigue; feelings of worthlessness or guilt; diminished ability to think; and recurrent thoughts of death or suicide ideation. The symptoms must cause distress or impairment in living and completing regular tasks, and the individual must not have experienced a manic episode. Each case of depression presents a varied combination of symptoms, and varied trajectories of outcomes, which has led to the notion that multiple distinct etiologies exist for this condition. For example, some with depressed mood may have pathophysiology associated with the medial prefrontal cortex, while others with predominant anhedonia and alterations in motivation may show a decrease in dopaminergic activity in the mesocortico-limbic pathway (7,8). These pathophysiological changes may also coexist in the same individual and may fluctuate over the illness course.

1.1.2 Impact of MDD

Depression affects over 168 million people around the globe, two thirds of whom are women, being one of the major contributors to reduced quality of life (9,10). In Canada, about 8% of adults will have had at least one MDD episode throughout their lives (11). Individuals with MDD are more likely to be hospitalized and are at increased risk of suicide. As well, they are two times more at risk of unemployment than those without MDD (12–14).

Depression causes noteworthy economic and social burden; in Canada, mental health spending is projected to rise into the trillion dollar range by 2031 (15). Depression causes patients to become less productive; those with depression will lose 5.6 hours of productive time at work per week compared to non-depressed workers who will lose 1.6

hours (16). This equates to 225 million lost workdays and $36.6 billion lost in salary per year in the US, and an additional cost of about $32.3 billion per year to the Canadian economy (4,17).

The negative effects are not limited to the social, as depression increases rates of morbidity and mortality. The physical impairments seen in depression are of a similar magnitude to those in chronic diseases such as diabetes and cancer (18). Depression is correlated with a subsequent cardiovascular episode, its associated mortality, as well as with increases in risk for cardiovascular disease, stroke, and hypertension; the risk of any cardiovascular event increases by 20% in individuals with four or more depressive symptoms as compared to those without (19–25). Individuals with depression are eleven times more likely to attempt suicide than healthy individuals, and about one in ten will attempt suicide (14,26,27).

1.1.3 Late-Life Depression (LLD)

While depression in the adult population has been somewhat better delineated, late-life depression (LLD) – depression occurring in an older population – is likely different in terms of etiopathogenesis and presentation. The specific criterion of age for LLD has not been widely agreed upon at this time, but the age of qualification for geriatric psychiatry-related services, i.e. greater than 65 years, has been considered to be most acceptable. The participants in the study were older (over the age of 50, with some over the age of 65), therefore the symptomatology specific to LLD should be considered. As is the case with MDD, individuals must have an episode lasting at least 2 weeks with one of the primary symptoms (anhedonia and depressed mood) and at least four of the secondary symptoms as previously described. The DSM-5 criteria seem to fail in diagnosis of late-life adults as complaints about failing bodily functions will be written off as expected for the age bracket (28). Help-seeking behaviour which comes in the form of persistent grievances with fatigue, pains, and headaches can be easily overlooked. As a result, depression in the elderly is underdiagnosed and undertreated. While there is growing interest in better defining LLD, there have simultaneously been attempts to develop screening tools to characterise LLD-specific symptoms of depression. Of these

the self-rated Geriatric Depression Scale (GDS) has been validated in capturing some of the typical somatic symptoms experienced by patients suffering from LLD.

1.1.4 Impact of LLD

More than 300,000 of the elderly in Canada are depressed, and this is associated with $5 billion in health care costs per year -- a number which ever-growing in today's aging population (29). 1 in 10 of older adults are diagnosed with LLD at some point in their life (30). Older adults in the community with depression have a prevalence of 11.2%, but institutionalized older adults have even higher rates (30). 12-45% of LLD patients in hospitals reported symptoms of depression, and 12.4% of those in care homes had major depression (31,32). Those in care homes tend to be placed there as a consequence of caregivers becoming overwhelmed with the increasing demand and mental load needed for elderly care.

LLD is associated with both non-suicide and suicide mortality in older adults (33,34). In a Statistics Canada study, it was found that 19% of the suicide victims in Canada were over the age of 60, and of those men made up the majority. LLD patients may be more likely to commit suicide through more violent and deadly means, with 26% of suicide victims over 60 using firearms compared to only 12% of those aged 15-39 (35).

1.1.5 Late-Life Depression vs. Adult Depression

LLD is symptomatically distinct from adult depression, with LLD patients, for example, having a pronounced fear of falling (36,37). The stressors specific to late-life -- forced retirement, chronic illness, cognitive decline, social isolation, caregiving, financial distress, loss of independence, and bereavement -- can create an environment which leads to the development of LLD (38). Views towards life and death itself will be unique to older individuals as compared to those who are younger (39). Those with greater harm avoidance, insecure attachment, low extraversion, neuroticism, low self-esteem, no sense of greater purpose, no social roles, and lower education will be at greater risk for developing LLD. As well, those of the female sex and with nutritional deficits, especially in vitamin B12 and folates, will likewise be predisposed to depression (40).

Unlike depression associated with adult age, LLD is oftentimes comorbid with all-cause dementia (1.85, 95% CI 1.67-2.04, P<0.001), Alzheimer's disease (1.65, 95% CI 1.42-1.92, P<0.001), and vascular dementia (2.52, 95% CI 1.77-3.59, P<0.001) (41). Depression can be a signalling factor for dementia, and some claim that LLD is a risk factor for mild cognitive impairment and neurodegenerative diseases (causation has not been determined in this case). Comorbidities with other medical illnesses in LLD can affect scores on rating scales; elevated scores for eight items from the Hamilton Rating Scale for Depression (HAMD, Hamilton, 1960) have been observed in individuals over the age of 70, likely due to such somatic comorbidities (42). In addition, it seems that the pathways underlying depression in older populations may differ. For example, white matter hyperintensities have been revealed to play some role in the development of depression while their presence in younger adults is uncommon (43). Despite knowledge of dissimilar physical symptoms in adult and late-life depression, differences between the treatment of these two groups have not been distinguished and refined, an oversight which has left many older adults with insufficient resources to manage their symptoms.

1.2 Treatment of Depression

1.2.1 Treatment Options

The treatment of depression can be divided into three broad categories: medication, psychotherapy, and alternative treatments. For the purposes of this thesis, we will be focusing on the former. Each type of treatment can be offered alone, but it is recommended to receive them in combination to gain maximum benefit. Medications, or antidepressants, are the most relied-upon treatment and come with a variety of options. Selective serotonin reuptake inhibitors (SSRIs) are the most prescribed of the antidepressants. They act by blocking the reuptake of serotonin and therefore increase serotonin signalling. Other types of antidepressants such as serotonin and norepinephrine reuptake inhibitors (SNRIs), 5-HT1A receptor antagonists, and dopamine reuptake blockers function similarly with the basic principle of regulating levels of neurotransmitters (namely serotonin, norepinephrine, and dopamine) in the brain. Most common forms of antidepressants target monoaminergic mechanisms which were

discovered many decades ago; no novel drug therapies have entered the market except for esketamine, a ketamine-derived glutamate receptor blocker which was FDA-approved in 2019, and agomelatine, a melatonin receptor agonist approved for use in Europe in 2009 and Australia in 2010. Interestingly, within the last decade the monoaminergic hypothesis of SSRIs has been questioned, and has even been speculated that their mechanism of actions may hinge upon anti-inflammatory pathways (44).

Anti-inflammatory approaches have been considered for the treatment of depression as either add-ons or monotherapy (to be further discussed in Section 1.5.6) (45). Nonsteroidal anti-inflammatory drugs (NSAIDs), the most studied of the anti-inflammatory drugs, have been found to have anti-depressant effects when compared to placebos and are inexpensive and easy to obtain (46). Their effectiveness relies upon the ability to reduce prostaglandins. One class of NSAIDs, selective cyclooxygenase-2 (COX-2) inhibitors, are considered to have a more direct anti-inflammatory effect than other classes and therefore are more effective as treatments for depression (47–49). NSAIDs, however, may increase the risk of cardiovascular events (45,50). Cytokine-inhibitors, which target cytokine synthesis, concentration, or related receptors, have a direct anti-depressant effect, although they may increase the risk of infection (46,51). Other groups of anti-inflammatory drugs which have shown anti-depressant effects include but are not limited to statins, corticosteroids, and poly-unsaturated fatty acids (PUFAs) (45).

Despite the potential promise, it is not clear who should receive this treatment and the overall response rate is minimal as we are not able to stratify patients with neuroinflammation from those who do not have such inflammatory processes; furthermore, it is not clear how inflammation, which is generally a short-lived, constrained defence process, can lead to pervasive depression in those affected. This mechanistic understanding will help us identify the profile of inflammatory depression as well as aid in developing more empirically informed therapies. Moreover, the above treatments, inclusive of antidepressants, may not eliminate the risk of relapse, worsening of symptoms, or treatment-resistance.

1.2.2 Treatment-Resistant Depression

The ultimate aim regarding the above therapies is for the patient to achieve full recovery– the almost complete absence of burdensome symptoms. However, while they can be effective for many individuals, antidepressants can have little to no effect in some. Of those who receive "adequate" treatment for MDD, about 70% will respond to treatment, yet only 50% will fully recover; the remaining patients will not respond at all (52,53). Other studies suggest that this value is even lower, with only 30% reaching the goal of full remission, and those previously classified as responsive experiencing residual symptoms such as fatigue and insomnia, contributing to increased risk of relapse (54–56).

Those who do not sufficiently respond are referred to as having treatment-resistant depression (TRD). No consistent definition of this phenomenon exists, and clinicians may diagnose a patient if they do not respond to antidepressants whatsoever, or if they do not reach full remission after adequate dosing and course duration. Despite these differences, a concurrence between each definition is that to be diagnosed with TRD, one must fail to adequately respond to at least 2 courses of different antidepressants within an appropriate period of time.

Of those who respond minimally, treatment resistance may arise from being prescribed a suboptimal dose, an unsuitable medication course, or discontinuing medication altogether. This is further complicated by comorbidities with illnesses such as anxiety and the heterogenous nature of depression itself, which make it difficult to develop universally effective treatments. Misdiagnosis due to these factors is not uncommon, and the inconsistency in TRD's definition contributes to improper prescriptions of pharmacotherapies.

The disregard for the disparity in symptomatology and disease progression between adult and late-life depression makes this issue even more pressing for the elderly. Atypical symptoms, somatization, cognitive decline, lack of education about drug therapies, and a lack of support from family can all contribute to cases of treatment-resistant late-life depression (TRLLD) (57). The spectrum of possible depressive symptoms in older adults can range to bodily complaints such as stomach-aches and palpitations, present in about 20-80% of older patients but not commonly described in younger patients, leading to the belief that these individuals are experiencing side effects and therefore prematurely stopping treatment (28). While the response rates for older

adults are similar to those below the age of 50, it has been shown that the same cannot always be said about the time course. In the first 6-12 weeks of treatment, over 80% of those with TRLLD will fail to respond sufficiently or will experience early relapse (58,59).

TRD is immeasurably onerous for those it affects. An inability to find relief from its physical and mental demands is taxing. It is easy to forget about the financial burden: those with TRD will be subject to increased medical costs compared to those who respond to treatment (60). They are more likely to be hospitalized and their hospital visits are six times the cost of a treatment-sensitive individual (61). It becomes apparent that establishing concrete means of diagnosing TRD and TRLLD is incredibly important.

1.3 Explaining Depression – Inflammation as a Factor in its Development

Social as well as biological factors such as genetics, aging, and physical diseases appear to have an effect on one's mental health. The interplay between all of these factors and the degree of their severity may ultimately be what leads to depression, but the impact of each alone cannot be discounted (62). Predisposing factors may act on brain systems such as the frontolimbic networks and may bring about the vulnerability for depression, and promote chronicity and relapse (63). Etiological factors such as vascular changes, amyloid deposits, inflammation, and genetics may cause direct damage to reward, salience, and cognitive control networks, or may further increase the severity of the predisposing factors. Social stressors may lead to inflammation, oxidative stress, altered functional connectivity, or neurogenesis (64). These stressors may impact the brain in the same way as aging or disease-related processes. Social withdrawal behaviours associated with depression are linked with a desensitization of the hypothalamus-pituitary-adrenal (HPA) axis due to dampening of a prolonged stress response (38). Stress may also lead to a direct neglect of health. All in all, the various ways through which depression may occur are diverse and numerous. The cause of depression cannot be explained by a single factor and requires a complete understanding of several interacting etiological factors. However, the mechanisms of each factor are still unclear and should be studied in depth in order to gain a better understanding of how each etiological factor

works independently or synergistically and additively with other factors to produce depression.

A widely considered mechanism for depression is neuroinflammation which is part of the body's immune response -- a line of defense against infection and injury. The immune response can be classified into two categories: innate or acquired. The innate system is driven by white blood cells, while the acquired system consists of an immunological memory involving antigens and T and B lymphocytes. The question of whether the immune response involved in depression, a component of which being inflammation, is acquired has long been debated, but research has shown that there are increased T cell factors, implicated in both innate and acquired responses, in depressed individuals, suggesting that both systems may be at play (65,66). Despite having some answers to this broad and undeniably elemental question, it is only a small facet of the system at large and is representative of the larger dilemma seen in this field: there is a current lack of understanding of the complexity of the relationship between inflammation and MDD.

Complicating the matter further, inflammation can occur throughout the body and is present in a number of conditions, such as allergies, autoimmune diseases, and infections. This is inclusive of depression, which seems to be the main neuropsychiatric illness explained by brain tissue inflammation, but brain inflammation may play a role in the aetiologies of several other brain illnesses including schizophrenia, Parkinson's, epilepsy, and Alzheimer's. The focus of this thesis is on neuroinflammation as a theory explaining the development of MDD. The following is an overview on neuroinflammation-related findings for MDD, including relevant tools for its measurement and mechanisms of action.

1.4 The Anterior Cingulate Cortex and Insula as Important Nodes of Functional Connectivity in Depression

A variety of brain networks involved in processing of emotional information are thought to be relevant to the pathophysiology of depression. Two areas of the brain which have been repeatedly linked with response to and diagnosis of depression and have

displayed evidence of involvement in neuroinflammation (to be further discussed in Section 1.5.8) are the insula and the anterior cingulate cortex (ACC). The insula, an area with connectivity to depression-related regions, including the subgenual ACC (sgACC), which is involved in processing salient stimuli and regulating interoception (67,68), exhibits dysregulated functional connectivity (or *temporal correlation with separate brain regions*) in individuals with MDD (69–73). Such dysregulation has been associated with the development of a variety of cardinal features of MDD, including loss of pleasure (74–76). More precisely related to treatment of depression, its activity is altered as a result of depression treatment and can predict treatment outcomes. Antidepressants reduced insular activity (77,78) while regional cerebral blood flow in the insula was reduced with repetitive transcranial magnetic stimulation (rTMS), a popular treatment for TRD (79). Additionally, the right anterior insula was identified as a key factor in responsiveness to CBT or antidepressants (80). In a particularly insightful study using the insula as a seed region, the effective connectivity, or *the influence one neural system exerts over another* (81), of the insula with the dorsomedial prefrontal cortex (DMPFC) and from the pulvinar and visual cortex to the insula were shown to be diminished, which suggests a debilitated connection between the insula and higher processing as well as sensory regions in MDD (82).

Marrying the concept of treatment response and effective connectivity using rTMS, Iwabuchi et al. (2019) demonstrated that pre-treatment fronto-insular effective connectivity predicted response to rTMS treatment at 1 month in those with TRD, while right anterior insula cerebral blood flow was negatively correlated with the same treatment outcome, further cementing the insula's role in depression-involved brain networks (83). The above studies highlight the differential relationship the insula has with regions such as the DMPFC in MDD, a relationship which is modulated by treatment response, and how insula physiology can be improved with depression-targeted treatments.

Similarly, the ACC and sgACC are dysregulated in MDD, but interestingly, dysregulation of the sgACC's connectivity with the insula has been consistently reported in MDD (71,84–88). The sgACC's connectivity can be improved with and can predict the response to rTMS application to the dorsolateral prefrontal cortex (DLPFC) (89,90). This

is not limited to rTMS; the baseline functional connectivity between the sgACC and superior frontal/temporal gyrus, as well as between the sgACC and the middle temporal gyrus predicted electroconvulsive therapy (ECT) response (91). In 2018, Weigand et al. found that depressive symptom improvement was predicted by the functional connectivity between the sgACC and the DLPFC, solidifying the sgACC's importance in depression symptomatology and outcomes (92). Of relevance to the topic of the current study, increased sgACC activity can predict treatment resistance (93), and symptom improvement with, for example, ketamine, can normalize its hyperactivity (94). Such findings have made the insula and the ACC attractive targets for depression studies but there is still much left unexplained, especially regarding their role in neuroinflammation. In the current study, we focus on inflammation present in the sgACC and insula, and how this relates to their connectivity in MDD.

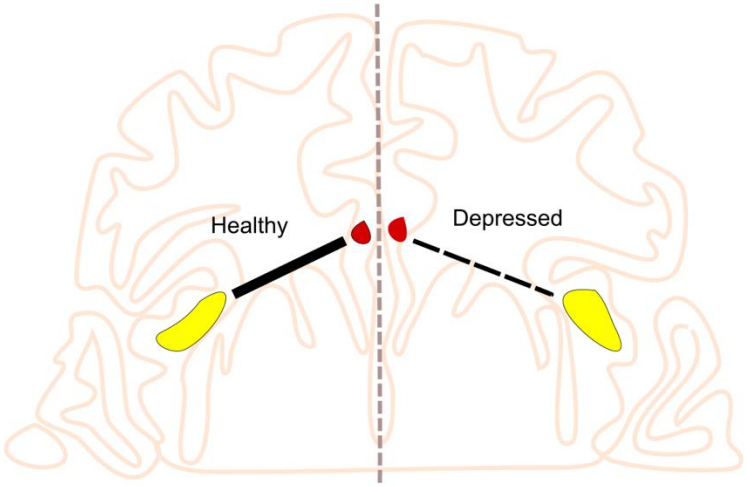

Figure 1.1. Axial view of the insula and sgACC. The right and left insula (yellow) are functionally connected to the left and right sgACC (red). We expect a weaker connectivity between the sgACC and insula in depressed individuals (associated with increased neuroinflammation) compared to healthy individuals.

1.5 Findings Concerning Inflammation and Depression

1.5.1 Mechanisms of the Neuroinflammatory Response

There exist many inter-related theories as to how inflammation increases depressive symptoms, but for the purposes of this overview we will focus on three: HPA axis-modulated neuroendocrine dysfunction, infection, and microglial activation. The first of these theories involves the stress hormone system, the HPA axis. In response to stressors, the HPA axis signals for the release of glucocorticoids by the adrenal glands, which act on organs throughout the body to regulate physiological processes and have a negative feedback effect at various levels of the system (95). In depressed individuals, this system can become dysregulated, demonstrating higher sensitivity to hormones, particularly adrenocorticotropic hormone (ACTH), and higher levels of glucocorticoids, which may be a result of disrupted negative feedback (96–100). Glucocorticoids are well known to have anti-inflammatory effects, notably through glucocorticoid receptor-mediated suppression of pro-inflammatory transcription factors, leading to decreased levels of pro-inflammatory cytokines and chemokines (101–104). These immunosuppressive effects can also occur through interaction with proinflammatory signalling pathways and can induce destruction of immune cells such as T lymphocytes (105). Although the anti-inflammatory effects of glucocorticoids have been studied in depth, they can likewise have pro-inflammatory properties through, for example, interactions with pro-inflammatory cytokines, an effect which seems to be exacerbated by stress (106–109).

MDD is more highly prevalent in individuals with inflammation-related disorders – including but not limited to arthritis, cardiovascular diseases, multiple sclerosis, and diabetes – and it is possible that chronic systemic inflammation as a result of illness may contribute to MDD pathophysiology (110). When an individual becomes sick, they will experience a set of symptoms very similar to those of depression: fatigue, loss of motivation, anhedonia, and loss of pleasure, among others. This is categorized as "sickness behaviour," and while different from depression itself due to its short-lived presence (only as long as the illness lasts), such symptomatology suggests that individuals who do develop MDD may be more susceptible or have been subjected to an intense or

long-lasting inflammatory response which has continued beyond the illness duration (111,112). During an illness, proinflammatory cytokines are produced at the site of infection and can proliferate throughout the body, traveling to the brain to cause depression-like symptoms and depression disorders through means such as the neural pathway which can activate primary afferent nerves like the vagus nerve or the humoral pathway which involves the choroid plexus (113–117).

An additional pathway exists in the brain through which systemic inflammation can exert its effects, and that is by activating microglia in the brain, thereby triggering a neuroinflammatory state. Due to the significant mounting evidence and new means of measuring microglia *in vivo* in humans, we are currently interested in this final theory. Microglia are involved in the neuroinflammatory response by acting as macrophages and can be measured as a proxy for inflammation. While there are multiple methods through which their activation can occur, a specific example of a mechanism describing the activation of microglia and how this results in negative outcomes involves the activation of indoleamine 2,3 dioxygenase (IDO), which breaks down tryptophan (TRP), a serotonin precursor, into kynurenine (KYN) (Figure 1) (111,118–120). Serotonin is well-known as an essential monoamine neurotransmitter involved in mood and cognition, and formation of KYN bypasses the pathway through which it is formed. This process results in decreased serotonin, which is similar to what individuals injected with interferon alpha (IFN-α), an inflammatory biomarker, who develop depression experience: decreased serotonin and increased KYN (121). KYN can also lead to the production of kynurenic acid in astrocytes and quinolinic acid in microglia (119). This has been implicated in causing oxidative stress, a process involving an increase in reactive oxygen species, leading to tissue damage (119,122). It has been reported that depressed suicide victims exhibit increased levels of quinolinic acid in the microglia of the ACC post-mortem (123). Those with chronic illnesses who are being treated with interferon alpha (IFN-α) manifest decreased TRP, increased KYN and KYN/TRP ratio, and increased depression scores (124). Healthy individuals, when faced with an inflammatory challenge, experience an activated KYN pathway, as well as increased IDO (125,126). Injection of quinolinic acid precursor 3-hydroxykyneurinine (3-HK) into mice leads to depression-like behaviours, and inhibition of IDO through an IDO inhibitor reduces these behaviours

(126–129). While the KYN pathway is not the only one which is associated with microglia, it seems to be strongly involved in the neuroinflammatory condition of depressed individuals.

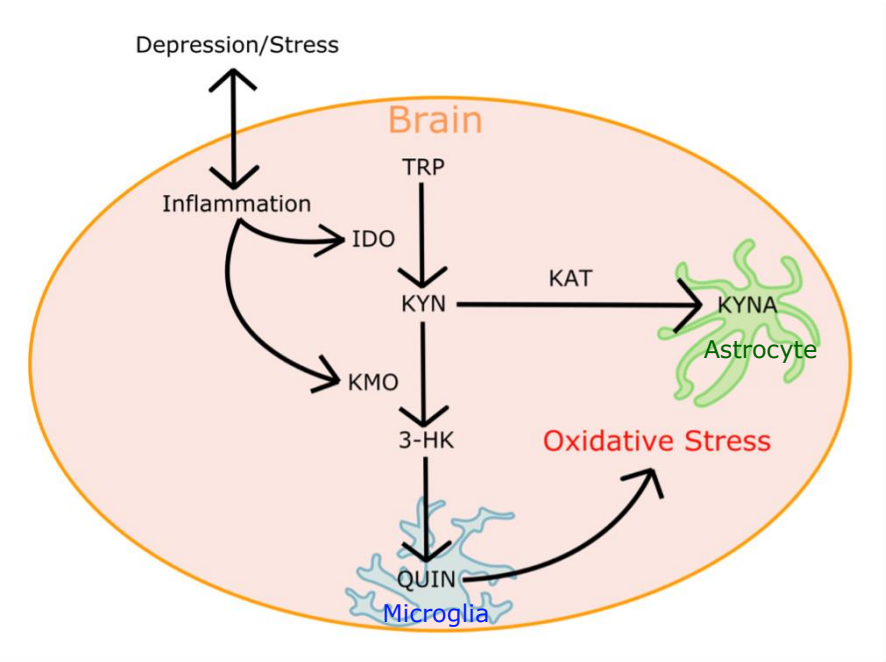

Figure 1.2. Kynurenine pathway of microglial activation – one of many contributors to neuroinflammation. Stress leads to increased inflammation in the body and brain, thereby increasing pro-inflammatory cytokines and leading to the activation of indoleamine 2,3 dioxygenase (IDO) and kynurenine-3-monooxygenase (KMO). IDO breaks down tryptophan (TRP) into kynurenine (KYN) while KMO facilitates the production of 3-hydroxykyneurinine (3-HK), eventually leading to quinolinic acid (QUIN) in microglia and oxidative stress. Kynurenine aminotransferase (KAT) catalyses the conversion of KYN to kynurenic acid (KYNA) in astrocytes.

1.5.2 Peripheral Inflammatory Markers

Measurements of peripheral inflammatory biomarkers are a simple method of assessing and confirming inflammation in the body. Inflammatory biomarkers, including but not limited to cytokines and acute phase proteins, are released by the body during immune processes and are exhibited in dysregulated amounts in those with neuropsychiatric illnesses. Previous studies over the last century have shown peripheral

increases in proinflammatory cytokines like INF-α and interleukin-6 (IL-6) in depression (130). Other inflammatory markers which are elevated in depression include acute phase proteins, chemokines, adhesion molecules, and inflammatory mediators like prostaglandins. These elevated levels even occur in those without any other recorded illnesses and in post-mortem brain tissue samples (131). Peripheral blood markers interleukin-1β (IL-1β), IL-6, tumour necrosis factor (TNF), and C-reactive protein (CRP) are the most reliable biomarkers of inflammation in individuals with MDD, as noted by numerous meta-analyses (130). Further, biomarkers such as TNF have been associated with genes involved in depression when involved in the immune response (132). Chronic elevation of these cytokines may lead to the adverse symptomatology of depression, and an increase in symptom severity is correlated with an increase in peripheral blood cytokine concentrations (133,134), although not every person with depression will have elevated levels of proinflammatory cytokines (130,135,136). This can be attributed to the heterogenous nature of depression. All in all, increases in proinflammatory cytokines observed in depression are the most basic means through which inflammation has been linked to depression.

Levels of certain inflammatory biomarkers can be modulated using antidepressants. In a meta-analysis containing 22 different studies pertaining to antidepressant effect in MDD, it was found that IL-1β and IL-6 and depressive symptoms were decreased in those who had undertaken antidepressant therapy, expressly SSRIs (137). Wang et al. (2019) demonstrated that this is also the case with TNF (138). These studies effectively proffer a relationship between inflammatory markers and the expression of depression – a relationship which can be managed in part with therapies specifically targeting depression.

In addition to increased innate levels of proinflammatory cytokines, administration of these cytokines can induce depressive symptoms. Administration of innate human cytokines such as TNF-α can lead to psychosis, delirium, and agitation, while administration, both acutely and chronically, of cytokine inducer lipopolysaccharide (LPS) in healthy volunteers leads to an increase in symptoms of depression (111,133,134,139). Individuals with established medical conditions see similar results; peripheral cytokine levels are increased in those exhibiting depressive symptoms

as a result of medical conditions, but who themselves were not originally clinically depressed (140,141). 35.6% of individuals with asthma experienced symptoms of depression, and those who were depressed had higher levels of TNF-α (142), while illness-induced increases in IL-6 and nuclear factor kappa B, which is involved in the initiation of the inflammatory response, are correlated with depressive symptoms such as fatigue and insomnia (143,144). These "sickness behaviours," as mentioned before, will disappear once the illness has ceased, but dysregulation of the inflammatory system may prolong their presence and this chronic exposure may contribute to MDD. In actuality, those with a medical illness are several times more likely to develop MDD (145).

In related experiments, blocking cytokines such as TNF or other units of the inflammatory pathway, such as COX-2, has resulted in a reduction of depressive symptoms in individuals with medical conditions such as arthritis and cancer, and in individuals with MDD (46,146). From these findings it is apparent that inflammation at the systemic level is deeply intertwined with depression symptomatology which lends credibility to the infection theory of inflammation. Again, this is not the case in every individual with increased inflammatory markers or an inflammatory medical condition, which suggests that a specific faction of individuals is vulnerable to inflammation-derived depression.

All this considered, the presence of inflammation alone is not enough to describe depression symptomatology. Inflammation is present in several neuropsychiatric illnesses including schizophrenia and obsessive-compulsive disorder, suggesting that it is possible inflammation has a multitude of neurological outcomes or a specific combination of biological factors must be present in order for the effects of inflammation to contribute to depression precisely.

1.5.3 Blood Brain Barrier Permeability

Until recently, it was not well understood how peripheral cytokines affected or were involved in the etiopathogenesis of depression at the level of the brain. As they exist in the plasma and are seemingly blocked from entry into brain parenchyma by the blood brain barrier (BBB), their connection to the brain was thought to be indirect (e.g., neural and humoral pathways which target the afferent nerve). Permeability in the BBB was

shown to be caused by chronic social stress in male mice. In the nucleus accumbens (Nac), an area associated with emotional regulation, claudin-5 (cldn5), a tight junction protein which acts as a cell adhesion molecule in the paracellular barrier between endothelial cells, is reduced and peripheral proinflammatory cytokines flow into the brain, leading to depression-like behaviours such as social avoidance, anhedonia, and helplessness. This process has been observed in post-mortem brain tissue samples of humans with MDD, effectively associating changes in the Nac endothelium with depression in humans (147,148). Other means through which peripheral cytokines may enter through the BBB include entrance of cytokines through leaky regions such as the circumventricular organ, passage of immune cells that generate cytokines into the brain, active transport, and increased inflammatory mediator signalling leading to production of inflammatory cytokines by microglia and subsequent BBB disruption (149–151).

1.5.4. Cytokines and Brain Activity

Since cytokines can enter the brain, it would be of interest to measure them in the brain along with their direct effects on brain activity. Unfortunately, levels of cytokines in the brain have been studied in most part using post-mortem samples (which are not fully generalizable), but the relationship between cytokines, brain activity, and brain connectivity has been a topic of interest with the development of more sophisticated neuroimaging technologies. The results of these studies demonstrate that there is an association between inflammation and dysregulation of both activity and connectivity of specific brain regions. Individuals treated with IFN-α have increased activation in the dorsal anterior cingulate cortex (dACC), increased arousal anxiety, and an increased alarm response (152). In an experiment, when subjected to a public speaking task subjects exhibited increased concentrations of oral IL-6 and soluble TNF receptor 2 (sTNF-R2), which was correlated with activation of the dACC in a social rejection task. In addition, activation of the amygdala was correlated with increased oral IL-6 expression in response to a social evaluation stressor, with participants who had the greatest IL-6 responses demonstrating the greatest connectivity in the fear network (amygdala, DMPFC).

On the contrary, increased IL-6β has been associated with decreased activity in the anterior cingulate and amygdala, and increased likelihood of treatment resistance

(153). Likewise, an increase in CRP was associated with a reduction in functional connectivity in a network including the ventral striatum, para-hippocampal gyrus/amygdala, orbitofrontal and insular cortices, and posterior cingulate cortex (154). These changes in connectivity were focused on the ventral medial prefrontal cortex (VMPFC), which mediates the relationship between CRP and anhedonia. While these results are the opposite of those mentioned previously, they all indicate dysfunction of the brain during inflammation. Increases in brain activation and connectivity in areas such as the ACC and amygdala being correlated with stress and inflammation create the foundation for our understanding of inflammation and its influence on the brain. While these studies do not include specific measures for depression or depressed subjects, and therefore cannot be directly related to its pathology, they reveal that inflammation has a very real effect on brain functionality. Of note, stress and inflammation are both predisposing factors for depression and their role in these studies allows for speculation on how depression may be involved (136).

Other studies have found dysregulation of the brain associated with both inflammation and depression. Functional connectivity between the striatum and the VMPFC was correlated with increased peripheral IL-6, IL-1B, and IL-1 receptor antagonists in MDD (155). The striatum is further involved as increased cytokines reduce its activation with reward networks, and an injection of the typhoid vaccine, an inflammatory stimulator, can decrease connectivity between it and the subgenual ACC, a brain area associated with depression (Section 1.5). This connection is mediated by IL-6. Moreover, the changes seen in the brain are not limited to the functional; the physical properties of the brain have been shown to be altered in those with MDD. A thinner MPFC was found in MDD patients with a greater number of depressive episodes, and CRP level was inversely associated with right MPFC thickness (156). Hippocampal volume is negatively associated with higher levels of IL-6 and CRP in MDD, a potentially debilitating effect but not surprising as memory deficits are a recurring and common symptom of depression (157). What is most meaningful about these results is their direct linkage to depression; they tie together depression, inflammation, and brain measures in single studies. This confirmation that there is a relationship between all three, with seemingly negative physical outcomes, bolsters the need for further investigations

into their relatedness, as questions pertaining to how cytokines or other inflammatory markers correlate with specific brain changes, for example, remain unanswered.

1.5.5 Late-Life Depression and Inflammation

Studies investigating factors of inflammation in older depressed populations are unfortunately relatively rare despite available studies indicating inflammation as a factor in LLD and age itself furthering an inflammatory state (158). Increased expression of cell adhesion molecules in the DLPFC (associated with ischemia), of free 8-isoprostane, a marker for oxidative stress which can lead to chronic inflammation, and other proinflammatory biomarkers have been demonstrated in LLD (159–162). Decreases in inflammatory cytokine levels in older adults (mean age 60.3) after practicing meditation, as compared to a music group, highlight the presence of inflammation in and its relatedness to depression symptomatology in older adults (163). As previously mentioned, somatic comorbidities are often predisposing factors of LLD, so it is interesting that symptoms often complained about in old age such as frailty have been linked to chronic inflammation (164,165). More recently, a 2020 study by Sonsin-Diaz et al. found that compared to those who maintained low levels of CRP, older individuals (mean age 75.5) who have elevated levels of CRP throughout the majority of a 21 year period experienced depression symptoms of greater severity (166). This is further supported by Torre-Luque A. in 2019 and Kokkekler MJE et al. in 2020 who both found that inflammatory dysregulation is associated with increased depression severity in older adults (167,168). Overall, inflammation seems to play a critical role in the furtherance of depression in these individuals, and age may intrinsically lead to such outcomes, although it is not clear the extent to which inflammation affects older adults and if these effects are more severe.

1.5.6 Antidepressant Effects of Anti-inflammatory Agents

Another link between inflammation and depression is the effect that anti-inflammatory agents can have on depressed individuals. A meta-analysis studied the effects of pro-inflammatory cytokine inhibitors on depressive symptoms, as compared to placebos. They found that these medications significantly decreased depressive symptoms

(169). In a study using infliximab, motor retardation, motivation, suicide ideation, and depressed mood were all significantly improved (170). Two separate meta-analyses found that in thousands of patients, anti-inflammatory drugs (including NSAIDs) improved depression symptomatology (171,172). It should be pointed out that these meta-analyses were performed in those with comorbid inflammatory conditions such as psoriasis, which are posited to have a separate mechanistic profile. Although these findings do suggest antidepressant effects of anti-inflammatory agents, the current evidence is not sufficient for progression to clinical use. It is unknown how anti-inflammatory agents affect specific depressive symptoms such as fatigue (45). Consideration of potential side effects, methodology for the identification of treatment-responsive patients, and comorbidities, a possible risk factor, will need to be completed. Most importantly, replicability of these findings will need to be achieved – a milestone which remains to be seen. Discrepancies in methodology have contributed to inconsistent research on the effects of anti-inflammatory agents such as NSAIDs, and a consensus on research methodology is crucial for lending credibility to their use as antidepressants (173). Nonetheless, their use in research and their ability to have some effect on depression symptomatology create further evidence associating depression with inflammation.

1.5.7 Treatment Resistance and Inflammation

Little evidence exists at this point to link treatment resistance in depression with inflammation as measures of inflammation are not commonly assessed in TRD despite inflammation being an indicator of treatment response and it contributing to the potential existence of an inflammatory "subtype" of depression (174). Recent research has suggested that inflammatory dysregulation may be the difference between a treatment responder and a non-responder (175,176). This is substantiated by low monocyte chemoattractant protein 1 (MCP1), an inflammatory cytokine, being found to be a predictor of treatment resistance (177), along with increased peripheral IL-6 in majorly depressed patients who did not respond to amitriptyline treatment (178). Other peripheral cytokines, such as TNF, sTNF-R2, and IL-6 are elevated in those with 3 or more failed treatment trials as compared to those with 1 or 0 failed trials (179), and a variety of

diverse proinflammatory biomarkers have been shown to be increased in those with TRD as compared to healthy controls (180,181).

Specific expression combinations of inflammatory biomarkers and genes may be what contributes to this subtype. This may be why inflammatory biomarkers can have opposite effects in certain contexts (although this has yet to be elucidated). Interleukin-2 (IL-2) detected before treatment has been associated with treatment resistance in the short term, but CRP, IL-6, MCP4 elevated post-treatment has been linked with poor long-term (3-12 months after hospital discharge) response to treatment (174). Furthermore, TRD patients with high inflammation (as indicated by elevated CRP levels) respond better to anti-inflammatory agents than those with low to average CRP (182). A follow-up study found these high inflammation patients displayed a unique lipid and glucose metabolism gene expression profile which is possibly an indicator of this inflammatory subtype of depression (183). If this subtype indeed exists, then treatment resistance in depression may be to a great degree influenced by inflammation or a distinct inflammation-related profile. Further research is needed to link inflammation and treatment resistance in depression.

1.5.8 Microglia and Inflammation

As previously mentioned, microglia play an important role in the inflammatory response as they mediate the cascade by acting as the macrophages of the brain. They release inflammatory mediators, and are involved in the overexpression of glutamate – leading to glutamate excitotoxicity – and in the production of quinolinic acid, a potent neurotoxin, from KYN (184). In chronically stressed mice, there is a decline in the number of microglia in the hippocampus, as well as decreased activation of said microglia (185). In individuals with multiple sclerosis, microglial activation is present and is associated with symptoms of depression (186). In MDD, microglial activity is linked with increases in levels of IL-6, IL-8, and TNF in the CSF and brain parenchyma, as well as with a reduction in astrocytes and oligodendrocytes (187,188). This reduction could be due to monocytes infiltrating the brain parenchyma as a result of increased permeability of the BBB (188). Suicidal individuals are known to have increased levels of monocytes in addition to increased activation of microglia and microglial priming in both grey and

white matter (189–191). This occurs in these patients regardless of if they fulfill DSM criteria. The above findings are reflective of cytokine data, wherein individuals with depressive symptoms as a result of other illnesses or inflammatory challenges experience microglial activation (133,192–194).

Microglial activation and density can be imaged using positron emission tomography (PET) tracers, wherein the tracer binds to translocator protein (TSPO) on the surface of mitochondria of microglia. Setiawan and colleagues reported that, while using tracer [^{18}F]N-2-(fluoroethoxyl)benzyl-N-(4phenoxypyridin-3-yl)acetamide ([^{18}F]FEPPA), TSPO total volume distribution (V_T), or TSPO density, was significantly increased by 26% in the prefrontal cortex, 32% in the ACC, and 33% in the insula in patients with a major depressive episode (195). In particular, the greater TSPO V_T in the ACC correlated with greater depression severity and duration (196). The severity and duration of unmedicated MDD were strong predictors of TSPO V_T, accounting for 50% of variance in TSPO V_T in the prefrontal cortex, anterior cingulate cortex, and insula. Richards et al repeated the initial results while using [^{11}C]PBR28 in major depressive episode (MDE) patients, finding that TSPO binding was significantly increased in unmedicated MDE patients, and could be decreased by psychotherapy (197,198). An additional [^{18}F]FEPPA study found increased microglial activity in the hippocampus, while a study using [^{11}C]CK11195 reported higher microglial activity in the ACC, the insula, and the prefrontal cortex in unmedicated MDD patients as compared to healthy controls (199,200). In LLD patients, increased uptake was correlated with levels of peripheral CRP (201). Overall, [^{18}F]- FEPPA studies indicate increased microglial activity in MDD, and studies using other TSPO-PET tracers reflect these findings.

On the contrary, Hannestad et al (2013) found that TSPO is not elevated in depression, although they used [^{11}C]PBR28 as their radioligand (202). Another study, also using [^{11}C]PBR28 as a radioligand, was performed in 10 depressed individuals using injected LPS, compared to 10 healthy controls. They found that TSPO binding did not correlate with inflammation or mood even though TSPO levels did increase (203). In another study, IFNα was administered which led to a decrease in V_T in the whole brain, but with correction this effect was eliminated. Mood changes were apparent in subjects given IFNα, but no association was found between inflammation and mood (204). This

could be attributable to differing study methodologies, as well as to differing TSPO tracer characteristics, or the potential suppression of microglia in specific conditions. Indeed, some studies have found decreased numbers of glial cells in the subgenual anterior cingulate cortex in depressed individuals compared to healthy controls (205–207), suggesting distinct roles for microglia. At this point in time there seems to be no clear consensus on how microglia should respond and how this may change in response to depression.

Considering the above, it becomes apparent that the status of microglial activation in TRD is still unknown. Particularly of note, it is not clear if key frontolimbic regions such as the insula and ACC exhibit higher microglial activation, and how such activation, if present, relates to the connectivity between these two regions. In healthy individuals administered an inflammatory challenge, the resulting depressive symptoms are mediated by activity of the insula and sgACC (67,194). To further understand neuroinflammation in the sgACC and the insula, microglial activation in these areas can be measured. In the subsequent chapter, we examine this issue in human subjects.

1.6 Imaging the Mechanisms of Depression

1.6.1 In Vivo Measures of Inflammation

Inflammation is frequently assessed through measurements of peripheral inflammatory biomarkers. This method is simple, cost-effective, and easy-to-complete, and as such has been well-established in literature. Biomarkers will be found at the site of inflammation, as well as circulating in plasma and serum, and can be measured from blood samples in addition to urine, saliva, and cerebrospinal fluid. A major drawback of this technique involves cases when the region of interest is the brain: one is unable to ascertain neuroinflammation, but rather a whole-body inflammatory state. Post-mortem brain tissue samples can be acquired to assess biomarker levels directly in the brain with a high degree of specificity, but these samples come from the endpoint of disease progression (which is not of interest) and are not generalizable.

1.6.2 PET Imaging of Inflammation

PET is the most highly sensitive imaging technique and is widely used in the research of neuropsychiatric disorders, movement disorders, and more. Microglial activation, and thus inflammation of the brain, can be quantified by detection of translocator proteins of 18 kDa (TSPO) which are expressed on the surface of mitochondrial membranes and are upregulated during the immune response. Many studies over the years have confirmed this upregulation during neuroinflammation (208) as in the brains of depressed individuals as described above.

The most commonly used TSPO radioligand to study microglia to date has been [^{11}C]PK11195 which poorly penetrates the blood brain barrier, has poor affinity for TSPO, high plasma protein binding, and a difficult process for synthesis. Second generation ligands – ligands developed from lessons learned from [^{11}C]PK11195 – such as [^{18}F]FEPPA, have a higher brain penetration rate as well as a high affinity for TSPO. [^{18}F]-labelled radioligands have improved counting statistics and image quality compared to [^{11}C], in addition to a longer half-life (20.39 minutes vs. 108.9 minutes) (209,210) which is better suited for clinical imaging. Other [^{18}F]-labelled radioligands such as [^{18}F]FEDAA1106 and [^{18}F]PBR06 have many favourable properties but have issues such as being overly lipophilic or can confound quantification through the production of brain-penetrating metabolites (211,212).

1.6.3 In Vivo Imaging of Brain Network Connections

Magnetic Resonance Imaging (MRI) offers good spatial resolution, is non-invasive, and has good spatial localization for both structural and functional imaging. Functional MRI (fMRI) applies radiofrequency pulses (RF) on a time-varying basis to measure variations in neural metabolism. The majority of fMRI imaging uses the blood-oxygen-level-dependent (BOLD) MRI technique to detect changes in levels of deoxygenated hemoglobin, a measurement which acts as a proxy for neuronal activation. When an area of the brain is activated, this area will have an increased metabolic rate, and therefore receive a greater influx of cerebral blood flow to replenish depleted stores of oxygen and flush out waste products. The oxyhemoglobin will then be converted to

deoxygenated hemoglobin as oxygen is delivered to the activated area. Initially, there will be a surge in deoxygenated hemoglobin as the affected area consumes the oxygen, but within a few seconds the deoxyhemoglobin will be overpowered by oxyhemoglobin as the body overcompensates for the amount of oxygen that is needed. This event is referred to as the hemodynamic response. Oxygenated hemoglobin is diamagnetic and indistinguishable from tissue, but deoxygenated hemoglobin is paramagnetic and creates gradients in the magnetic field which affect specific MRI signal parameters -- T2 and T2* relaxation times -- in the blood. The changes can be measured using BOLD fMRI while the subject is in a "resting state" (in the scanner with no particular thoughts) or in a task-based situation with a specific set of rules to which the subject must adhere.

Functional connectivity is the statistical probability of two or more regions of the brain being functionally related in time (213). These distant regions will show correlated activity in a resting state or in response to a task at the same moment. When regions are shown to be functionally connected consistently, they are considered to be components of a network. As a strength, this approach is relatively straightforward and without bias due to its lack of *a priori* assumptions. It is important to note that since this method is correlational, causation cannot be immediately assumed.

1.6.4 In Vivo Imaging of Brain Macrostructure Connections

Along with functional connectivity, depression symptomatology is linked with alterations in structural connectivity. White matter tracts associated with and connected to the ACC have been implicated in depression. A prominent white matter tract with projections from the cingulate gyrus towards the temporal lobe, forming a C shape around the corpus callosum, is the cingulum bundle. This tract has been linked with emotion, pain, and executive dysfunction (214,215) and, using diffusion tensor imaging (DTI), its integrity has been measured in depressed individuals, identifying decreases in fractional anisotropy and axial diffusivity, and increases in radial diffusivity (216–220). Such patterns of microstructure alterations have been associated with familial risk for depression (221), and reductions in fractional anisotropy have been correlated with depression severity and duration in the cingulum (222). Although the cingulum bundle is

of great interest for those studying MDD, it is currently unknown how neuroinflammation is related to its integrity in those with the condition.

DTI is an MRI technique which measures the diffusion of water three-dimensionally across the brain using diffusion weighted image (DWI). The diffusion tensor captured from DWI represents the three eigenvectors (which each correspond to an eigenvalue, a scaling factor) that characterize water diffusion direction and magnitude, along the three dimensions, which provides insight into white matter integrity. Diffusion measured in tissue will be anisotropic (vary depending on direction), and in white matter anisotropy can be caused by cellular membranes, myelination, and axon density. By modelling the diffusion tensor, diffusion tensor parameters that describe several aspects of water diffusion direction and magnitude can be calculated to characterize white matter macrostructure. The DTI parameters are 1) fractional anisotropy – the relative difference between the largest eigenvalue and the others, representing microstructural integrity, 2) mean diffusivity – the average of all three eigenvalues, representing membrane density, 3) axial diffusivity -- the value of the primary eigenvalue, representing diffusion along the main white matter tract, and 4) radial diffusivity -- the average of the 2^{nd} and 3^{rd} eigenvalues, representing demyelination. Statistical analyses of these parameters using ROIs, voxel-based analyses, or tract-based spatial statistics (TBSS) can be used to quantify brain macrostructure. ROI analyses can be biased and accurate parcellations of white matter regions are difficult to achieve. Voxel-based analyses are automated, spatially-specific, and avoid bias, but registration of individual brain images to a common space for statistical analysis requires complex algorithms and may not be accurate (223). TBSS is a recent method which eliminates issues with registration algorithms and the need to use spatial smoothing to reduce noise, therefore increasing statistical power (223). Here, TBSS was used to quantify connectivity in the cingulum bundle in relation to levels of inflammation measured with TSPO PET. However, DTI can be limited by challenges with tissue type differentiation (partial volume effects) as diffusion is averaged over a relatively large voxel, and by difficulty resolving crossing fibers – fibers from separate pathways -- as the tensor can only model a single major fiber direction (224). Thus, DTI can be difficult to interpret, and depending on the tissue type, brain region, and sample, the same measure may represent separate outcomes entirely. Overall, DTI is particularly

useful in the study of neurodegenerative disorders, movement disorders, neurodevelopment, and aging, but special care must be taken in its interpretation.

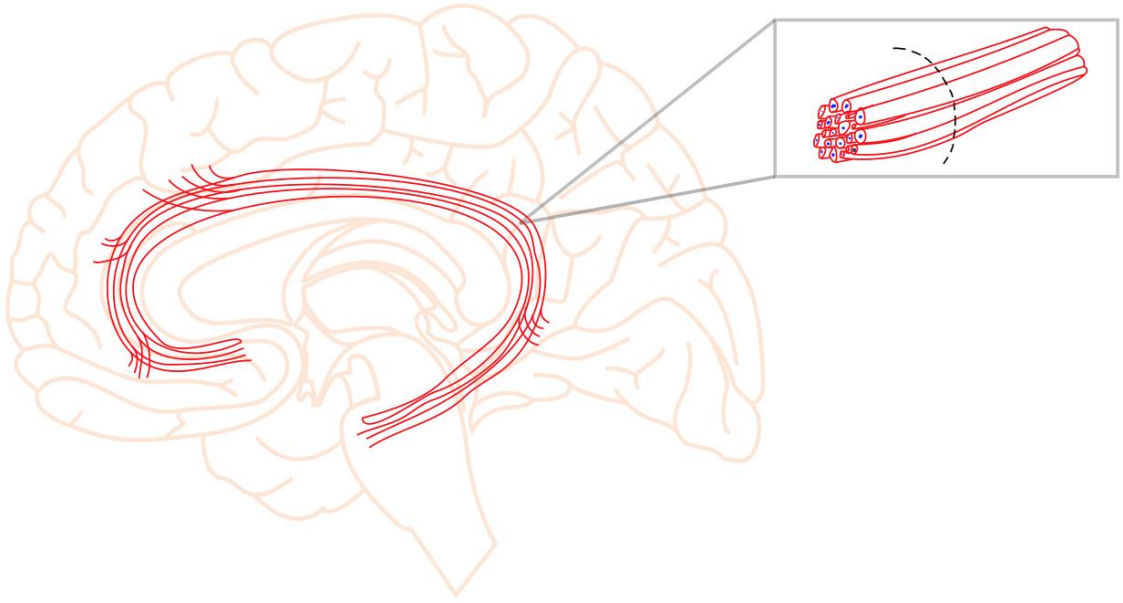

Figure 1.3. The cingulum bundle. The cingulum bundle (red) is a prominent white matter tract which forms a C-shape around the corpus callosum. It has been repeatedly associated with the pathophysiology of depression. We expect the neuroinflammation to reduce its white matter axonal integrity.

1.7 Study Outline

Individuals aged 50 years and older with treatment-resistant MDD were compared to healthy controls. After [^{18}F]FEPPA injection, all individuals were scanned by a hybrid PET/MR system in order to assess microglial activity and functional/structural connectivity. PET images were used to obtain mean relative SUV data for the subgenual ACC and the insula. Functional images were used to assess functional connectivity with the ACC as a seed. DTI data was used to assess structural connectivity of the cingulum bundle. The goal was to relate network data to inflammation.

1.7.1 Study Objectives

1. To assess [^{18}F]FEPPA-traced microglial activation findings in treatment-resistant older MDD patients as compared to healthy controls.

2. To investigate the relationship between microglial activity and functional connectivity between the sgACC and the insula.

3. To investigate the relationship between microglial activity and white matter structural connectivity in the cingulum bundle.

1.7.2 Study Hypotheses

We hypothesized that subjects with MDD would demonstrate increased microglial activity in the insula and sgACC, and this aberration would be associated with alterations in functional connectivity between these two regions and with structural connectivity of the cingulum bundle.

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Chapter 2

2 The Functional and Structural Consequences of Aberrant Microglial Activity in Major Depressive Disorder

This chapter has been adapted from the following manuscript submitted to the Journal of Psychiatry and Neuroscience: The Functional and Structural Consequences of Aberrant Microglial Activity in Major Depressive Disorder.

2.1 Introduction

Major depressive disorder (MDD), characterised by recurrent cognitive and emotional dysfunction, is one of the most prevalent psychiatric disorders and a major contributor toward reduced quality of life (1). While the pathophysiology of MDD is not fully understood yet, various brain networks that facilitate cognitive and emotional information processing are thought to be relevant. Two critical brain regions for predicting therapeutic response and prognosis in MDD are the subgenual anterior cingulate cortex (sgACC) and the insula. Increased sgACC activity can predict resistance to treatment -- this hyperactivity normalizes with an improvement in symptoms (2,3) – and be altered by treatment itself (4–6). Similarly, the metabolic state of the insula, modifiable through MDD therapies, appears to be a critical determinant of response to various forms of treatment in MDD (7–9). Insula, through its extensive connectivity with other brain regions including sgACC, functions to assign salience to emotional and sensory stimuli and regulates interoception (10,11), the disruption of which has been associated with the emergence of several cardinal features of MDD (12–14).

Dysfunction of the immune system, and the resulting aberrant load of neuroinflammation, is increasingly considered to be an important pathway towards depressive illness, at least in a subgroup of patients with MDD. Aberrant neuroinflammation has major implications for treatment outcomes; conventional antidepressants that have little to no anti-inflammatory properties may not be effective,

thus resulting in a treatment-resistant profile (15,16). Microglia, which have an integral role in driving the neuroinflammatory response in the brain, have been shown to have increased activity in MDD (16–19), by using PET ligands (e.g. [^{18}F]N-2-(fluoroethoxyl)benzyl-N-(4phenoxypyridin-3-yl)acetamide or [^{18}F]FEPPA) that bind to receptors expressed on the mitochondrial membrane of activated microglia. Microglia may alter brain network connectivity through means such as synaptic pruning (20); this likely has downstream consequences on connectivity among brain regions. Prior studies have linked peripheral and post-mortem markers of inflammation with alterations in resting-state functional connectivity in MDD (21,22). While excessive microglial activity has been demonstrated in vivo in MDD, we do not know if this has functional and structural consequences on brain networks relevant to the pathophysiology of depression.

Subjective depressive consequences resulting from neuroinflammatory triggers in healthy volunteers are mediated by the neural activity in the insula and sgACC (10,23). The dysfunction of ACC, in particular its subgenual division, and its altered connectivity with the insula have been consistently reported in MDD (24–29). The insula, ACC as a whole, and the sgACC show increased microglial activity and density in MDD (16,18,19). In the current study, we focus on the microglial activity of insula and sgACC and the connectivity anchored on these nodes in MDD.

Several white matter (WM) tracts have been implicated in depression. The cingulum bundle is a WM tract associated with pain, emotion, and executive function (30,31) that carries a large fraction of projections from the ACC, towards regions that are critical in the pathophysiology of depression such as the hippocampus (32) and parahippocampal gyrus (33). In MDD, alterations in integrity of this tract have been reported and associated with axonal degeneration, familial risk, illness severity and persistence (34–40). In particular, patients with later-life depression are observed to have a higher load of WM lesions affecting the cingulum (41); deep-brain stimulation of the cingulum bundle is a promising treatment option for treatment-resistant MDD (42). We investigated the potential relationship between microglial activity and cingulum integrity in MDD.

We hypothesized that in MDD increased [^{18}F]FEPPA -measured microglial activity in the sgACC and insula would relate to both disrupted connectivity among these

two nodes, and the severity of MDD. This current study investigated this relationship in four parts using a hybrid PET/MRI approach: (1) demonstrating aberrant microglial activity in sgACC and insula in MDD compared to healthy individuals, (2) relating microglial activity to the observed severity of depression, (3) evaluating the functional connectivity between the sgACC and the insula in MDD using functional MRI, (4) establishing the relationship between aberrant microglial activity and the structural and functional connectivity of the affected regions in MDD in comparison to the healthy individuals.

2.2 Methods

2.2.1 Participants

Twelve depressed individuals aged 45 years and older referred to the Therapeutic Brain Stimulation clinic at the Parkwood Institute Mental Health Care Building (London, Ontario, Canada) were recruited between December 2017 and March 2019 for the study. PET/MRI data from 23 healthy volunteers recruited for an ongoing dementia imaging study were included as controls (Table 2.1). Patients were assessed by their treating psychiatrist per the Diagnosis and Statistical Manual of Mental Disorders, 5th Edition - Structured Clinical Interview for DSM-5 to confirm their diagnosis (43). Symptomatology was reassessed at 6 to 12-month intervals. Up to two weeks before the scan, patients were administered the Montreal Cognitive Assessment (MoCA), the Beck Depression Inventory (BDI) test, Clinical Global Impression Severity scale (CGI-S), and the 17-item Hamilton Depression Rating Scale (HAM-D17) (44–47). The medication history, level of education, onset of depression, and treatment response were collected at study enrollment.

All participants were free from confounding neurological or psychiatric disorders, or other conditions that could confound PET and MR imaging including known history of infection and autoimmune disorder. Given the known differences in TPSO binding affinity common to second-generation TSPO PET ligands, participants with low-affinity binding, which represents < 10% of the population, were excluded to reduce intersubject variability (48). Genotyping of the TSPO-related polymorphism was performed at The

London Regional Genomics Centre at Robarts Research Institute and individual TSPO polymorphism status was included as a covariate in statistical analysis. This study was approved by the Western University Health Sciences Research Ethics Board and conducted in accordance with the Declaration of Helsinki ethical standards. All participants provided written informed consent.

2.2.2 PET/MR Imaging

Individuals were scanned once using a 3T Biograph mMR (Siemens Healthineers, Erlangen, Germany) utilising a 12-channel PET-compatible head coil in order to obtain both PET and MRI data in tandem. Each subject was injected with an intravenous bolus of [^{18}F]FEPPA (185±5 MBq (5±0.1mCi)), synthesized at Lawson Health Research Institute following a reported method (49). Dynamic PET images were acquired for 90 minutes in list mode, starting immediately following tracer injection. The 60 to 90 minute post-injection frames were reconstructed to six 5-minute frames using Siemens e7 tools and an iterative algorithm (ordered subset expectation maximization algorithm; 3 iterations, 21 subsets, 2 mm full-width at half-maximum (FWHM) Gaussian filter, 2.5 zoom factor, 344 × 344 × 127 matrix representing 2 × 2× 2 mm^3 voxels), and corrected for decay, scatter, dead-time, and attenuation using an MR-based approach (50).

A T1-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence (slice thickness = 1mm, repetition time = 2000 ms, echo time = 2.98 ms, flip angle = 9 degrees, acquisition matrix = 256 × 256, and field of view = 256 mm) was used for generation of 1mm isotropic-voxel-tissue masks for PET attenuation and partial volume error correction, masking, co-registration of MRI with PET, and spatial normalization of images into Montreal Neurological Institute (MNI) space. fMRI images were acquired during rest with eyes opened in a 2 mm isotropic resolution covering the whole brain with 64 interleaved axial slices (slice thickness = 3 mm, repetition time = 2500 ms, echo time = 30 ms, flip angle = 90 degrees, acquisition matrix = 80 mm × 80 mm, and field of view = 240 mm). A total of 164 volumes were obtained; the first 6 were discarded to account for magnet stabilization. A 10-min diffusion-weighted imaging (DWI) scan was acquired using a single-shot EPI sequence with the following parameters: 64 diffusion-encoding directions; *b*-values = 0 and 1000 s/mm^2; 2x2x2 mm^3

isotropic voxels. Two b_0 images were acquired with opposite phase-encoding directions to correct for susceptibility-induced distortions in DWI.

2.2.3 PET/MR Image Processing

Preprocessing of PET images was done using the SPM12 toolbox (www.fil.ion.ucl.ac.uk) and in-house MATLAB (2018b; The MathWorks, Natick, MA) scripts. To compensate for motion, the PET time frames were aligned to the first frame and averaged to one image volume using the SPM realignment function and converted to standardized uptake value (SUV) images. T1-weighted images were segmented into grey matter, WM, and CSF using the segmentation tool in the computational anatomy toolbox (CAT12) (51). The grey matter segment was then smoothed using an 8 mm kernel to permit improved alignment of MRI to PET. A whole-brain mask generated from the cumulative sum of the gray matter, WM, and CSF segments were applied to the PET SUV images to remove extracerebral voxels. The SUV images were corrected for partial volume effects (PVE) using the Müller-Gartner method implemented in the PETPVE12 toolbox in SPM (52). To ensure WM and CSF signal contamination were well-compensated, the WM and CSF segments were eroded in MATLAB with morphological filtering and connected component analysis using a 2 × 2 voxel size. Point-spread function (PSF) for PVE correction was set at 5 mm isotropic to model the PSF of the PET/MRI scanner. The SUV images were spatially normalized to MNI space following the unified segmentation method (53), smoothed using a Gaussian filter with a FWHM of 10 mm, and count-normalized by the mean SUV in the primary motor cortex to create relative SUV (rSUV) maps. Masks for the grey regional analyses were generated from the Automated Anatomical Labeling (AAL3; https://www.gin.cnrs.fr/en/tools/aal/) atlas. The masks were eroded by 2 voxels as described above to restrict inclusion of voxels outside of the ROI and minimize misalignment of ROI to individual PET images. Mean rSUV values were extracted from left and right sgACC and insula for statistical analysis. The sgACC has been distinctly reported to have higher microglial activity in voxelwise studies of MDD, while the insula has been reported to have higher microglial activity in ROI studies (16,18,19). In addition, we also explored other subregions of ACC (pregenual and supracallosal ACC) in an exploratory manner, and report uncorrected results.

fMRI images were pre-processed using the default direct processing pipeline in CONN toolbox (Matlab2018b; www.nitrc.org/projects/conn, RRID:SCR_009550) and motion parameters were included as regressors. A seed-based analysis with the sgACC as a seed region was performed between patients and controls (excluding four control individuals due to frontal artifacts) including covariates (age and sex). Pearson's r-to-z-scores representing functional connectivity between the sgACC and the insula were extracted for statistical analysis. DWI data were preprocessed using an in-house pipeline to generate diffusion tensor imaging (DTI) scalar maps; fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) maps (54). The DTI scalar maps were spatially normalized to MNI space using a three-step registration algorithm (consisting of rigid, affine, and deformable transformations to a standard MNI T1 1-mm template) in ANTs (55). Region-based analyses of DTI scalars were performed by calculating mean FA, MD, AD, and RD values in the cingulum bundle WM pathway using regional masks from the John Hopkins White Matter Atlas (56). While there are multiple metrics available from DTI, increased AD has been associated with axonal integrity (57,58) and increased extracellular water content; increased extracellular water content is a biomarker of neuroinflammation (59). Although metrics such as FA, MD, and RD may likewise be sensitive to water content changes, the specific activity of microglia may be more closely related to AD as they aid in engulfing axonal debris (60,61). We chose AD as the primary DTI index of interest for this analysis.

2.2.4 Statistical Analysis

Covariates of no interest included age, sex, and TSPO status. Multiple linear regressions including covariates of no interest were performed to assess differences in regional microglial activity (rSUV) and in functional connectivity (z-scores) to determine the effect of diagnostic status. Given the 4 ROIs chosen *a priori*, a Bonferroni-corrected statistical threshold of 0.0125 was used as the threshold for significance. A multiple linear regression per ROI was performed with non-linear locally estimated scatterplot smoothing (LOESS) to assess the relationship between [^{18}F]FEPPA uptake and HAM-D scores, and between [^{18}F]FEPPA uptake and DTI measures of the cingulum bundle, adjusting for covariates as above (threshold p=0.05).

To test the effects of functional connectivity on microglial activity, first a single factor representing all functional connectivity variables (left sgACC to left insula; left sgACC to right insula; right sgACC to left insula; right sgACC to right insula; left sgACC to right sgACC; left insula to right insula) was derived using principal components analysis (PCA). Next, a non-linear regression predicting left sgACC microglial activity with the PCA-derived connectivity variable and covariates of no interest was run across the whole sample. The connectivity variable and age were included as non-linear (LOESS) predictors while the categorical variables of diagnostic status, sex, and TSPO status were linear. The same models (excluding diagnostic status as a predictor) were run on patients and controls separately. Non-linear regressions were used to relate functional connectivity with [^{18}F]FEPPA uptake, as both increased (62) and decreased (63,64) connectivity has been reported in preclinical and clinical studies of inflammatory markers, indicating the likelihood of a U-shaped relationship between microglial activity and functional connectivity. Including age and the PCA-derived factor as non-linear in the model reduced the model error significantly compared to the linear model. The number of knots included in the non-linear model was selected based on the generalized cross-validation (GCV) method, which uses leave-one-out cross-validation to minimize the model's prediction error, thus minimizing overfitting. All statistical tests were run using SAS 9.4M7 (Raleigh, North Carolina) with a statistical threshold of p=0.05.

2.3 Results

We found a significant increase in [^{18}F]FEPPA uptake in patients when compared to controls in the left sgACC (t=2.684; p=0.012) while all other regions except the right pregenual ACC showed higher [^{18}F]FEPPA uptake than controls but did not survive correction (Figure 2.1). [^{18}F]FEPPA uptake from the left and right insula were significant predictors of HAM-D scores in a linear model including non-linear age (left p=0.039; right p=0.037) (Figure 2.2). Z-scores representing functional connectivity between the sgACC and the insula revealed no significant reduction in pairwise connectivity (Figure 2.3; right sgACC-right insula p=0.053; left sgACC-left insula p=0.474; left sgACC-right insula p=0.059; right sgACC-left insula p=0.209).

Table 2.1. Participant Demographics.

Variable[a]	**Depressed (n=12)**	**Healthy (n=23)**
Sex	8 F, 4 M	11 F, 12 M
Age (yr.)	54.9 ± 4.5	60.3 ± 8.5
TSPO Status[b]	8 HAB, 4 MAB	12 HAB, 11 MAB
MoCA[c]	26.5 ± 2.1	N/A
HAMD-17	18.9 ± 7.1	N/A
Age of Onset[d] **(yr.)**	30.4 ± 13.8	N/A

[a] All means include ± SD.
[b] HAB=high affinity binder, MAB=mixed affinity binder.
[c] Missing data from 1 subject.
[d] Missing data from 2 subjects.

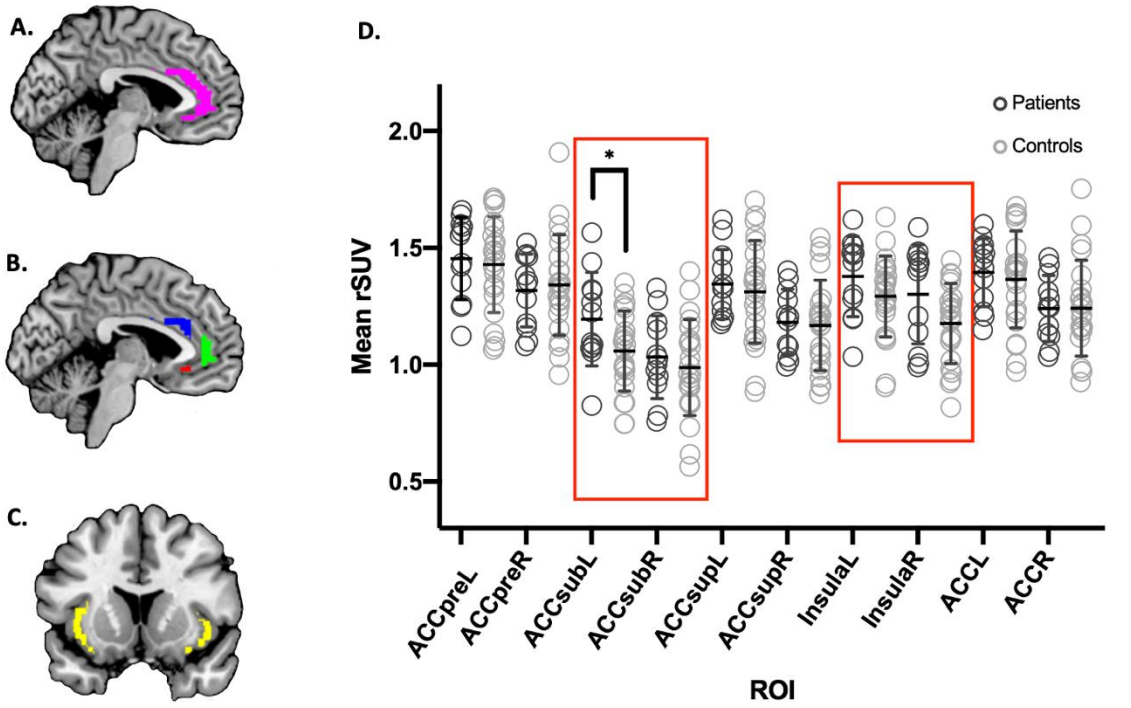

Figure 2.1. Differences in mean relative standard uptake values (rSUV) per region of interest (ROI). ROIs included left and right sides of (A) the whole anterior cingulate cortex (ACC; in violet), (B) its pregenual (green), subgenual (red), and supracallosal (blue) subregions, as well as (C) the insula. (D). ROIs chosen *a priori* for hypothesis testing are highlighted in the red box. Linear regressions were run for each ROI (including covariates), finding significant differences between patients (N=12) and controls (N=23) in the left subgenual ACC (p=0.012) when partial volume correction is used.

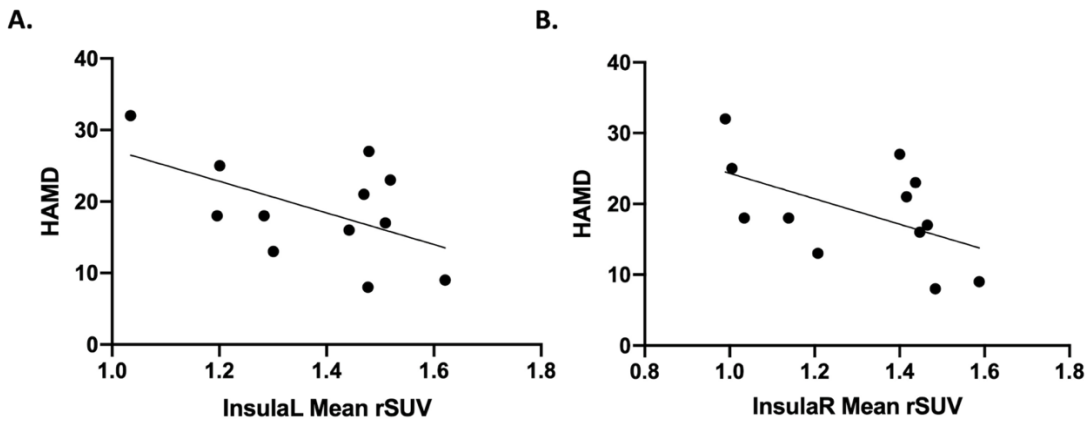

Figure 2.2. The relationship between mean relative standard uptake values (rSUV) from the insula and HAM-D scores. A linear regression including age as a non-linear factor (LOESS) and sex as covariates found that both **(A)** left insula [^{18}F]FEPPA activity (represented by rSUV; p=0.039) and **(B)** right insula [^{18}F]FEPPA activity (p=0.037) significantly predicted HAM-D scores in major depressive disorder patients (N=12).

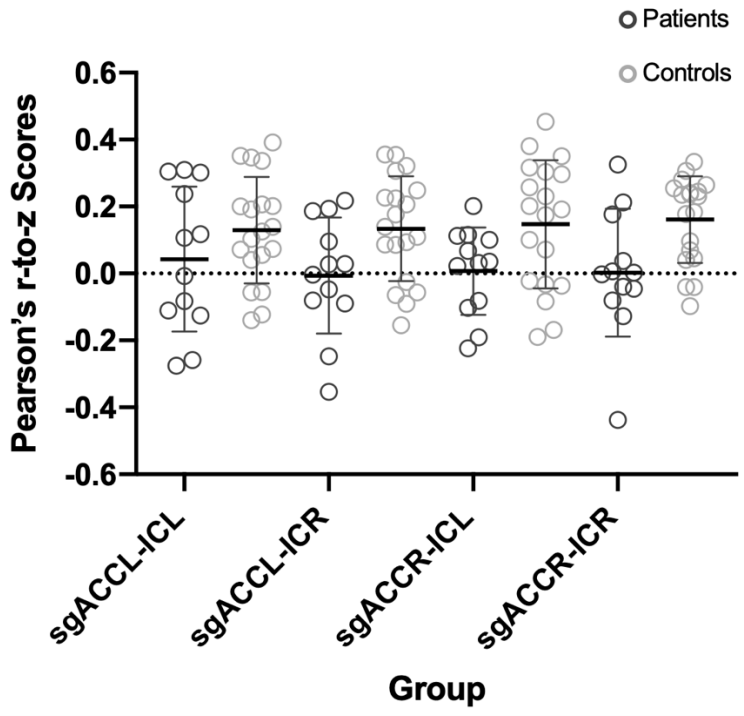

Figure 2.3. Differences in subgenual anterior cingulate cortex-insula functional connectivity. A linear regression including age and sex as covariates found that functional connectivity (represented by Pearson's r-to-z scores) did not significantly differ between patients (N=12) and controls (N=19) but was borderline significant in the connection between the right subgenual anterior cingulate cortex (sgACC) and the right insula (p=0.053) and between the left sgACC and the right insula (p=0.059). sgACCL is left subgenual anterior cingulate cortex; ICL is insular cortex left; sgACCR is right subgenual anterior cingulate cortex; ICR is insular cortex right.

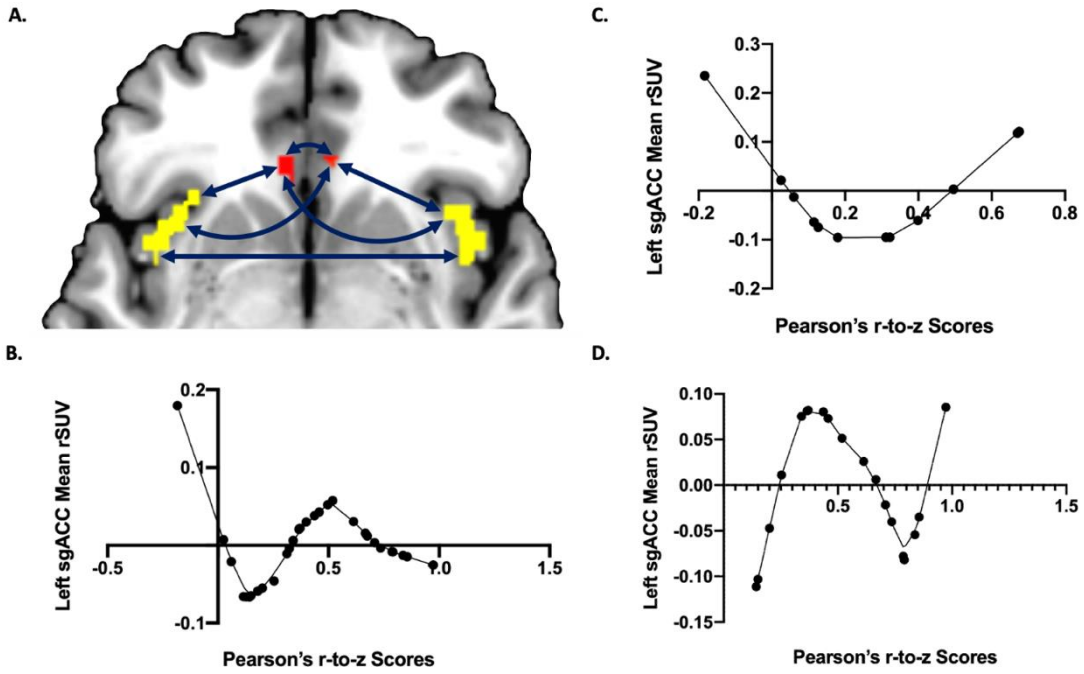

Figure 2.4. The relationship between mean relative standard uptake values (rSUV) and functional connectivity. Non-linear regressions including a PCA-derived functional connectivity factor and age as non-linear factors (LOESS), and linear covariates of no interest were run in order to assess the impact of functional connectivity (represented by Pearson's r-to-z scores) between the subgenual anterior cingulate cortex (sgACC) and insula on [^{18}F]FEPPA activity (represented by rSUV). **(A)** All six pairwise connectivity variables between bilateral sgACC (red) and insula (yellow) were used to create a single

principal factor. **(B)** When using the whole sample (N=31), increasing functional connectivity was associated with a sinusoidal-like change in [^{18}F]FEPPA activity (p=0.044). **(C)** In patients (N=12), increasing functional connectivity was associated with first a decrease, then an increase in [^{18}F]FEPPA activity (p=0.015) while **(D)** in controls (N=19) there was again a sinusoidal relationship between connectivity and activity (p=0.035).

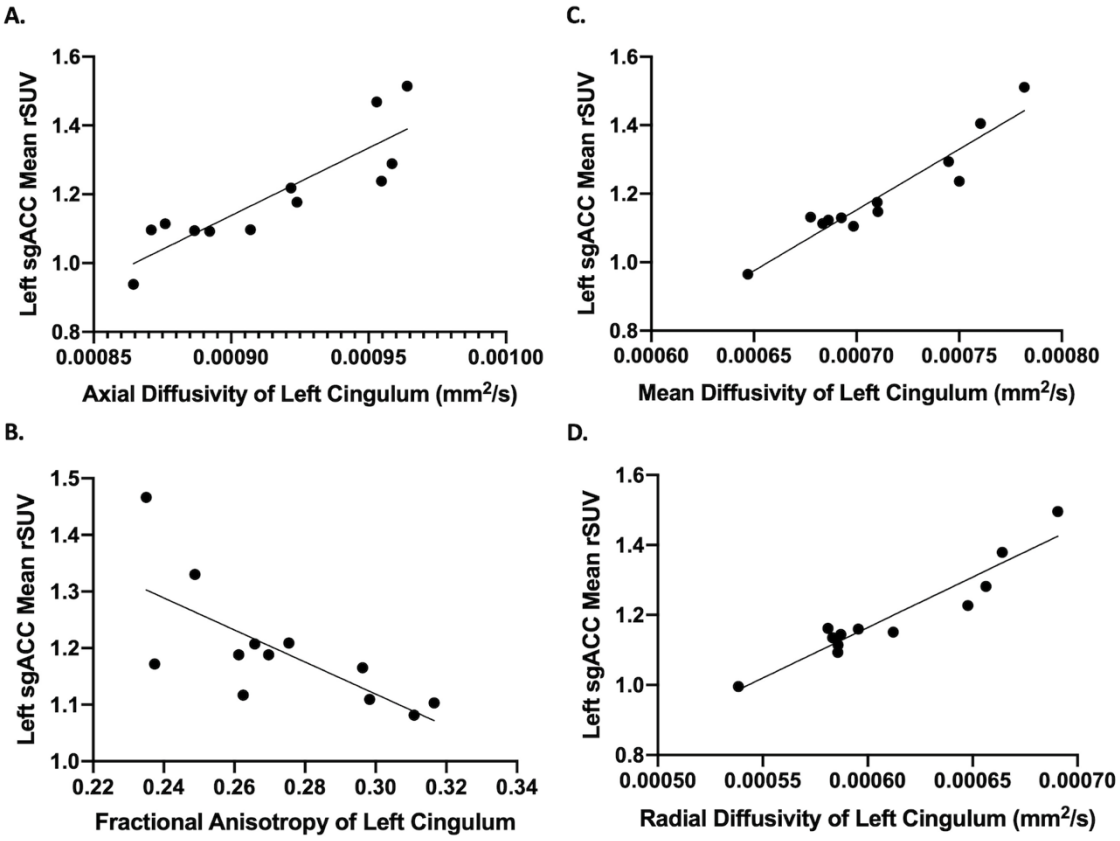

Figure 2.5. The relationship between microglial activity and structural connectivity. Linear regressions including age as a non-linear covariate, and sex, TSPO status, and diffusion tensor imaging metrics of white matter integrity as linear predictors were run in order to assess the impact of white matter integrity of the cingulum bundle on microglial activity in MDD patients (N=12). **(A)** Increasing axial diffusivity was associated with an increase in microglial activity (p=0.023) **(B)** Increasing fractional anisotropy was not significantly associated with microglial activity (p=0.843). **(C)** Increasing mean diffusivity was not significantly associated with microglial activity (p=0.072). **(D)**

Increasing radial diffusivity was not significantly associated with microglial activity (p=0.173).

We then tested if functional connectivity between the sgACC and the insula (derived from a single principal factor explaining most of the variance of six pairwise connectivity variables between bilateral sgACC and insula = $\binom{4}{2} = 6$) is a significant factor explaining [^{18}F]FEPPA uptake in the left sgACC, the region with higher uptake in MDD. This was first done across the whole sample, finding that functional connectivity was a significant non-linear predictor of microglial activity (p=0.044; Figure 2.4B). When investigating patients and controls separately, functional connectivity was a significant non-linear factor for both groups in explaining left sgACC [^{18}F]FEPPA uptake (patients p=0.015; controls p=0.035). Interestingly, higher [^{18}F]FEPPA uptake related to optimal (near-average) connectivity in the healthy subjects, while in MDD, both reduced and increased connectivity occurred with higher [^{18}F]FEPPA uptake (Figure 2.4B & 2.4D).

Finally, we assessed the relationship between microglial activity of the left sgACC and WM integrity of the cingulum bundle. We found that AD, a measure of unidirectional diffusion, from the left cingulum bundle significantly predicted microglial activity of the left sgACC in a linear model (p=0.023) (Figure 2.5A) but FA, MD, and RD did not significantly predict microglial activity (FA p=0.843; MD p=0.072; RD p=0.173) (Figure 2.5B,C,D).

2.4 Discussion

In the current study, we demonstrated 3 key findings relevant to the neuroinflammation hypothesis of MDD: (1) a significantly increased microglial activity in the left sgACC occurs in patients being treated for MDD; (2) the higher the microglial activity in the insula, the less severe the HAMD scores; (3) left sgACC microglial activity influenced the functional connectivity between the sgACC and the insula and the axonal integrity of the cingulum bundle in MDD.

Setiawan and colleagues (2015) previously found increases in microglial density in the prefrontal cortex, the insula, and the ACC using [^{18}F]FEPPA in MDD, and other

groups have replicated these results using analogous [^{11}C]-PBR28 (16,18). Here, we localized these previous findings to the sgACC as a specific site of increased [^{18}F]FEPPA uptake. The findings of Su and colleagues (2016) using a voxel-wise search in LLD (19) corroborate our localisation to sgACC.

We found that microglial activity from the left and right insula predict HAM-D scores, but this relationship is negative. Prior studies have reported an increase in severity of depression with increasing microglial activity (16), but individuals with increased neuroinflammation showed better treatment outcomes as well (65). Our sample consisted of patients receiving long-term treatment, wherein depression severity was low among those with higher microglial activity. It is possible that the inverse relationship we report is more reflective of the post-treatment improvement in outcome in a subset of patients inclined to have higher neuroinflammation, as reported by (65), or of neuroprotective microglial activity (66). The relationship between symptom severity and microglial activity may differ according to stage of treatment, phase of illness, or treatment-resistant profile. It is also possible that different measures of microglia (rSUV vs. V_T) between studies may have affected results, although the extent of this effect is unclear.

Levels of sgACC-insula functional connectivity were likewise dependent on diagnostic status, which has previously been elucidated (67). As hypothesized, we then found that functional connectivity between the sgACC and the insula is significantly associated with microglial activity from the left sgACC in both patients and controls. To our best knowledge this is the first study to use PET/MR to investigate the functional consequences on [^{18}F]FEPPA uptake in MDD. A study investigating the network pathophysiology as it relates to neuroinflammation (also using PET/MR) in Alzheimer's dementia found that those with increased radioligand binding have abnormal functional connectivity (68). Although this study was done in Alzheimer's patients, later-life MDD and cognitive impairment are highly correlated (with depression being an early risk factor of dementia), and the current results highlight a possible neuroinflammatory basis for both conditions.

We note that patients with MDD have a V-shaped relationship between microglial activity and functional connectivity that is distinct from healthy controls. The healthy

individuals showed an initial increase in microglial activity with increasing functional connectivity, reaching a peak, with a subsequent decrease. In patients, both an increase and decrease in functional connectivity was seen in conjunction with higher levels of microglial activity. On a speculative note, these observations may reflect 2 different states of the microglia – neurotoxic state, seen in patients, where higher levels relate to extremes of functional connectivity, and a neuroprotective or surveillance state, seen in healthy controls, where optimal functional connectivity requires sufficiently high microglial activity, maintaining neuronal communication (69). A dynamic pattern of microglial activation, particularly the early increase and later switch to decreased activity, have been identified in a previous study chronicling the transition from cognitively normal to mild cognitive impairment to Alzheimer's disease (69). The speculation presented here needs further confirmation in a longitudinal hybrid PET/MR study using a larger cohort of MDD with age-matched and medication-naïve healthy adults.

When relating WM macrostructural connectivity to microglial activity, we found that AD (diffusion in a direction parallel to the WM tract) of the cingulum bundle was significantly associated with microglial activity in the sgACC. Currently, there is no consensus on the findings of specific DTI metrics in MDD, and meta-analyses have posited that characteristic patterns may be restricted to subsets of the disease (30,36). An increase in AD with increasing microglial activity may denote alterations in WM microstructure (i.e., axonal integrity), and since AD is associated with neuroinflammation (59) and microglia in particular (60,61), it is then, perhaps, that neuroinflammation may negatively impact WM integrity of the cingulum bundle. This relationship may signal increased axonal injury, prompting microglia to clear up axonal debris, or chronic activation of microglia which may facilitate axonal degeneration. Furthermore, although FA, MD, and RD were not significantly associated with microglial activity, their general trends are reflective of age-related patterns of axonal degeneration (70,71). This may then indicate that microglia have a similar effect as age which lends added support to a shared mechanism between Alzheimer's disease and depression.

While the specifics of how microglia are able to influence brain connectivity are ambiguous at best, it is possible that microglia may alter connectivity through synapse

pruning. Remodeling of neuronal circuits is an important function of microglia along with their role in the immune response. Abnormalities in this remodeling function can have negative impacts upon synaptic plasticity as well as behaviour (20). The remodeling function is mediated by microglia-neuron crosstalk, a reciprocal communication which when dysregulated may be involved in depression (20). Manipulation of the C-X3-C Motif Chemokine Receptor 1 (CX3CR1) pathway, wherein microglia-neuron communication is prominent, can affect and induce depressive-like behaviour in mice (72–74). Reduced CX3CR1 signaling, and therefore reduced transmission between the two cell types, can affect the efficiency of synapses to transmit signals and may result in modified connectivity, although there are many microglia-neuron signalling pathways through which this can occur; this includes microglial decline and senescence (75,76). While microglia have numerous states of activation, activation specific to inflammation has been linked with altered synaptic current in hippocampal neurons, meaning there is some precedent to these findings (77,78).

Our study has a number of strengths including the use of hybrid PET/MR that mitigates the interval-related confounds that can affect multimodal acquisitions (improved spatial and temporal co-registration), correcting for genetic variations in TSPO status, as well as the use of a second-generation TSPO ligand. But we were limited by the sample size (increasing risk for type II error for exploratory analyses), a lack of task-encoded fMRI data to further validate the relevance of the observed functional dysconnectivity, and use of a MDD cohort which was not medication-naïve. Medications could have anti-inflammatory properties which could alter intrinsic brain activity and influence functional states. Nevertheless, we were sufficiently powered to detect the group differences as reported. Using previously reported effect sizes for the association between microglial activity and depression severity (r=+0.63) (16) and assuming a power of 80%, with a type I error rate of 5% and a type II error rate of 20%, we estimated that a minimum of 12 patients would be sufficient to detect regional-level differences. Secondly, the use of SUV and reference regions as compared to other methods such as kinetic modelling and supervised cluster analysis further limits the strength of our findings and employing these methods in the future will be necessary for confirmation of our results (79).

TSPO imaging is a promising non-invasive tool for research of *in vivo* responses to inflammation; nevertheless, there are inherent limitations to its use. Microglial activation is described as pro-inflammatory or anti-inflammatory, but this idea is shifting due to emerging research on the ever-changing inflammatory state/morphology of glial cells in response to the environment. An increase in TSPO expression has been associated with both pro-inflammatory and anti-inflammatory states, and TSPO-specific radioligands are unable to distinguish the different states (80,81). This could be due to the states existing in a continuum rather than a binary. It may also be that these TSPO-radioligands measure microglial density rather than microglial activation, and that they represent a combination of microglial as well as astrocytic signalling (82,83). This lack of specificity in inflammatory states may also be a challenge for other radioligands designed to image neuroinflammation by targeting other inflammatory processes besides TSPO, including emerging radioligands that specifically bind to microglia and not astrocytes (84). In addition, TSPO imaging is constrained by a rs6971 gene polymorphism, which we have adjusted statistically in the current work.

2.5 Conclusions

In general, our study first highlights the central role that activated microglia may play in key brain circuits to influence the mechanistic processes (axonal integrity, functional connectivity) and behavioural phenotypes (symptom severity) relevant to depression. Secondly, our work reinforces the need for hybrid PET/MR as a translational neuroimaging tool. Linking mechanistic hypothesis at a receptor/cellular level observed using PET, to network-level dysfunctions observed using DTI/fMRI is a crucial step to generate a more systemic understanding of the pathophysiology of psychiatric disorders.

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Chapter 3

3 Conclusions

3.1 Summary

Throughout the current thesis we have aimed to understand the relationship between FEPPA-measured microglial activity and both functional and white matter connectivity in MDD as this has not been previously elucidated. We have concluded that (1) microglial activity from the left sgACC is significantly increased in MDD, (2) insular microglial activity is negatively associated with depression severity, (3) functional connectivity between the sgACC and insula is decreased in MDD, (4) microglial activity from the left sgACC is non-linearly related to functional connectivity and (5) white matter connectivity is linearly associated with microglial activity.

The diagnostic status-dependent differences in microglial activity and functional connectivity have been well-established in previous literature. The disparity in microglial activity between patients and controls reflects our current understanding of microglia as having an important role in depression symptomatology. The negative association between microglial activity and depression severity may be a post-treatment improvement in outcomes in those with higher neuroinflammation as our sample consisted of individuals receiving long-term treatment (1).

The double peaks in microglial activity in relation to functional connectivity may be the result of two microglial phenotypes of activation (2): a neurotoxic state which occurs in patients at the lowest extreme of functional connectivity, and a neuroprotective state in healthy controls which requires high microglial activity at optimal functional connectivity to maintain microglia-to-neuron crosstalk.

Increased AD of the cingulum bundle is associated with decreased microglial activity. When considering all DTI metrics (decreased FA and increased MD, AD, and RD with increasing microglial activity) together, it becomes apparent that the effect of microglial activity on white matter (or vice versa) is similar to age-related effects, possibly signalling white matter axonal degeneration (3,4).

3.2 Significance

MDD is a heterogeneous condition which cannot be fully explained at the time of this thesis. This makes finding the most effective treatment difficult, especially in those with TRD; in some cases, the most effective treatment has yet to be recognized as a viable option. MDD diagnosis is further complicated by age, through which we see distinct differences in those with MDD and those with LLD. These distinct differences have not been widely studied to date, contributing toward a large gap in knowledge. It becomes imperative that a better-cultivated understanding of MDD is acquired for development of novel treatments which are suited for specific subsets of depression. Considering this, the results of the current study are significant. We have further studied the specifics of microglial activity in depression and related this with functional and structural connectivity. This is completely novel as no other groups, to the best of our knowledge, have related microglial activity with both functional and structural outcomes in MDD. What is especially important about this development is that we are able to better understand the interplay between different brain systems, something which has not been done extensively in depression. These separate systems function in tandem to produce what we know as clinical depression, and while we know a great deal about each one, with limitations in current research methods, the ways in which they work together are relatively unknown.

Building on this further, current research has singled in on the ability of anti-inflammatory agents to reduce severity of depression symptomatology. While at this moment anti-inflammatory agents and the phenomenon of neuroinflammation itself are nowhere near assiduously studied, their recent rise in research fascination has made our study apt for filling this gap in knowledge. It therefore becomes possible that anti-inflammatory agents can be recognized as viable MDD adjuvant treatment options in the future. Understanding this relationship may lead to a breakthrough in the future of depression treatment – something which is sorely needed considering the current lack of novel options.

Our results are reflective of previous studies of Alzheimer's disease, further establishing a link between the two conditions. It has been previously found that those

with late-life depression are at risk for dementia; since the microglial activity in our population reflects that of the decline into Alzheimer's, it may be that microglia play a pivotal role in disease progression, and likewise in the concurrent development of Alzheimer's. At this point this is speculation, but it illuminates a potential avenue for research into dementia prevention and treatment.

MDD is the leading cause of disability today. It affects one's productivity, one's mood, and can lead one to taking their own life. It is not limited in who it affects, being the second leading cause of death among young Canadians (5). Such an illness cannot be taken lightly and any advancements in our understanding of it can aid in improving and saving lives. The importance of this cannot be understated; depression is isolating and deadly and with additional research we get closer to exceeding the current limitations of treatment.

3.3 Limitations

While the results shown are promising, as is the case with most studies they do not come without certain considerations. First and most glaringly, the sample size used in the current study is low. While our calculations of power do suggest that this sample size is enough to see significant differences, this is the absolute minimum. The choice in individuals within our sample is flawed as well. Those in the depressed cohort were not restricted based on their medications, and we cannot be sure what anti- or pro-inflammatory effects they may have. While the controls were age-matched (+/- 5 years), in later life such an age disparity can have a significant impact. As previously mentioned in Chapter 1, there are physical and symptomological differences between MDD and LLD. This can completely change how the illness presents itself and how it arises.

Secondly, the detection of microglial activity from TSPO in itself is challenging. TSPO expression is not limited to microglia, as it can be overexpressed in reactive astrocytes (6), withal in peripheral macrophages which may be able to cross the BBB to reach the brain (Dupont A.C. et al., 2017). It should be noted that levels of TSPO in astrocytes can become overexpressed during glutamate excitotoxicity, which

may pose problems as glutamate excitotoxicity is posited to be a significant factor in depression outcome (7). But this worry seems to not be of great consequence, as the contribution of TSPO from microglia may be about 7x greater than that of astrocytes, and the contribution of TSPO from these astrocytes has not yet been found to make a significant contribution toward the PET image (8). Nonetheless, the specificity of TSPO PET tracers in microglial binding should be considered in the future.

Specificity of TSPO tracers to the type of microglial activation is another confounding factor. Microglia, previous to activation, are highly susceptible to environmental signals. Depending on the environment, when activated they can be described as either pro-inflammatory (M1) or anti-inflammatory (M2); the M2 state is thought to be less prevalent and can be activated by anti-inflammatory cytokines in addition to apoptotic cells and myelin debris, leading to tissue repair. The fact that it is possible for both of these responses to be active and that TSPO tracers seemingly do not distinguish between the two confounds interpretations of microglial activation. Moreover, the idea of concrete categories of microglial activity is shifting as it is theorized that the states exist in a continuum rather than a binary. It may also be that these TSPO-radioligands measure microglial density rather than microglial activation (9).

Thirdly, the choice in radiotracers and study design seems especially important, as there is no single convincing answer to the direction of microglial activity in MDD. Each study uses a different TSPO tracer, and studies are able to both replicate and completely deny previous findings depending on the tracer used. Considering that FEPPA has a greater specificity to TSPO than many other radioligands, it can be assumed that studies using FEPPA are more reliable and accurate. The effectiveness of TSPO tracers is further questioned when considering which ones may be impeded by MDD pathophysiology. A recent study by Turkheimer et al. found that in states of low-grade inflammation (in MDD and during inflammatory inducer injection in healthy individuals) uptake of TSPO tracers into CSF and brain parenchyma is decreased as compared to healthy controls due to a reduction in BBB permeability (10). It should be noted that the tracers used in this study were [^{11}C]PK11195 and [^{11}C]PRB28, not FEPPA. [^{11}C]PK11195, while the first and most widely used TSPO radiotracer, has a short half-life and low signal to noise ratio due to high nonspecific binding and

lipophilicity, and may not reflect FEPPA activity. [^{11}C]PRB28 presents much of the same issues as [^{11}C]PK11195 despite being an analogue to FEPPA.

In conjunction with the issues related to choice of TSPO tracer, another consideration in interpretation of these results is that TSPO tracer uptake increases with age. A study done by Kumar and colleagues in healthy children and adults (age range of adults 20-49) found that adults overall have increased uptake of [^{11}C]PK11195 while the distribution of TSPO tracer is not altered between groups (11). Given the difference in age between our control group and our patient group (with controls being about 5 years older and more definitively in a LLD categorization), it may be that the difference we see in microglial activity between patients and controls is diminished by the disparity in age. It is known that increased inflammation is associated with older age, but the exact origin of such inflammation is not fully understood.

Finally, the choice of the reference region method of data normalization for the PET data is controversial. Reference regions must be picked carefully; they must have no significant differences in tracer uptake between patients and controls, and they must have relatively little TSPO tracer uptake. There exists no area of the brain with no tracer uptake, and as such there is no perfect reference region. A few reference regions have been validated in depression: the cerebellum is a common choice due to its large size, lending itself to greater stability. Reference regions are typically used as a simple alternative in the absence of arterial blood data. In the current study this data was not available, and we were unable to accurately model the tracer kinetics. Supervised cluster analysis methods are available to create supervised reference regions, but their development is time consuming.

3.4 Future Directions

While we have found a connection between functional connectivity and neuroinflammation-related molecular activity in depression, this is only the beginning of fully understanding this relationship. Due to the limitations above, the results must be supported by future work and replicated by other groups in order to confirm their authenticity.

In the future, it would be prudent to explore methods for absolute quantification of microglia that do not rely on reference region normalization. Absolute quantification of PET requires measurement of the input function – the time-dependent concentration of the PET tracer in blood plasma – to quantify the PET tracer concentration in tissue using tracer kinetic models. Although PET tracer kinetic modelling would allow for increased accuracy of PET measurements with fewer confounders, the need for serial arterial blood sampling makes this approach relatively invasive. Less invasive absolute quantification alternatives such as supervised cluster analysis would eliminate some of the issues associated with reference region-based normalization, as it uses a data-driven approach to identify the most suitable reference region.

As well, it would enrich the current findings if serum inflammatory biomarkers were measured as to indicate levels of whole-body inflammation. Peripheral inflammatory biomarkers have been linked with dysfunction in connectivity in MDD (12), so bringing all of these factors together would solidify our conclusions.
An interesting finding was the relationship between microglial activity and functional connectivity and how this reflected previous results found in Alzheimer's disease.

Although our subjects were older, they were not fully at risk for Alzheimer's or other forms of dementia/cognitive impairment. This means it is possible that we are seeing a trend in microglial activity associated with age which may predict Alzheimer's prognosis. In the future it would be intriguing to explore whether there are subtypes of microglial activation in the years leading up to being at risk for dementia, and if this is related to factors such as stress exposure. In order to do so, a larger sample size would be needed, as well as longitudinal data.

Finally, we would be interested in relating the current findings to alternative imaging parameters, such as glutamate and glutathione, and in other brain regions, such as the striatum, in order to build a more cohesive story about depression as a whole.

3.5 Concluding Remarks

The results of this thesis suggest that inflammation is critical in depression and its manifestation. It moderates the severity of depressed mood and can affect both functional

and structural connectivity; thus, evanescent inflammation may have a longstanding impact of mood changes, as seen in post-viral depressive episodes. These findings are reminiscent of previous dementia and aging studies, giving insight into the interplay between separate systems and how their interactions contribute to clinical diagnostic status at large. Our work opens the possibility of using less expensive, more accessible, and less invasive DTI/fMRI metrics as a measure of the effect of reducing inflammation to treat depression.

3.6 References

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Appendix A Ethics Approval

Western Research

Date: 22 May 2019

To: Dr. Lena Palaniyappan

Project ID: 108342

Study Title: Molecular Imaging of Brain Inflammation in Depressive Disorders

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 04/Jun/2019

Date Approval Issued: 22/May/2019

REB Approval Expiry Date: 12/Jun/2020

Dear Dr. Lena Palaniyappan,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix B Clinical Evaluation

REB 10012862 APPENDIX D

Comprehensive Clinical Assessment:

Date of initial assessment:

Patient Number (unique study ID, not hospital number):

Assessor(s):
1.
2.

Section 1. Current Medications:

Allergies:

Psychotropic Medications

Medication	Dose	Duration-months

Physical Health Medications

Medication	Dose	Duration-months

MEDICAL HISTORY: Known Medical Conditions:

Section 2. PSYCHIATRIC HISTORY

First onset of depression: Month ---- (if available)/ Year -----
Course of depression

- Severe with suicidality (Yes/No); Psychosis (Yes/No);
- Requiring hospitalization (Yes/No)
- Full interepisode recovery (Yes/No)

Complicated by

- Substance use (Y/N) Name of the street drug: _____
- Alcohol (Y/N)
- Other psychiatric diagnoses: _____

PRE MORBID PERSONALITY

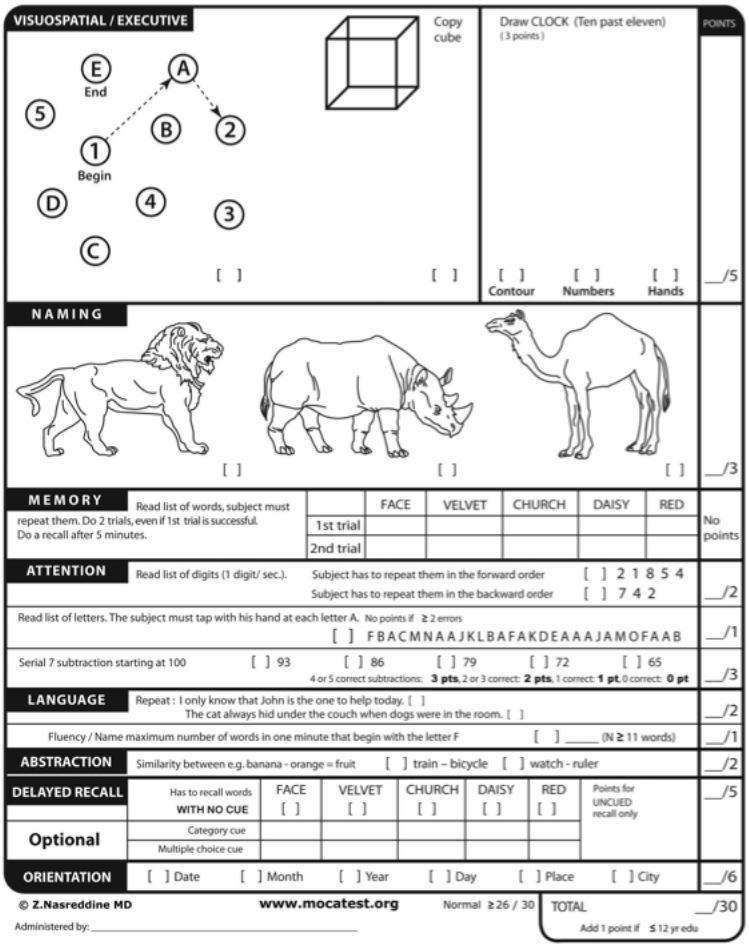
SUBSTANCE USE:

Description	**Smoking**	**Alcohol**	**Cannabis**	**Others**
How long				
Total amount per day				
Past Withdrawal symptoms				
Ever attempted to stop				
Ever had treatment				
Current motivation:				
Is this habit problematic				

Section 3. General Physical Examination:

Height		Weight	BMI	
Pulse:	BP:	Handedness:	RR:	Temp:

Section 4. MONTREAL COGNITIVE ASSESSMENT (MOCA)



Section 5.: Clinical Global Impression (CGI)

1. Severity of illness

Considering your total clinical experience with this particular population, how mentally ill is the patient at this time?

0 = Not assessed
1 = Normal, not at all ill
2 = Borderline mentally ill
3 = Mildly ill
4 = Moderately ill
5 = Markedly ill
6 = Severely ill
7 = Among the most extremely ill patients

Section 6. Beck Depression Inventory

A (Mood) 0 I do not feel sad 1 I feel sad 2a. I am blue or sad all the time and I can't snap out of it 2b. I am so sad or unhappy that it is very painful 3 I am so sad / unhappy that I can't stand it	**B (Pessimism)** 0 I am not particularly discouraged about the future 1 a. I feel discouraged about the future 2 a. I feel I have nothing to look forward to b. I feel that I wont ever get over my troubles 3 I feel the future is hopeless and that things cannot improve
C (Sense of failure) 0 I don't feel like a failure 1 I feel I have failed more than average person 2 a. I feel I have accomplished very little that is worthwhile or that means anything b. As I look back on my life all I can see is a lot of failure 3 I feel I am a complete failure as a person (parent, husband, wife)	**D (Lack of satisfaction)** 0 I am not particularly dissatisfied 1 a. I feel bored most of the time b. I don't enjoy things the way I used to 2 I don't get real satisfaction out of anything any more 3 I am dissatisfied or bored with everything
E (Guilty feeling) 0 I don't feel particularly guilty 1 I feel bad or unworthy a good part of the time 2 a. I feel quite guilty b. I feel bad or unworthy practically all the time now 3 I feel as through I am very bad or worthless	**F (Sense of punishment)** 0 I don't feel I am being punished 1 I have a feeling that something bad may happen to me 2 I feel I am being punished or will be punished 3 a. I feel I deserved to be punished b. I want to be punished
G (Self hate) 0 I don't feel disappointed in myself 1 a. I am disappointed in myself b. I don't like myself 2 I am disgusted with myself 3 I hate myself	**H (Self accusations)** 0 I don't feel I am any worse than anybody else 1 I am very critical of myself for my weaknesses or mistakes 2 a. I blame myself for everything that goes wrong b. I feel I have many bad faults

I (Self-punitive wishes) 0 I don't have nay thoughts of harming myself 1 I have thoughts of harming myself but I would not carry them out 2 a. I feel I would be better off dead b. I have definite plans about committing suicide c. I feel my family would be better off if I were dead 3 I would kill myself if I could	**J (Crying spells)** 0 I don't cry anymore than usual 1 I cry more than I used to 2 I cry all the time now I cant stop it 3 I used to be able to cry but now I can't cry at all even though I want to
K (Irritability) 0 I am no more irritated now than I ever am 1 I get annoyed or irritated more easily than I used to 2 I feel irritated all the time 3 I don't get irritated at all at the tings that used to irritate me	**L (Social withdrawal)** 0 I have not lost interest in other people 1 I am less interested in other people now than I used to be 2 I have lost most of my interest in other people and have little feeling for them. 3 I have lost all my interest in other people and don't care about them at all
M (Indecisiveness) 0 I make decisions as well as ever 1 I am less sure of myself now and try to put of making decisions 2 I can't make decisions any more without help 3 I can't make any decisions at all any more	**N (Body Image)** 0 I don't feel that I look any worse than I used to 1 I am worried that I am looking old or unattractive 2 I feel there are permanent changes in my appearance that make me look unattractive 3 I feel that I am ugly or repulsive looking
O (Work inhibition) 0 I can work about as well as before 1 a. It takes an extra effort to get started at doing something b. I don't work as well as I used to 2 I have to push myself very hard to do anything 3 I can't do any work at all	**P (Sleep disturbance)** 0 I can sleep as well as usual 1 I wake up more tired in the morning than I used to 2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep 3 I wake up early every day and cant get more than 5 hours sleep
Q (Fatigability) 0 I don't get any more tired than usual 1 I get tired more easily than I used to 2 I get tired from doing almost anything 3 I get too tired to do anything	**R (Loss of appetite)** 0 My appetite is no worse than usual 1 My appetite is not as good as it used to be 2 My appetite is much worse now 3 I have no appetite at all anymore
S (Weight loss) 0 I haven't lost much weight, if any, lately 1 I have lost more than five pounds 2 I have lost more than ten pounds 3 I have lost more than fifteen pounds	**T (Somatic preoccupation)** 0 I am no more worried about my health than usual 1 I am concerned about aches and pains or upset stomach or constipation or other unpleasant feelings in my body 2 I am so concerned with how I feel or what I feel that it's hard to think of much else 3 I am completely absorbed in what I feel

U (Loss of libido) 0 I have not noticed any recent change in my interest in sex 1 I am less interested in sex than I used to be 2 I am much less interested in sex now 3 I have lost interest in sex completely	

Section 7. Hamilton Depression Rating Scale (HDRS)

1 DEPRESSED MOOD*(sadness, hopeless, helpless, worthless)* 0 |__| Absent. 1 |__| These feeling states indicated only on questioning. 2 |__| These feeling states spontaneously reported verbally. 3 |__| Communicates feeling states non-verbally, i.e. through facial expression, posture, voice and tendency to weep. 4 |__| Patient reports virtually only these feeling states in his/her spontaneous verbal and non-verbal communication.	**10 ANXIETY PSYCHIC** 0 |__| No difficulty. 1 |__| Subjective tension and irritability. 2 |__| Worrying about minor matters. 3 |__| Apprehensive attitude apparent in face or speech. 4 |__| Fears expressed without questioning.
2 FEELINGS OF GUILT 0 |__| Absent. 1 |__| Self reproach, feels he/she has let people down. 2 |__| Ideas of guilt or rumination over past errors or sinful deeds. 3 |__| Present illness is a punishment. Delusions of guilt. 4 |__| Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations	**11 ANXIETY SOMATIC (physiological concomitants of anxiety) such as:** gastro-intestinal – dry mouth, wind, indigestion, diarrhea, cramps, belching cardio-vascular – palpitations, headaches respiratory – hyperventilation, sighing urinary frequency sweating 0 |__| Absent. 1 |__| Mild. 2 |__| Moderate. 3 |__| Severe. 4 |__| Incapacitating
3 SUICIDE 0 |__| Absent. 1 |__| Feels life is not worth living. 2 |__| Wishes he/she were dead or any thoughts of possible death to self. 3 |__| Ideas or gestures of suicide. 4 |__| Attempts at suicide (any serious attempt rate 4).	**12 SOMATIC SYMPTOMS GASTRO-INTESTINAL** 0 |__| None. 1 |__| Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen. 2 |__| Difficulty eating without staff urging. Requests or requires laxatives or medication for bowels or medication for gastro-intestinal symptoms.
4 INSOMNIA: EARLY IN THE NIGHT 0 |__| No difficulty falling asleep. 1 |__| Complains of occasional difficulty falling asleep, i.e.more than 1/2 hour. 2 |__| Complains of nightly difficulty falling asleep.	**13 GENERAL SOMATIC SYMPTOMS** 0 |__| None. 1 |__| Heaviness in limbs, back or head. Backaches, headaches, muscle aches, loss of energy and fatigability. 2 |__| Any clear-cut symptom rates 2.

5 INSOMNIA: MIDDLE OF THE NIGHT 0 |__| No difficulty. 1 |__| Patient complains of being restless and disturbed during the night. 2 |__| Waking during the night – any getting out of bed rates. 2 (except for purposes of voiding).	**14 GENITAL SYMPTOMS (symptoms such as loss of libido, menstrual disturbances)** 0 |__| Absent. 1 |__| Mild. 2 |__| Severe.
6 INSOMNIA: EARLY HOURS OF THE MORNING 0 |__| No difficulty. 1 |__| Waking in early hours of the morning but goes back to sleep. 2 |__| Unable to fall asleep again if he/she gets out of bed.	**15 HYPOCHONDRIASIS** 0 |__| Not present. 1 |__| Self-absorption (bodily). 2 |__| Preoccupation with health. 3 |__| Frequent complaints, requests for help 4 |__| Hypochondriacal delusions.
7 WORK AND ACTIVITIES 0 |__| No difficulty. 1 |__| Thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies. 2 |__| Loss of interest in activity, hobbies or work – either directly reported by the patient or indirect in listlessness, indecision and vacillation (feels he/she has to push self to work or activities). 3 |__| Decrease in actual time spent in activities or decrease in productivity. Rate 3 if the patient does not spend at least three hours a day in activities (job or hobbies excluding routine chores. 4 |__| Stopped working because of present illness. Rate 4 if patient engages in no activities except routine chores, or if patient fails to perform routine chores unassisted	**16 LOSS OF WEIGHT *(RATE EITHER a OR b)*** a. When Rating by History: 0 |__|No weight loss 1 |__|Probable weight loss associated with present illness 2|__|Definite (according to patient) weight loss b. On weekly ratings by ward psychiatrist, when actual changes are measured: 0 |__|Less than 1 lb. weight loss in week 1 |__|Greater than 1 lb. weight loss in week 2 |__|Greater than 2 lb. weight loss in week
8 RETARDATION(slowness of thought and speech, impaired ability to concentrate, decreased motor activity) 0 |__| Normal speech and thought. 1 |__| Slight retardation during the interview. 2 |__| Obvious retardation during the interview. 3 |__| Interview difficult. 4 |__| Complete stupor.	**17 INSIGHT** 0 |__| Acknowledges being depressed and ill. 1 |__| Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc. 2 |__| Denies being ill at all.
9 AGITATION 0 |__| None. 1 |__| Fidgetiness. 2 |__| Playing with hands, hair, etc. 3 |__| Moving about, can't sit still. 4 |__| Hand wringing, nail biting, hair-pulling, biting of lips.	Total score: |__|__|

Curriculum Vitae

Name	Jasmine Deniz Cakmak
Post-secondary Education and Degrees	Honours BSc in Biomedical Science University of Ottawa Ottawa, Ontario, Canada 2015-2019 MSc Candidate in Neuroscience The University of Western Ontario London, Ontario, Canada 2019-present
Honours and Awards	Western Graduate Research Scholarship 2019-2021 Dean's Honour Roll 2018-2019 University of Ottawa Entrance Scholarship 2015
Related Work Experience	Teaching Assistant The University of Western Ontario 2019-2021
Publications	**Cakmak, Jasmine D.** et al. 2021. The functional and structural consequences of aberrant microglial activity in major depressive disorder. *J Psychiatry Neurosci. Submitted.* Cakmak, Sabit; Hebbern, Chris; **Cakmak, Jasmine D;** Dales, Robert E. 2017. The influence of polycyclic aromatic hydrocarbons on lung function in a representative sample of the Canadian population. *Environmental Pollution 228:1-7 DOI. 10.1016/j.envpol.2017.05.013* Cakmak, Sabit; Hebbern, Christopher; **Cakmak, Jasmine D;** Vanos, Jennifer. 2016. The modifying effect of socioeconomic status on the relationship between traffic, air pollution and respiratory health in elementary schoolchildren *Journal of environmental management 177:1-8*