Novel approaches to the pathophysiology of late-life depression

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Abstract

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The growing impact of under-recognised and under-treated late-life depression (LLD) stands to negatively affect our societies within the context of an ageing world. LLD is a complex disorder where past studies have explored a narrow set of characteristics in isolation (e.g. clinical, neuropsychological, brain imaging, genomics and proteomics). These isolated analyses have yielded useful findings, and continue to do so, however they are limited given the neurobiological mechanisms of LLD are complex and involve interplay between many brain systems, and can manifest in various investigative modalities. Fortunately, there are novel methods for advancing mental health research. In this dissertation, a variety of novel approaches are used to develop a more comprehensive understanding of the pathophysiology of LLD. This is achieved by exploring discreet studies of peripheral biomarkers (i.e. immunology and genomics), as well as neuroimaging biomarkers (i.e. functional and molecular imaging), and contextualising them against each other. Novel applications of these principles and research tools including machine learning may yield more effective diagnostic, treatment and preventive options for LLD.

Thesis declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and, where applicable, any partner institution responsible for the joint award of this degree.

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Table of Contents

List of tables

List of figures

List of Abbreviations

2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)-amino]-2-naphthyl}ethylidene) malononitrile ([^{18}F]FDDNP)

Alzheimer's disease (AD)

Amyloid β (Aβ)

Anterior cingulate cortex (ACC)

Apathy Evaluation Scale (AES)

Beck Depression Inventory (BDI)

Blood brain barrier (BBB)

Body mass index (BMI)

cAMP responsive element binding protein (CREB)

Central nervous system (CNS)

Cerebrospinal fluid (CSF)

Chronic traumatic encephalopathy (CTE)

Citalopram (CIT)

Clinical Global Impression (CGI)

Cognitive control network (CCN)

Connor-Davidson Resilience Scale (CD-RISC)

C-reactive protein (CRP)

Cumulative Illness Rating Scale-Geriatric (CIRS-G)

Cytometric bead array (CBA)

Database for Annotation, Visualization and Integrated Discovery (DAVID)

Default Mode Network (DMN)

Diagnostic and Statistical Manual (DSM)

Diffusion tensor imaging (DTI)

Dopamine (DA)

Dorsal anterior cingulate cortex (dACC)

Dorsolateral prefrontal cortex (DLPFC)

Echo-planar imaging (EPI)

Effect size (ES)

Electrocardiogram (ECG)

Enzyme-linked immunosorbent assay (ELISA)

FMRIB Software Library (FSL)

Fractional anisotropy (FA)

Geriatric Depression Scale (GDS)

Global burden of disease (GBD)

Glucocorticoid receptor (GR)

Hamilton Anxiety Scale (HAS or HAM-A)

Hamilton Depression Rating Scale (HDRS or HAM-D)

Hypothalamus-pituitary-adrenal (HPA)

Hypoxia-inducible factors (HIF)

Independent components analysis (ICA)

Indoleamine 2,3 dioxygenase (IDO)

Induced pluripotent stem cells (iPS)

Institutional Review Board (IRB)

Interferon (IFN)

Interferon γ-induced protein (IP)

Interleukin (IL)

International Classification of Diseases (ICD)

Janus kinase (JNK)

Late-life depression (LLD)

Macrophage inflammatory protein (MIP)

Macrophages migration inhibitory factor (MIF)

Major depressive disorder (MDD)

Major histocompatibility complex, class II, DR β 5 (HLA-DRB5)

Medial prefrontal cortex (mPFC)

Medial temporal lobe (MTL)

Medical Outcomes Study Short Form 36-Item Health Survey (SF-36)

Methylphenidate (MPH)

Mild cognitive impairment (MCI)

Mini-Mental State Examination (MMSE)

Mitogen-activated protein kinase (MAPK)

Monocyte chemotactic protein (MCP)

Montgomery-Asberg Depression Rating Scale (MADRS)

Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC)

Neural stem cells (NSCs)

Neurofibrillary tangles (NFTs)

Noradrenaline (NA)

Nuclear factor-κB (NF-κB)

Nucleus accumbens (NAcc)

Peripheral blood mononuclear cells (PBMC)

Pittsburgh Compound B (PiB)

Positron emission tomography (PET)

Posterior cingulate cortex (PCC)

Posterior superior temporal sulcus (pSTS)

Preferred reporting items for systematic reviews and meta-analyses (PRISMA)

Reactive oxygen species (ROS)

Regions of interest (ROIs)

Relative distribution volume (DVR)

Relative risk (RR)

Resting-state functional magnetic resonance imaging (rs-fMRI)

Selective serotonin reuptake inhibitors (SSRIs)

Selenium binding protein 1 (SELENBP1)

Serotonin (5-HT)

Serotonin noradrenaline reuptake inhibitors (SNRIs)

Sialic acid binding immunoglobulin-like lectin, pseudogene 3 (SIGLECP3)

Signal transducer and activator of transcription (STAT)

SMA- and MAD-related protein 7 (SMAD 7)

Standard deviation (SD)

Statistical parametric mapping (SPM)

Stress-activated protein kinase (SAPK)

TGFβ activated kinase-1 (TAK-1)

T-helper (T_h)

Tumour necrosis factor (TNF)

Tumour, node, metastasis (TNM)

Udvalg for Kliniske Undersogelser (UKU)

Uncinated fasciculus (UF)

White matter lesions or hyperintensities (WMH)

World Health Organization (WHO)

Years lived with disability (YLD)

PREFACE

There are many, many people to thank in the completion of this PhD dissertation, which has taken me from tropical Townsville, to Adelaide, Los Angeles and finally Melbourne.

Firstly, I would like to thank Professor Bernhard T Baune for his support and mentorship not only during this PhD period, but also since the initiation of my engagement in research some 7 years ago. Prof Baune has supported me tirelessly through my development as a medical student, researcher, intern and now psychiatry registrar. During this time, I have developed not only as a researcher and clinician but also on a personal level. If it was not for his ongoing support, I may not have made it this far. It is through Prof Baune's work in psychiatric neuroscience that I have found tremendous meaning – the complexities of the brain, the importance of high quality science, and the benefits of a rich convergence or transdisciplinary approach to enquiry. Prof Baune has been supportive in enabling me to pursue my interests in travelling to the United States of America on a Fulbright Scholarship, as well as my settling back in Melbourne, Australia. This kind of unwavering support is very rare as I have asked a lot through this unique research career, and I will be forever grateful.

Following, I would like to thank Prof Helen Lavretsky for her generosity in supporting my Fulbright Scholar period at the UCLA Division of Geriatric Psychiatry with her research group. My goal was to spend 12 months living the USA working with world class experts in a variety of fields. I certainly found this in spades within Prof Lavretsky's group. This period at UCLA and in California has spurred me on in my career to continue exploring novel pathways of 'adding

value' to patient care. Through Prof Lavretsky's group, I have particularly taken stock of innovations in positive psychiatry, as well as evidence generation in novel fields (e.g. integrative psychiatry). This has been fascinating and enriching to observe, and in small part, contribute to.

Also, I would like to thank A/Prof David Merrill for his support through the articulation of the convergence psychiatry concept, which has developed through much iteration. A/Prof David Merrill has been supportive in helping me understand convergence psychiatry as it applies to research, as well as clinical medicine. A/Prof Merrill and I have many years of interesting work ahead of us.

To all collaborators, I am most grateful. From collaborators within the Discipline of Psychiatry at the University of Adelaide to those from Prof Lavretsky's research group and the Brain Mapping Centre at UCLA.

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1.0 Introduction

The world's population is ageing at a rate unprecedented in human history [1], which places substantial pressure on health systems around the globe. Co-occurring with population ageing is the rise in chronic diseases (e.g. obesity, heart disease, diabetes, cancer and dementia) and common mental disorders (e.g. depression) [2]. Late-life depression (LLD) is the focus of this research, and is a major public health issue. Community-based studies find the prevalence of total depression in cohorts of older adults between 4.8 and 13.7% when using Diagnostic and Statistical Manual (DSM)-IV criteria [3]. In addition to this substantial prevalence, a recent meta-analysis of clinical trials suggests a response rate of 48% and a remission rate of 33.7% were found for antidepressant treatments in LLD, both similar to response and remission rates found in adult patients [4]. Clearly, there are major issues with the rising prevalence of LLD and modest treatment efficacy.

The current method for classifying mental disorders in psychiatry is via the use of the DSM 5th edition (DSM-5) [5]. The DSM-5, as with previous editions, uses diagnostic classification systems based on symptoms experienced by the patient and those observed by the diagnostician. The DSM-5 is criticised for this approach given concerns with reliability and validity. One method for addressing these concerns is to identify diagnostic and prognostic systems which utilise biomarkers. Such biomarkers would represent the underlying neurobiological changes occurring in the disorder, and would be targets to develop novel treatment and preventive approaches, and predict disorder development. There are a number of promising lines of enquiry for biomarkers in LLD.

Key areas of interest and advancement in biomarker development and the pathophysiology of LLD revolve around peripheral biomarkers (i.e. immunology and genomics) and neuroimaging biomarkers (i.e. functional and molecular neuroimaging). With immunology, there is an increasing recognition of the role of inflammatory cytokines in the development of depression, with negative effects on neurotransmission, neuroplasticity and neuroendocrine systems [6, 7]. There is a small, emerging body of literature of the effect of chemokines in depression pathophysiology [8], however no quantitative analyses have been conducted to date. Genetics is increasingly used to understand the pharmacogenetics of treatment resistance, with candidate genes relating to monoaminergic neurotransmission, neuroplasticity, blood brain barrier (BBB) mechanisms and immune modulation [9]. However, to date no genome-wide expression biomarker study has been conducted in an LLD sample. Functional neuroimaging is a modality which has received attention in the adult depression literature as it allows the measurement of brain activity by detecting associated changes in blood flow [10]. The literature in LLD requires further development, specifically with resting-state functional magnetic resonance imaging (rs-fMRI). Neurotoxicity associated with amyloid and tau protein aggregation may represent a pathophysiological cascade that, along with vascular compromise, may predispose individuals to LLD. This therefore represents an important area of scientific inquiry, particularly given *in vivo* amyloid and tau neuroimaging are possible using positron emission tomography (PET) methods. Finally, biomarkers for antidepressant treatment resistance will be explored to support our understanding of LLD pathophysiology.

The majority of past studies exploring the aetiology, pathophysiology and diagnosis of LLD have focused on a relatively narrow set of characteristics often

in isolation. These characteristics include clinical, socio-demographic, neuropsychological, structural brain imaging, functional brain imaging, molecular brain imaging, genomics or proteomics. This type of analysis has yielded useful findings, and continues to require further attention as there is room for development. However, this approach is limited given the neurobiological mechanisms of LLD are complex and likely to involve interplay of changes which manifest in brain structure and function, neurochemistry and neuropathology [11-14]. Some studies are using novel statistical analysis techniques, such as machine learning, to incorporate data from multiple modalities, i.e. neuropsychology, molecular imaging, structural imaging and other peripheral biomarkers [11].

1.1 Specific studies

The goal of this dissertation is to introduce and establish value for novel approaches to psychiatric research, and to specifically establish the value for the pathophysiology of LLD. We pursued this goal in the below studies with neuroimaging and peripheral biomarkers:

Peripheral biomarkers studies:

Study 1: To quantitatively meta-analyse the data on concentrations of all chemokines in patients diagnosed with major depressive disorder (MDD) versus healthy controls.

Study 2: In this pilot study, leukocyte genome-wide transcriptional alterations were examined in remitters versus non-remitters with antidepressant treatment.

- Hypothesis: Genome-wide transcriptional analyses will reveal potential predictors of remission in the dopaminergic pathways based on our pilot data.

Neuroimaging studies:

Study 3: To use a data-driven, pilot case-control study to compare rs-fMRI data between age-matched depressed and non-depressed older adults.

- Hypothesis: LLD would be associated with aberrant connectivity within the Default Mode Network (DMN); therefore, this network was targeted in our primary analysis. An exploratory analyses of LLD-related differences in functional connectivity was conducted in other resting-state networks to determine whether LLD is associated with broader dysfunction.

Study 4: In this cross-sectional pilot study, PET scans were performed after injection of 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)-amino]-2-naphthyl}ethylidene) malononitrile ([^{18}F]FDDNP), an *in vivo* amyloid and tau neuroimaging, in patients with LLD to explore neural correlates of apathy.

- Hypothesis: Greater severity of apathy will correlate with greater [^{18}F]FDDNP binding in the anterior cingulate regions based on our previous reports with other imaging modalities.

1.2 Outline of chapters

This dissertation will provide an overview of the novel approaches to the pathophysiology of LLD, via a combination of literature reviews and primary papers (all either accepted or submitted for peer-reviewed journals). In chapter 2, an overview and introduction is provided of the pathophysiology of LLD. The diagnosis and definitions are outlined, followed by epidemiological data, risk factors and the pathophysiology. Following this, chapter 3 overviews the role of peripheral biomarkers in the pathophysiology of LLD. The aim of this chapter is to set the scene and rationale for the two primary studies of this dissertation which approach the pathophysiology of LLD with peripheral biomarkers (i.e. immune and genomic). Chapter 4 is a meta-analysis exploring concentrations of chemokines in MDD. This meta-analysis has not been conducted previously in the literature. Our aim is to critique this novel and promising field of enquiry and provide strong recommendations for further development of this early stage field. Genomic predictors of remission to antidepressant treatment in geriatric depression using genome-wide expression analyses are examined. This is the first such genome-wide study conducted in LLD. In chapter 5, an overview of the role of neuroimaging biomarkers for the pathophysiology of LLD is provided, with the aim of setting the scene for the final two primary studies of this thesis. Chapter 6 is the rs-fMRI study whereby rs-fMRI methods are used to compare functional connectivity between LLD subjects and healthy controls. This type of study is novel as no other authors have compared current LLD patients with healthy controls. We believe this study will provide a novel contribution to the literature. In chapter 7, we explore the neural correlates of apathy in LLD with the amyloid and tau binding PET ligand, [^{18}F]FDDNP. This is the first amyloid or tau ligand study in LLD looking for the

neural correlates of apathy in a cohort of LLD subjects. Throughout this dissertation we justify the complimentary approaches of the four primary studies. In chapter 8, innovative approaches to exploring the pathophysiology of LLD with multimodal methods are outlined including machine learning. Finally in chapter 9, we summarise our findings and provide future directions for this important field.

2.0 Introduction and overview of the pathophysiology of late-life depression

This chapter provides a background review of LLD and its diagnostic procedures, epidemiology and risk factors associated with LLD. The chapter provides an overview of pathophysiological models.

2.1 Diagnosis and definition of late-life depression

LLD can be defined as a MDD that develops after the age of 60 years, however onset and definition may vary (see for review [15]). There are no established DSM-5 diagnostic criteria for LLD, so MDD criteria are often used. In the DSM-5, MDD [5] is diagnosed when five or more of the following symptoms have been present during a 2-week period (at least one of the symptoms must be either depressed mood or loss of interest or pleasure): depressed mood, loss of interest or pleasure, significant weight change (increase or decrease), sleep disturbance (insomnia or hypersomnia), psychomotor change (agitation or retardation), fatigue, feelings of worthlessness or inappropriate guilt, impaired concentration and recurrent thoughts of death. Other diagnostic criteria include a significant impairment in social, occupation or other life functioning, and the episode not attributable to substances or other medical conditions. There are a number of other psychometric scales which can be used to diagnose or screen for LLD (reviewed elsewhere [16]). The most common psychometric scales used include the self-rated Beck Depression Inventory (BDI) and the Geriatric Depression Scale (GDS), as well as the clinician-rated Hamilton Depression Rating Scale (HDRS or HAM-D) and Montgomery-Asberg Depression Rating Scale (MADRS) [17]. It is important to distinguish early-onset depression with recurrent depressive episodes in late years from LLD, whereby the depressive illness

develops for the first time in later life. There is some evidence to suggest these two depression categories differ in terms of presentation, neuropsychological features, neurobiological factors and treatment response [18-22], however this remains to be accepted by expert peer-group consensus or meta-analysis. Continued research into understanding subtypes, trajectories and patterns of LLD are important [23]. Subtypes of unipolar depression often considered in the literature include: vascular, melancholic, non-melancholic, atypical, psychotic, minor/subthreshold, seasonal affective disorder and dysthymia. This dissertation will focus on major unipolar LLD [5, 15].

2.2 Population ageing projected to increase the prevalence and burden of late-life depression

World population ageing in the 21st century is unprecedented in human history, and will place substantial pressure on health systems across the world with concurrent rises in chronic diseases, particularly late-life affective disorders. A recent United Nations World Population Ageing Report provides the most current and projected figures in relation to world ageing [1]. The report suggests the global share of older people (aged ≥ 60 years) increased from 9.2% in 1990 to 11.7% in 2013 and will continue to grow as a proportion of the world population, reaching 21.1% by 2050. Globally, the number of older persons (aged ≥ 60 years) is expected to more than double from 841 million people in 2013 to more than 2 billion in 2050. Moreover, the share of older persons aged ≥ 80 years (the 'oldest old') within the older population was 14% in 2013 and is projected to reach 19% in 2050. These global trends in population ageing are mirrored in Australia. The number of Australians aged ≥ 65 years is expected to increase rapidly, from around 2.5 million

in 2002 to 6.2 million in 2042 [24]. For those aged ≥ 65 years, the growth is even more rapid, from around 300,000 in 2002 to 1.1 million in 2042.

2.3 Burden, prevalence and incidence of late-life depression

The burden, prevalence and incidence of LLD are considerable and are logically expected to increase with population ageing. Community-based studies find the prevalence of total depression between 4.8 and 13.7% when using DSM-IV criteria [3]. In considering only MDD cases, the prevalence is between 1 and 5.37% [25]. When considering depression determined by psychometric scales, the prevalence is higher as compared to expert-led comprehensive psychiatric interviews. A community sample of 359 older Italian adults (≥ 74 years) found an overall prevalence of 25.1% (95% CI: 20.6–29.6) when utilising the International Classification of Diseases (ICD) 10 criteria [26]. Prevalence of mild, moderate and severe depression was 16.4% (95% confidence interval (CI): 12.6–20.2), 7.5% (95% CI: 4.8–10.2) and 1.1 (95% CI: −0.4–2.6) respectively. A rate of 5.6% of the population complained of subthreshold depressive symptoms. A systematic review has been conducted exploring the incidence of depressive disorders in late-life, persons aged ≥ 70 years [27]. A total of 20 studies were found with 14 using categorical and six using continuous diagnoses. The incidence rates of MDD were 0.2–14.1/100 person-years and the incidence of clinically significant symptoms was 6.8–100 person-years. The most recent global data on MDD comes from a contemporary analysis of the World Health Organization's (WHO) Global Burden of Disease (GBD) Study 2010 [28]. This study identified depressive disorders as a leading cause of burden internationally, and suggests all-age MDD was also a contributor of burden allocated to suicide and ischaemic heart disease. Depressive

disorders were the second leading cause of years lived with disability (YLD) in 2010. MDD accounted for 8.2% (5.9–10.8%) of global YLDs and dysthymia for 1.4% (0.9–2.0%). The burden of depressive disorders was highest in adults of working age (15–64 years) with 60.4 YLDS in 2010 versus adults ≥ 65 years with 6.1 million YLDs. The burden of depression arises from negative impacts on quality of life, productivity, caregiving function, relationship stability, job loss and diminished financial achievement [23].

2.4 Risk factors for late-life depression

When exploring the pathophysiology of LLD, it is important to also consider the risk factors which may contribute to the disorder itself. For a thorough review of this topic, see [18, 29]. The risk factors for LLD can be broadly broken down to biological and psychosocial categories. Biological risk factors can be considered in terms of vascular, general medical disorders, dementia-related and other genetic factors. Vascular risk factors noted in the literature include cardiovascular diseases like coronary heart disease, myocardial infarction, cerebrovascular disease and white matter hyperintensities (WMH). General medical disorders include obesity, diabetes mellitus and hypertension. Dementia-related risk factors include vascular dementia, Alzheimer's disease (AD) and Parkinson's disease. Other genetic factors can be explored elsewhere. Psychosocial risk factors can be broadly considered as personality-related, life- and social stressors. Personality-related risk factors include the presence of a personality disorder, high neuroticism, low self-efficacy, presence of learned helplessness and obsessional traits. Life stressors include medical illness and disability, poor

functional status, trauma and bereavement. Social stressors include impaired social support, loneliness, low income and lesser education.

2.5 Clinical staging in late-life depression

Clinical staging and profiling in LLD is a model that may be useful for biomarker research. Clinical staging explores where a patient lies along a continuum of the course of an illness; it may allow for earlier treatment which is more effective and less harmful [30]. Successful examples of staging come from oncology, where the use of tumour, node, metastasis (TNM) classification is commonly used, and congestive heart failure, where the New York Heart Association classification system is commonly used [31]. In the depression literature, a clinical staging model has been developed by McGorry et al. [30, 32]. This model ranges from stage 0 (at risk, but symptomatic) through to stage 4 (severe and unremitting illness). Stage 1 is defined as an initial stage of undifferentiated general symptoms of distress (stage 1a) followed by a state more suggestive of the disorder, albeit subthreshold (stage 1b). Stage 2 then represents a first episode of the disorder which may be then remits or is followed by the development of persistent symptoms, frequent relapses or ongoing impairment (stage 3). Stage 4 in this model may include severe dementia-level cognitive impairment. McGorry et al. [30, 32] also suggest biomarkers should be integrated into this model, and these biomarkers may reflect causal mechanisms (e.g. genetic and oxidative stress markers), consequences of the pathophysiology (e.g. cognitive, structural and physiology), and some may reflect both (e.g. inflammation). These biomarkers may assist in diagnosis and the development of novel therapeutic interventions (e.g. anti-inflammatory agents). There are no published staging systems for LLD [31].

2.6 Depression as a risk factor for dementia

As our global population ages, the rates of dementia are expected to rise to record levels. For example, in 2013 world-wide 44.4 million people with dementia were estimated, and this is expected to rise to 135.5 million by 2050 [33]. One of the key risk factors for dementia is depression [34-37]. Data suggests that one in 10 cases of dementia world-wide can be attributed to depression based on a 13.2% prevalence and 7.9% (95% CI: 5.3–10.8) as a population attributable risk based on modelling (and a relative risk (RR) of 1.65) [34]. Three meta-analyses have been conducted in this domain and reported a RR of depression for dementia range between 1.65 and 1.90 [38-40]. Clearly, there is some variation in the literature exploring the role of depression as a risk factor for dementia [41]. This may be partly due to the heterogeneity of depression phenotypes studied. One way to enhance the research in depression is by investigating commonly shared clinical and neural features between depression and dementia (discussed in chapter 6).

2.7 Reviewing the pathophysiology of late-life depression

Research into the pathophysiology of depression has historically encompassed many interrelated systems including the hypothalamus-pituitary-adrenal (HPA) axis, neurotransmitter systems, neurotrophins and neuroplastic processes and neuroinflammation [42-46].

The monoamine hypothesis of depression is the most established hypothesis in depression and suggests the underlying pathophysiological changes occurring in depression are the depletion of central nervous system (CNS) monoamines, serotonin (5-HT), noradrenaline (NA) and dopamine (DA). This hypothesis has been extensively reviewed elsewhere [47, 48].

Many studies have also investigated the dysregulation of the HPA axis in depressive disorders (see for reviews [44, 49, 50]). One of the most consistent deficits described is the loss of glucocorticoid receptor (GR) mediated negative feedback on the secretion of the classical 'stress hormone', cortisol. This loss of negative feedback is commonly assayed by non-suppression of cortisol or adrenocorticotrophic hormone secretion in response to the dexamethasone suppression test [49]. Polymorphisms of GR genes have also been associated with depressive disorders [51].

The neurogenesis hypothesis of depression posits: (a) neurogenesis in the subgranular zone of the dentate gyrus of the hippocampus is negatively affected by stress; and (b) alterations in the rate of neurogenesis play a significant role in the pathophysiology of depression [52]. Anti-neuroplastic changes which occur in depression include a decrease in proliferation of neural stem cells (NSCs), decreased survival of neuroblasts and immature neurons, impaired neurocircuitry (cortical–striatal–limbic circuits), reduced levels of neurotrophins, reduced spine density and dendritic retraction [52-54]. Evidence from neuroimaging studies consistently suggests patients with depression demonstrate reduced volumes in structures relevant to the pathophysiology of this disorder, such as the hippocampus [55, 56].

In the field of psychiatric immunology, much of the focus on the role of the immune system in depression has been placed on the innate immune response and inflammation. Innate immune cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and interferon (IFN)-γ have been repeatedly shown to exert effects on key processes, such as neuroplasticity, neurotransmission, oxidative stress and neuroendocrinological functions that are considered to be

central to the development of depression [57-61]. The seminal meta-analysis [62] of 24 studies found significantly higher concentrations of the pro-inflammatory cytokines, TNF-α and IL-6, in depressed subjects compared with control subjects. An updated meta-analysis [63] of IL-6, C-reactive protein (CRP) and TNF-α found higher levels of IL-6 and CRP in depressed patients versus controls (29 studies for IL-6 and 20 for CRP). These studies strengthen evidence that depression is accompanied by activation of the inflammatory response system [62].

The vascular hypothesis of LLD suggests that cerebrovascular disease predisposes and precipitates certain symptoms [64]. A clinical presentation for vascular depression is characterised by cognitive deficits, psychomotor retardation, lack of insight and disability disproportional to the depression severity [65]. The hypothesis evolved out of the well established relationships between LLD, WMHs (vascular dysfunction markers noted on brain MRI) and vascular risk factors [12]. WMHs are associated with advanced age and vascular risk factors, such as diabetes, cardiac disease and hypertension. LLD is associated with the severity of WMHs and volumes of WMHs.

Amyloid and tau are key neuropathological hallmarks in the development of AD [66, 67], however they are increasingly studied in LLD. One group has developed and used the [^{18}F]FDDNP *in vivo* probe that binds to cerebral aggregates of amyloid β (Aβ) plaques and tau neurofibrillary tangles (NFTs) [68]. The FDDNP biomarker has been found to differentiate individuals with mild cognitive impairment (MCI), AD and normal cognition, wherein global [^{18}F]FDDNP binding is highest in patients with AD, intermediate in those with MCI, and lowest in normal comparison subjects [68]. This group is the first to explore this probe in LLD. In a 2011 paper by Kumar et al. [13], [^{18}F]FDDNP levels were compared

between 20 LLD patients and 19 healthy controls. When compared with controls, [^{18}F]FDDNP binding was significantly higher overall and in the posterior cingulate cortex (PCC) and lateral temporal regions in the MDD group. The authors suggested neurotoxicity due amyloid and tau protein aggregation may represent a pathophysiological cascade which, along with vascular compromise, may predispose individuals to LLD.

3.0 Peripheral biomarkers and their role in understanding the pathophysiology of late-life depression

3.1 Reviewing the role of inflammation in the pathophysiology of late-life depression

As mentioned above, the most established immune-based model of depression is the inflammatory or cytokine model of depression [57, 59, 69]. The following section outlines how the inflammatory model of depression is suggested to affect neurotransmission, the HPA axis and neurogenesis.

3.1.1 The effect of inflammation on neurotransmission

In recent years, immune dysfunction as observed in depression has been shown to influence the monoamine system through various mechanisms [46]. These mechanisms include the activation of the enzyme indoleamine 2,3 dioxygenase (IDO) by pro-inflammatory cytokines altering metabolism of tryptophan into neurotoxic metabolites and depleting tryptophan availability for 5-HT synthesis [70, 71]. These cytokines may also stimulate the reuptake of monoamines from the synapse by increasing the activity and density of 5-HT, NA and DA transporters [72-74]. Moreover, these cytokines may also be involved in the therapeutic response to conventional antidepressant pharmacotherapy targeting these receptors [75]. Some evidence also suggests immune mechanisms interacting with other neurotransmitter systems, most notably glutamatergic neurotransmission may be relevant to the pathogenesis and pathophysiology of depression (see for reviews [45, 76, 77]).

3.1.2 The effect of inflammation on the hypothalamo-pituitary-adrenal axis

The mechanisms by which immune dysfunction may influence the HPA axis are complex and bi-directional. For example, excess inflammatory activity may lead to disruption of the capacity of the GR to translocate to the nucleus where it would have acted to suppress the activity of pro-inflammatory transcription factors, such as nuclear factor-κB (NF-κB) [78]. The result of these impairments is a disruption of the potent anti-inflammatory and immunomodulatory effects of glucocorticoids on both peripheral and central immune cells which may be salvaged by antidepressant pharmacotherapy [79, 80]. The disruption of the glucocorticoid system may have additional effects on neuroplastic processes leading to a reduction in volume of key anatomical structures related to depressive disorders, such as the hippocampus [81]. Conversely, treatment with conventional antidepressant pharmacotherapy may act to increase hippocampal volume by mechanisms that are, at least in part mediated by activation of the GR [82].

3.1.3 The effect of inflammation on neurogenesis

Neuroinflammatory mechanisms in depression are thought to negatively affect neurogenesis leading to apoptosis and reduced neurotrophic production [60]. In rodent models of depression, IL-1, IL-6 and TNF are associated with reduced hippocampal neurogenesis; mechanisms which are associated with this include the stress-activated protein kinase (SAPK)/Janus kinase (JNK) pathway, hypoxia-inducible factors (HIF)-1α, JAK-signal transducer and activator of transcription (STAT) pathway, mitogen-activated protein kinase (MAPK)/cAMP responsive element binding protein (CREB) pathway, Ras-MAPK, PI-3 kinase, IKK/NF-κB and TGFβ activated kinase-1 (TAK-1) (see for review [60]).

3.1.4 The effect of inflammation on amyloid and tau deposition

Elevated pro-inflammatory cytokines (i.e. TNF-α, IL-6, IL-1β and IFN-γ) have been implicated in impaired cognitive function in clinical and pre-clinical studies [59, 83-85]. To this effect, an amyloid-neuroinflammation hypothesis has been recently suggested stating that neurodegenerative changes in late-onset AD are the result of an uncontrolled, chronic intra-cerebral inflammatory reaction triggered by the accumulation/aggregation of Aβ protein in plaques [83-85]. Pro-inflammatory cytokines have been found to enhance the accumulation of Aβ protein plaques, which in turn promote further neuroinflammation leading to progressive neurodegeneration [83-85]. Additional to effects on Aβ proteins, neuroinflammation is found to induce tau protein-based pathology (i.e. NFTs) [84, 86] and can lead to increases in reactive oxygen species (ROS) [69, 87-91]. Neuroinflammation is found to be associated with neurotransmitter dysfunction (i.e. reduced acetylcholine production/function, glutamate-induced excitotoxicity and increased quinolinic acid production) [92-99].

3.1.5 Limitations of the inflammatory hypothesis of depression

While the inflammatory pathophysiology of depression is useful, the involvement of immune factors in the pathophysiology of depression is now considered to be far greater than that of only the innate immune system, inflammation and glia [100]. This preliminary conclusion can be drawn from clinical observations, such as the lack of a commensurate increase in rates of depression with the ageing-associated neuroinflammatory state (rates are highest between 25 and 45 years) [101, 102] and the paucity of efficacy of anti-

inflammatory drug compounds (i.e. non-steroidal anti-inflammatory agents [103, 104] and anti-cytokine molecules, such as infliximab [105]) in depression. Moreover, immunomodulatory factors, such as systemic immune cells (e.g. chemokines, CNS-specific autoreactive CD4+ T-cells, M2-type blood-derived macrophagesa and T-regs) and microglia and astrocytes exert neuroprotective effects relevant to molecular mechanisms of neuroplasticity [60]. Indeed, a complex interaction is suspected to occur in the CNS between parts of the innate and adaptive immune system [106]. Chemokines are suspected to be involved in depression pathophysiology, likely through neuromodulatory effects, neurotransmitter-like effects, as well as regulation of neurogenesis and axon sprouting [8].

3.2 Introduction to the role of chemokines in the pathophysiology of depression

Recent advances in basic neuroscience have begun to describe novel roles for chemokines in neurobiological processes relevant to depression [8]. In looking beyond traditional roles in chemotaxis of immune cells, these novel processes may include regulating the migration, proliferation, and differentiation of NSCs/progenitor cells; regulation of axon sprouting and elongation; regulating the infiltration and activation states of central and peripheral immune cells; control of BBB permeability; regulation of neuroendocrine functions; pre- and post-synaptic modulation of traditional neurotransmitter systems; and possibly direct neurotransmitter-like effects [107-110].

The term ‘chemokine’ is a portmanteau of ‘chemotactic cytokine’ and first coined in 1992 to accommodate a growing list of related proteins with chemotactic functions [111]. With the notable exception of CX3CL1, which has a membrane

bound form, these proteins are present in a secreted soluble form. Receptors for these chemokines are primarily located on leukocyte subsets, and many receptors bind multiple ligands with variable affinity [111, 112]. Chemokines are known to be important for leukocyte migration and activation under both physiological and pathological conditions. These processes are vital to physiological immune surveillance as well as inflammatory responses. Chemokines have also long been recognised to have additional functions including inducing the release of pro-inflammatory mediators and control of T-helper (T_h)-1/T_h-2 polarisation [112].

The chemokine receptors and their ligands are broadly expressed throughout both the developing and adult CNS (see for reviews [108, 113-115]). Several of these chemokines are expressed under physiological conditions, including: CCL2, CCL3, CCL19, CCL21, CXCL8, CXCL12, CX3CL1 [113]. Other chemokines are upregulated in response to injury or inflammation. Chemokines known to be expressed by various cell types in the CNS under either basal or inflammatory conditions [113].

The disruption of these functions in vital neurodevelopmental periods or in later life may be mechanistically relevant to the pathophysiology of depression, while restoration of homeostasis in these functions may be relevant to recovery [8]. From a clinical study perspective, we are aware of a number of cross-sectional studies (monocyte chemoattractant protein (MCP)-1/CCL2) [116-127]; macrophage inflammatory protein (MIP)-1α/CCL3 [119, 128]; CXCL1 [128, 129]; IL-8/CXCL8 [14, 116, 119-121, 124, 125, 130-135]; MCP-3 [129]; TNF-β [129]; IL-16 [129]; CTACK [129]; macrophages migration inhibitory factor (MIF) [129]; CCL11 [118, 136]; CXCL11/I-TAC [39]; MEC/CCL28 [39]; TECK/CCL25 [39]; Interferon γ-induced protein (IP)-10/CXCL10 [125, 137]; RANTES/CCL5 [117,

118]. There are only two prospective studies exploring associations between chemokines and depression [138, 139]. In one study, the associations between serum CXCL8 (IL-8) and depressive symptoms were explored in a large cohort of population-based, elderly participants for 2 years (age 70–90 years) [139]. Results indicated that serum IL-8 was positively associated with depressive symptoms on the GDS at baseline ($p = 0.025$), at 2 years follow-up ($p = 0.038$), and an increase in depressive symptoms from baseline to 2 years ($p = 0.021$). This study is the only known study on subjects with LLD.

The association between chemokine dysfunctional and depression has been documented in individual studies of various chemokines, however the association is not consistently significant in all studies or for all chemokines. Thus, a generalisable pattern of chemokine dysfunction remains to be defined. Fortunately, the results from individual studies can be combined quantitatively using meta-analytical techniques to improve the strength of the evidence.

3.3 A meta-analysis on the role of chemokines in the pathophysiology of depression

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3.3.1 Statement of authorship

Principal Author

Name of co-author:	Dr Harris A Eyre
Contribution to the paper:	Co-designed research; co-performed research; co-analysed data; primarily wrote the paper.
Overall percentage	40% contribution
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 3/2/16

Co-author contribution

By signing the Statement of Authorship, each author certifies that:

I. The candidate's stated contribution to the publication is accurate (as detailed above);

II. Permission is granted for the candidate in include the publication in the thesis; and

III. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of co-author:	Tracy Air
Contribution to the paper:	Co-designed research; co-performed research; primarily analysed data; co-wrote the paper. 20% contribution.
Signature	Date: 15/1/16

Name of co-author:	Alyssa Pradhan
Contribution to the paper:	Co-performed research; approved final version of the paper. 5% contribution.
Signature	Date: 15/1/16

Name of co-author:	James Johnston
Contribution to the paper:	Co-performed research; approved final version of the paper. 5% contribution.
Signature	Date: 15/1/16

Name of co-author:	Prof Helen Lavretsky
Contribution to the paper:	Co-analysed data; co-wrote and approved final version of the paper. 5% contribution.
Signature	*H. Lavretsky (electronic signature)* Date: 15/1/16

Name of co-author:	Michael J Stuart
Contribution to the paper:	Co-designed research; co-performed research; co-analysed data; co-wrote the paper. 10% contribution.
Signature	Date: 15/1/16

Name of co-author:	Prof Bernhard T Baune
Contribution to the paper:	Co-designed research; co-performed research; co-analysed data; co-wrote the paper. 15% contribution.
Signature	!!!!! !! Date: 15/1/16

3.3.2 Abstract

Background: Chemokines are increasingly recognised as playing a role in depression, and LLD. There is, however only one study exploring chemokine concentrations in association with LLD (outlined previously [139]), therefore this meta-analysis incorporates studies on depression in all ages. We meta-analyse the data on concentrations of all chemokines in patients diagnosed with a major depression versus healthy controls.

Methods: We included studies which utilised DSM-IV diagnostic criteria for major depression, participants free from major medical conditions, studies with healthy controls, and unstimulated measurements of chemokines. We only included chemokines which had ≥ 3 studies performed.

Results: Two chemokines and 15 studies in total met criteria for this meta-analysis; eight for MCP-1 (N = 747) and seven for IL-8 (N = 560). There were significantly higher concentrations of MCP-1 in depressed subjects compared with control subjects – overall mean difference of 36.43 pg/mL (95% CI: 2.43–70.42). There was significant heterogeneity across these studies (I^2 = 98.5%). The estimates of mean difference between the control and depression groups did not remain significant when the trim-and-fill procedure was used to correct for publication bias. There was no significant difference in concentrations of IL-8 in depressed subjects compared with control subjects. Significant heterogeneity was found across these studies (I^2 = 96.7%). The estimates of mean difference between the control and depression groups remained non-significant when the trim-and-fill procedure was used to correct for publication bias. This meta-analysis reports significantly heterogeneity in this field among studies.

Conclusions: There are higher concentrations of the chemokine MCP-1 in depressed subjects compared with control subjects, and no differences for IL-8. More high quality research and consistent methodologies are needed in this important area of enquiry.

3.3.3 Aims and rationale

The association between chemokine dysfunction and depression has been documented in individual studies of various chemokines, however the association is not consistently significant in all studies or for all chemokines. Thus, a generalisable pattern of chemokine dysfunction in depression remains to be defined. Fortunately, the results from individual studies can be combined quantitatively using meta-analytical techniques to improve the strength of the evidence. Taken together, this study reports the results of a meta-analysis conducted to determine whether the concentrations of specific cytokines differs quantitatively between patients diagnosed with a major depressive episode and control subjects.

3.3.4 Methods and materials

Data sources

We (AP and JJ) searched Embase, PsycINFO, Ovid Medline, ScienceDirect, Google Scholar and the Cochrane Central Register of Controlled Trials database up to September 2015. We also manually scrutinised references cited in the systematically searched articles. To optimise sensitivity in searching clinical studies, we used the following basic terms: XCL*, CX3C*, CCL*, CXCL*, IL-*, MCP, scya, scyb, NAP, GCP, depression, MDD and depressive symptoms.

Study selection

Studies were selected for data extraction and analysis based on the following inclusion criteria: (a) original research studies measuring chemokine concentrations in depressed and non-depressed subjects; (b) subjects met DSM-IV criteria for major depression; (c) studies were in English; (d) participants were free from major medical comorbidities (e.g. cancer, heart disease and arthritis); (e) psychiatrically healthy subjects were used as controls; and (f) unstimulated chemokine analyses were used. We excluded studies including participants with stimulated chemokine-based analyses or non-serum/plasma markers.

Data extraction

Two independent reviewers (AP and JJ) used a custom data extraction template to summarise the selected articles. Abstracted information included age, gender, sample size, depression metrics, comorbidities, chemokines analysed, method and source, and concomitant drug use. Where possible, we also sought key data that were missing from the original reports through correspondence with the investigators.

Quality assessment

This meta-analysis was carried out according to the PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines [140]. We used the Newcastle-Ottawa Quality Assessment Scale [141] for observational studies to assess quality; this scale is recommended by the Cochrane Collaboration. With this method, each study can obtain a maximum of nine points in three categories: *selection* of study participants (adequate definition, validation and representativeness of cases and controls), *comparability* of cases and controls, and

the ascertainment of *exposure*. For this study, our quality points for exposure were adapted from Haapakoski et al. [63]. These included method of assay, consistency of assay used, and three or more immune markers analysed. Given the small number of studies in this field, we included all in our meta-analysis (see table 1).

Table 1: Quality assessment of studies included in the meta-analysis

Study	**Selection**	**Comparability**	**Exposure**	**Total**
Song et al., 1998 [133]	*	*	***	**5**
Motivala et al., 2005 [126]	***	**	**	**7**
O'Brien et al., 2007 [132]	**		***	**5**
Sutcigil et al., 2007 [122]	**	*	***	**6**
Simon et al., 2008 [125]	**	*	**	**5**
Eller et al., 2008 [138]	**	*	***	**6**
Jonsdottir et al., 2009 [121]	**	**	***	**7**
Piletz et al., 2009 [123]	***	*	**	**6**
Lehto et al., 2010 [120]	***	*	***	**7**
Hocaoglu et al., 2012 [134]	**	**	***	**7**
Bai et al., 2014 [127]	**	**	***	**7**
Carvalho et al., 2014 [124]	***	**	**	**7**

Statistical analysis

Weighted mean differences between controls and cases were used to calculate the effect size (ES) of each study. A spreadsheet containing the extracted study data and the calculated ES was imported into Stata 12.0 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP) to perform the additional analyses. Random-effects models were used to estimate the overall ES. We measured statistical heterogeneity using the I^2 statistic for statistical variation across studies; values of 25% are low, 50% moderate and 75% high [142]. In addition, we used the metareg command in Stata 12.0, to conduct random-effects meta-regression analyses to assess for the source of heterogeneity. Univariate models were used to examine for the effects for the following study characteristics: mean age of the sample, gender (% male) and assay type (Cytometric bead array (CBA) versus enzyme-linked immunosorbent assay (ELISA)). If two or more of these analyses were significant, multivariate meta-regressions were planned. The possibility of publication bias was assessed through a Begg funnel plot graph and testing for asymmetry using the Egger weighted regression test. The non-parametric 'trim-and-fill' method was used to estimate the number of hypothetical studies that were missing due to possible publication bias using the metatrim command in STATA.

3.3.5 Results

Study inclusion

The utilisation of the PRISMA guidelines and a systematic search of electronic databases and manual searching through literature reviews yielded a total of 504 studies. A total of 358 duplicates were removed leaving 146 studies. After reviewing titles and abstracts, 118 studies were again excluded. Twenty-eight studies were then examined via review of full-text articles. Thirteen studies were excluded given the following. Five studies were excluded as the chemokines analysed were not explored in ≥ 3 studies [39, 128, 129, 136, 137], one study was excluded due to a lack of control group [131], one study was excluded as the authors did not respond to requests for data, and standardised means or standard deviations (SDs) were not available [135], five studies were excluded as they did not include a DSM-IV-based clinician assessment of depression [14, 118, 119, 130, 143], and one study was excluded as genetic markers for chemokines were utilised [116]. Finally, 15 studies were included in the meta-analysis and these studies included only two chemokines, MCP-1/CCL2 and IL-8/CXCL8 (see figure 1 and tables 2, 3, 4 and 5 for details).

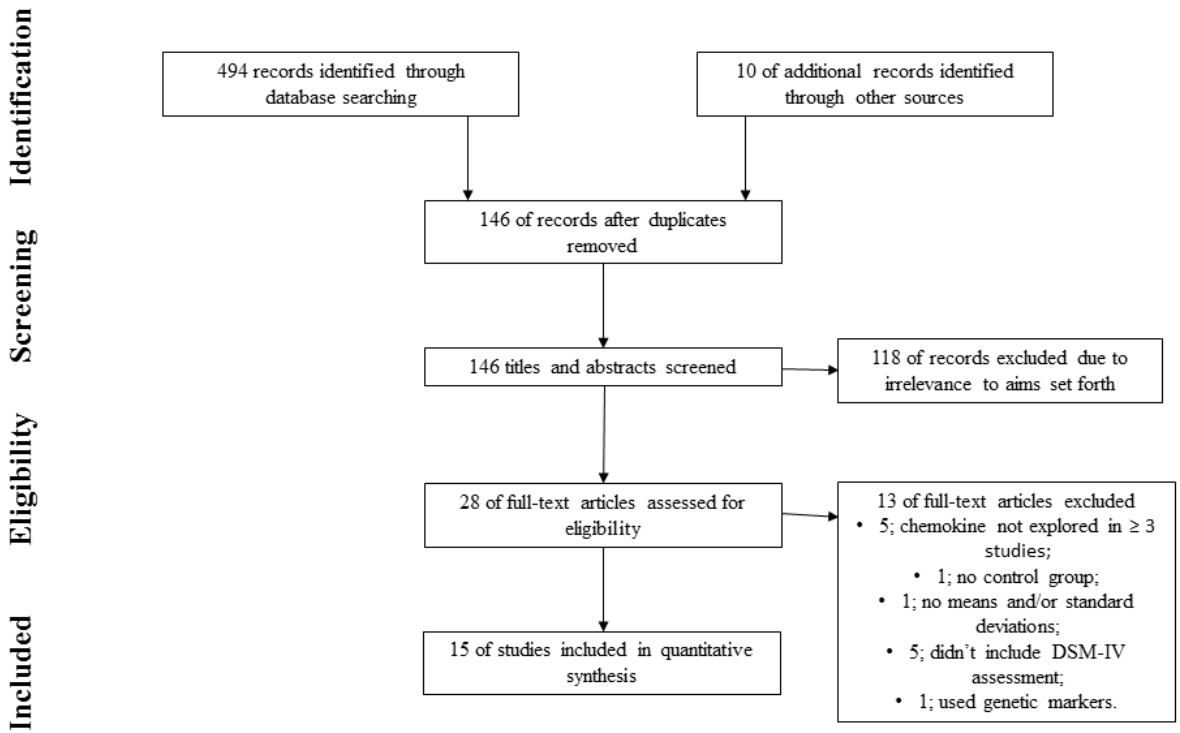

Figure 1: Study selection and inclusion process for meta-analyses

Studies of MCP-1/CCL2

Eight studies, involving 747 participants, were included in the MCP-1/CCL2 meta-analysis. There were significantly higher concentrations of MCP-1/CCL2 in depressed subjects compared with control subjects with an overall mean difference of 36.43 pg/mL (95% CI: 2.43–70.42; $p = 0.036$) (see figure 2). There was significant heterogeneity across studies ($I^2 = 98.5\%$). Our meta-regression explored heterogeneity in included studies with regard to the mean age of the sample, gender (% male), and assay used (CBA vs. ELISA). None of these variables significantly influenced our estimates of the ES ($p = 0.599$, $p = 0.485$, $p = 0.603$ respectively). Funnel plots showed evidence of asymmetry (not shown here), and there was evidence of bias using the Egger (weighted regression) method (p for bias = 0·05). The estimates of mean difference between the control and depression groups did not remain significant when the trim-and-fill procedure was used to correct for publication bias. Adjustment for publication bias according to Duval & Tweedie's trim-and-fill procedure resulted in a mean difference of −8.98 (95% CI: −38.27–20.30; $p = 0.548$) with four studies imputed.

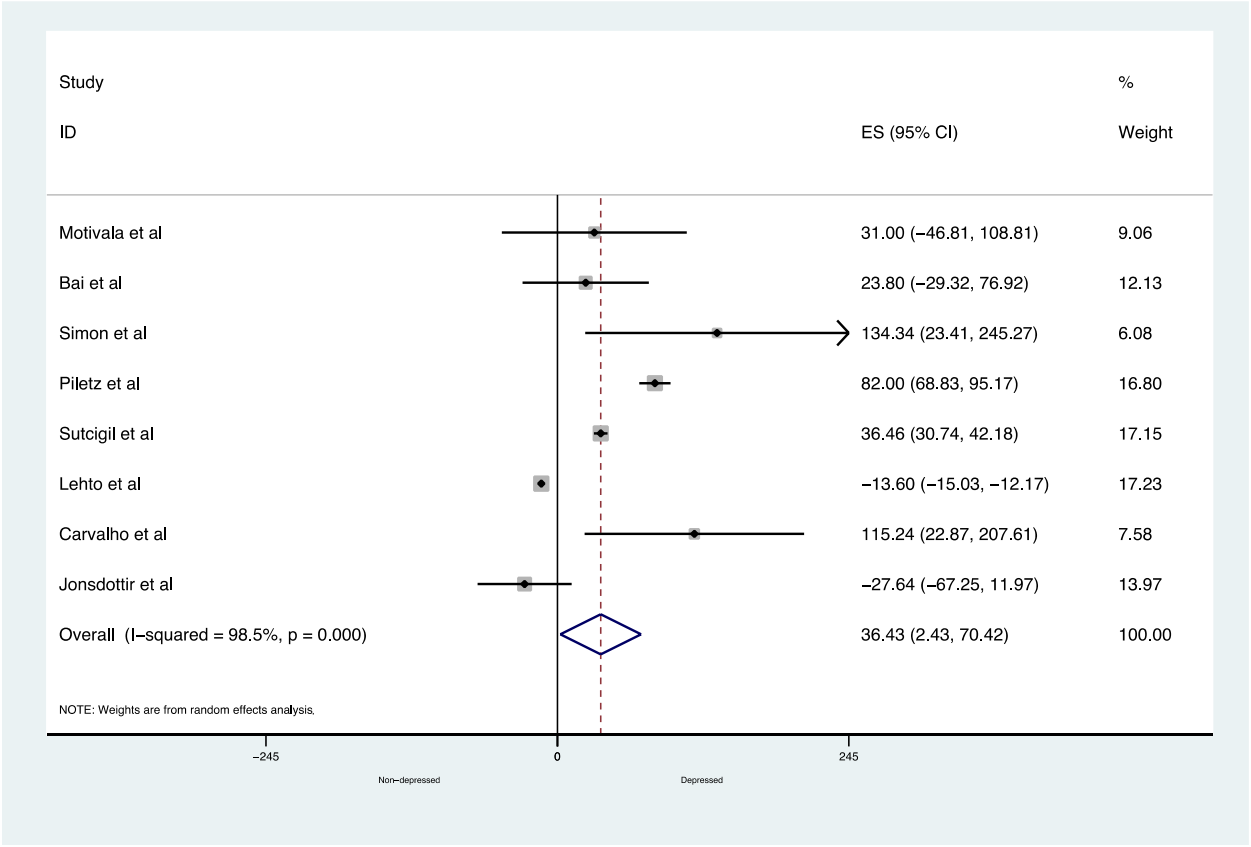

Figure 2: Forest plot showing individual and combined effect size estimates and 95% CIs for all trials in the analysis for MCP-1

Table 2: Sociodemographic characteristics of included studies of looking at MCP-1/CCL2 concentrations in depression

Study/ year	**N (D, ND)**	**Gender (% male) (D, ND)**	**Age (D, ND)**	**Comorbidities**
Motivala et al, 2005 [126]	40 (22/18)	100%/100%	44.4+/- 7.5 and 40.3 +/- 9.1	No underlying medical condition that might influence sleep disturbance or depression. No recent viral infections (past 10 days). No chronic medical conditions (diabetes mellitus, cancer, COPD). No hypertension or antihypertensives being taken.
Sutcigil et al, 2007 [122]	55 (30/25)	52.2%/52%	34.78 +/- 7.42 and 34.32 +/- 7.8	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Simon et al, 2008 [125]	98 (49/49)	59.18%/57.14%	41.65 +/- 11.07 and 41.69 +/- 11.28	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Piletz et al, 2009 [123]	39 (22/17)	14%/17%	39.4 +/- 1.9 and 39 +/- 2.1	No endocrinological or general medical conditions (e.g. diabetes, heart disease).

Jonsdottir et al, 2009 [121]	84 (42/42)	0/0	42.1 +/- 9.4 and 42.7 +/- 7.5	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Lehto et al, 2010 [120]	122 (61/61)	31.3%/31.3%	53.74 +/- 1.19 and 53.77 +/- 1.2	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Bai et al, 2014 [127]	235 (109/126)	22.9%, 31%	41.96 +/-13.8, 41.88 +/- 10.0	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Carvalho et al, 2014 [124]	89 (47/42)	43%/50%	54 (32-82) and 49 (31-74)	No endocrinological or general medical conditions (e.g. diabetes, heart disease).

Abbreviations: D, depressed; ND, non-depressed; DSM, Diagnostic and Statistical Manual; BDI, Beck Depression Inventory; HDRS, Hamilton Depression Rating Scale; SCID, Structured Clinical Interview for Depression; MADRS, Montgomery Asberg Depression Rating Scale; IL, interleukin; MCP, monocyte chemoattractant protein; DSSS, Depression and Somatic Symptom Scale.

Table 3: Clinical characteristics of included studies of looking at MCP-1/CCL2 concentrations in depression

Study/ year	Inpatient or outpatient	Depression diagnosis (scales)	Depression score (D/ND)	Duration of illness	Study type	Medication status	Assay: source, kit, fasting conditions, time of day
Motivala et al, 2005 [126]	Outpatient	DSM-IV/SCID; HDRS-17	19.3 +/- 4.2 / 1.2 +/- 1/3	n/a	Cross-sectional	Nil	Serum; 9 pm; not fasting; ELISA.
Sutcigil et al, 2007 [122]	Outpatients	DSM-IV/SCID; HDRS-17	28.39 +/- 4.53 / 4.2 +/- 1.8	n/a	Open label trial	Sertraline	Serum; fasting; 9 am; ELISA.
Simon et al, 2008 [125]	Outpatient	DSM-IV/SCID/ HDRS.17	19.3 +/- 5.3/0	6.22 +/- 8.81/0	Cross-sectional	None for ≥ 1 wk	Serum; multiplex; time not given; unclear if fasted
Piletz et al, 2009 [123]	Outpatient	DSM-IV/SCID; HDRS/21	26.2 +/- 1.0 / 1.0 +/- 0.4	n/a	Open label trial	Venlafaxine	Serum; fasting; ELISA; time not given.
Jonsdottir et al, 2009 [121]	Outpatient	DSM-IV/SCID; HAD	32% had HDRS >10	n/a	Cross-sectional	31% on antidepressants	Serum; before 9:30 am; fasting; multiplex.

Lehto et al, 2010 [120]	Outpatient	DSM-IV/SCID; HDRS-29	14.79 +/- 0.95 / 3.59 +/- 0.40 (HDRS-29)	n/a	Cross-sectional	Mixed antidepressants	Serum; multiplex; 8 am; 12 hr fasting;
Bai et al, 2014 [127]	Unclear	DSM-IV/SCID; BDI-II/DSSS-22	27 +/- 12.1 / 3.3 +/- 3.3 (BDI)	n/a	Cross-sectional	Mixed antidepressant	Serum; ELISA; fasting; time not given.
Carvalho et al, 2014 [124]	Inpatient	DSM-IV/SCID; HDRS-17	24.4 (range 18 – 30) / 0	1 episode (range 0 – 4) / o	Cross-sectional	Nil for ≥ 1 wk	Serum; 8 – 10 am; cytometric bead assay; unclear if fasted.

Abbreviations: D, depressed; ND, non-depressed; DSM, Diagnostic and Statistical Manual; BDI, Beck Depression Inventory; HDRS, Hamilton Depression Rating Scale; SCID, Structured Clinical Interview for Depression; MADRS, Montgomery Asberg Depression Rating Scale; IL, interleukin; MCP, monocyte chemoattractant protein; DSSS, Depression and Somatic Symptom Scale.

Studies of IL-8/CXCL8

Seven studies, involving 560 participants, were included in the IL-8/CXCL8 meta-analysis. There was no significant difference in concentrations of IL-8/CXCL8 in depressed subjects compared with control subjects with an overall mean difference of −0.58 pg/mL (95% CI: −1.53–0.36, $p = 0.228$) (see figure 3). There was significant heterogeneity across studies (I2 = 96.7%). Our meta-regression explored heterogeneity in included studies with regard to the mean age of the sample, gender (% male), and assay used (CBA vs. ELISA). None of these variables significantly influenced our estimates of the ES ($p = 0.984$, $p = 0.374$, $p = 0.865$ respectively). There was evidence of asymmetry in funnel plots (not shown here), however the Egger test (weighted regression) was not significant (p for bias = 0·69). The estimates of mean difference between the control and depression groups remained non-significant when the trim-and-fill procedure was used to correct for publication bias. Adjustment for publication bias according to Duval & Tweedie's trim-and-fill procedure resulted in a mean difference of −0.594 (95% CI: −1.56–0.37; $p = 0.227$) with nine studies imputed.

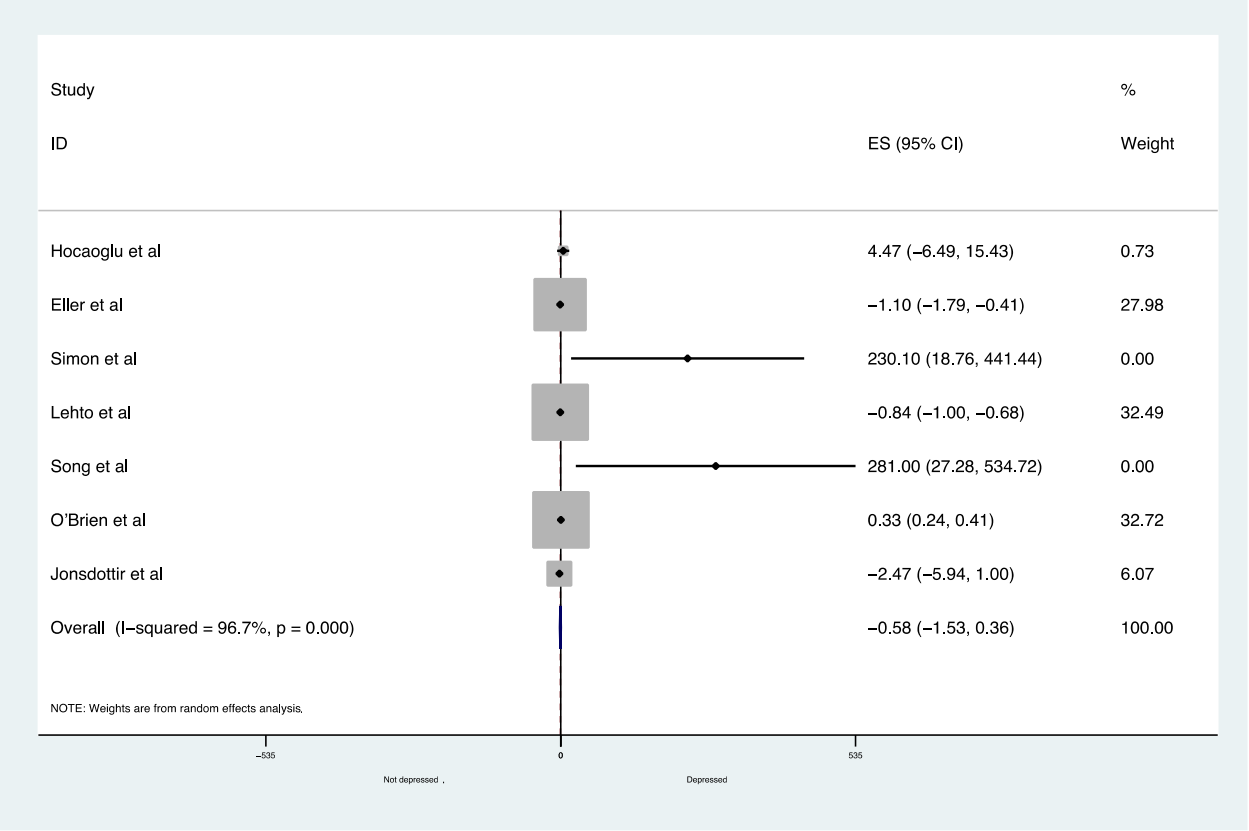

Figure 3: Forest plot showing individual and combined effect size estimates and 95% CIs for all trials in the analysis for IL-8

Table 4: Sociodemographic characteristics of included studies of looking at IL-8/CXCL8 concentrations in depression

Study/ year	**N (D, ND)**	**Gender (% male) (D, ND)**	**Age, years (D, ND)**	**Comorbidities**
Song et al, 1998 [133]	20 (6/14)	33.3%/57%	50.3+/-15.3 and 45.5 +/- 15.5	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
O'Brien et al, 2007 [132]	52 (28/24)	32%/41.7%	44.15 +/- 13.20 and 35.58 +/- 8.98	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Simon et al, 2008 [125]	98 (49/49)	59.18%/57.14%	41.65 +/- 11.07 and 41.69 +/- 11.28	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Eller et al, 2008 [138]	145 (100/45)	35%/42.2%	32.1 +/- 11.9 and 32.9 +/- 14.1	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Jonsdottir et al, 2009 [121]	84 (42/42)	0/0	42.1 +/- 9.4 and 42.7 +/- 7.5	No endocrinological or general medical conditions (e.g. diabetes, heart disease).

Lehto et al, 2010 [120]	122 (61/61)	31.3%/31.3%	53.74 +/- 1.19 and 53.77 +/- 1.2	No endocrinological or general medical conditions (e.g. diabetes, heart disease).
Hocaoglu et al, 2012 [134]	60 (30/30)	20%/53.3%	38 +/- 13 and 30 +/- 9	No endocrinological or general medical conditions (e.g. diabetes, heart disease).

Abbreviations: D, depressed; ND, non-depressed; DSM, Diagnostic and Statistical Manual; BDI, Beck Depression Inventory; HDRS, Hamilton Depression Rating Scale; HAD, Hospital Anxiety and Depression Scale; SCID, Structured Clinical Interview for Depression; MADRS, Montgomery Asberg Depression Rating Scale; IL, interleukin; MCP, monocyte chemoattractant protein.

Table 5: Clinical characteristics of included studies of looking at IL-8/CXCL8 concentrations in depression

Study/ year	**Inpatient or outpatient**	**Depression diagnosis (scales)**	**Depression scale score, years(D/ND)**	**Duration of illness, months**	**Study type**	**Medication status**	**Assay: source, kit, fasting conditions, time of day**
Song et al, 1998 [133]	Not mentioned.	DSM-IV/SCID	n/a	n/a	Cross-sectional	None for 6 wks prior	Serum; 7:45 am; fasting; ELISA
O'Brien et al, 2007 [132]	Outpatient	DSM-IV/SCID; HDRS-17	25.44/0	n/a	Cross-sectional	SSRI, SNRI, lithium + SSRI.	Serum; 9 – 11 am; ELISA; unclear if fasted
Simon et al, 2008 [125]	Outpatient	DSM-IV/SCID/ HDRS-17	19.3 +/- 5.3/0	6.22 +/- 8.81/0	Cross-sectional	None for ≥ 1 wk	Serum; multiplex; time not given; unclear if fasted

Eller et al, 2008 [138]	Outpatient	DSM-IV/SCID; MADRS	28.5 +/- 5.9 / 0	10.8 +/- 14.3	Prospective	Citalopram	Serum; 9 - 11:30 am; enzyme labelled, chemiluminescent sequential immunometric assay
Jonsdottir et al, 2009 [121]	Outpatient	DSM-IV/SCID; HAD	32% had HDRS >10	n/a	Cross-sectional	Mixed antidepressants	Serum; before 9:30 am; fasting; multiplex.
Lehto et al, 2010 [120]	Outpatient (population based sample)	DSM-IV/SCID; HDRS 29	14.79 +/- 0.95 / 3.59 +/- 0.40 (HDRS-29)	n/a	Cross-sectional	Mixed antidepressants	Serum; multiplex; 8 am; 12 hr fasting;
Hocaoglu et al, 2012 [134]	Outpatient	DSM-IV/SCID; HDRS-17	n/a	n/a	Cross-sectional	Treatment as usual	Serum; fasting; no time given (morning); ELISA.

Abbreviations: D, depressed; ND, non-depressed; DSM, Diagnostic and Statistical Manual; BDI, Beck Depression Inventory; HDRS, Hamilton Depression Rating Scale; HAD, Hospital Anxiety and Depression Scale; SCID, Structured Clinical Interview for Depression; MADRS, Montgomery Asberg Depression Rating Scale; IL, interleukin; MCP, monocyte chemoattractant protein.

3.3.6 Discussion

After a number of narrative and systematic reviews, this study is the first quantitative analysis of chemokines in depression. After exploring all chemokines, the meta-analysis was only possible for two, MCP-1/CCL2 and IL-8/CXCL8, given stringent inclusion and exclusion criteria. The analysis reports higher concentrations of MCP-1/CCL2 in depressed subjects compared with control subjects. It highlights the state of this field and outlines both positive and negative findings hence providing direction for future research.

MCP-1/CCL2 was found to be elevated in depressed versus non-depressed individuals, however significant heterogeneity was found and levels were non-significant when publication bias was corrected for. When considering the clinical significance of chemokines, it is important to also appreciate their background neurobiological functions and associated research initiatives. MCP-1/CCL2 is known for its pleiotrophic actions with functions in chemotaxis, activating function on monocytes/macrophages, T-lymphocytes and dendritic cells and CNS-specific functions [8]. In the CNS, the functions are only beginning to be understood. MCP-1/CCL2 and its receptor (CCR2) are expressed on astrocytes, microglia, neurons and NSCs [144, 145]. The expression is under both basal conditions and upregulated in response to inflammatory cytokines [8]. MCP-1/CCL2 has been found in pre-clinical models to regulate the inflammatory activation state of CNS resident microglia [146]; neuromodulation in electrophysiological models [147]; and mediating the migration and proliferation of NSCs after release from BBB cells [148]. A recent study highlighted the importance of CCL2/CCR2 signalling in a pre-clinical depression model [149]. In this study mice were provided peripheral immune challenge (lipopolysaccharide), and then a detailed neuroimmune assessment was conducted including analysis of brain cytokines, chemokines, immune cells and neurons.

Lipopolysaccharide (LPS) caused a pro-inflammatory state in the brain, and caused microglia and CNS-associated phagocytic activation characterised by a marked overproduction of CCL2, TLR4/CD14, CD80 and IL-4Rα. The LPS administration also caused a selective increase of CCR2+ inflammatory monocytes within the brain. Finally, CCL2 hyperpolarised serotonergic raphe neurons in the mid-brain possibly suggesting a reduced 5-HT tone in projection areas.

IL-8 was not found to be elevated in depressed versus non-depressed individuals, and studies were found to carry significant heterogeneity. IL-8 is also known for pleiotropic actions given functions in chemotaxis and pro-inflammatory effects supporting activation and degranulation of neutrophils, basophils and monocytes/macrophages [8, 111]. In recent times, the CNS-specific effects of IL-8 have begun to be explored [8]. In the CNS, it appears IL-8 has both immune and non-immune functions. It is constitutively expressed on neurons, astrocytes, microglia, oligodendrocytes, BBB endothelial cells and NSCs [150, 151]. From an immune perspective, IL-8 is thought to be involved in regulating the activity of infiltrating peripheral immune cells in states of significant BBB compromise [151]. From a non-immune perspective, it has been shown to enhance neurotransmitter release and inhibit Long-Term Depression (LTD) in cultured rodent Purkinje neurons [152]. Finally, Kelland et al. [153] has found a role for IL-8 on stem cell biology *in vitro*. IL-8 was found to cause CXCR-1-mediated death of NSC, but not oligodendrocyte progenitor cells. IL-8 also acted as a potent chemoattractant to both cell types. This suggests a role for IL-8 in the CNS for recruiting these cells types to sites of inflammation which may have an impact in depression pathophysiology.

A recent systematic review explored the role of chemokines in clinical and pre-clinical populations in depression [8], and another explores the pathophysiology mechanisms in depression [154]. These reviews demonstrate the majority of published studies including

measurement of chemokines in these psychiatric disorders have concerned the prototypical 'pro-inflammatory' chemokines IL-8/CXCL8 and MCP-1/CCL2. There is, however, evidence to suggest a wide range of chemokines are involved in the pathophysiology of depression and depression-like behaviour. The chemokine CXCL12 has effects of depression-related pathophysiological mechanisms as it may enhance the activity of GABA and glutamate on serotonergic neurons, enhance the proliferation and direct the migration of human neural progenitor cells [107]. CX3CL1 also has several non-immune mechanisms which may be relevant to depression including inhibition of serotonergic neurotransmission by enhancement of the activity of GABA on serotonergic neurons, inhibition of glutamatergic activity in hippocampal neurons, and regulation processes of neuroplasticity such as long term potentiation (LTP) [107]. It is important to note that evidence from systematic reviews shows early mechanistic evidence does associate select chemokines with the neurobiological processes (neurogenesis, neuroinflammation, HPA axis, neurotransmission), however, as with the current clinical evidence, this early evidence does not clearly demonstrate any specificity for a certain psychiatric disorder, but is primarily relevant to mechanisms which are shared across disorders i.e. bipolar disorder, schizophrenia [154].

The relative lack of significant findings in this study is useful to consider. One explanation for this may be due to the small sample size of these studies. The average was around 60 participants (minimum 20 and maximum 245). Another reason is the relatively inconsistent time of day chemokine were collected across these studies, most in the morning and after fasting, however some without fasting and at least one in the evening. This is critical given the circadian-based expression of cytokines and chemokines [155]. From a mechanistic perspective, there is emerging data to suggest a significant role for chemokines in depression-

related pathophysiological processes and depression-like behaviour [156, 157]. These results from mechanistic studies may not be translating into human studies for a number of reasons. The CNS immune milieu in human depression may not be adequately sampled from peripheral chemokine levels. This may be due to chemokines being expressed at very low levels in the CNS, particularly under basal conditions [110]. Deranged levels of chemokines may not be detected in the periphery if their function is autocrine or paracrine within the CNS [109]. This is an argument for measuring chemokine levels via cerebrospinal fluid (CSF) sampling, and via human CNS cell lines from induced pluripotent stem cells (iPS) derived from peripheral somatic cells of depressed subjects [158]. Fourthly, there are a variety of depression subtypes which haven't been parsed out in this study e.g. melancholic, non-melancholic, atypical. Therefore, this is an important area for further study given there is some evidence to suggest inflammatory profiles differ among depression subtypes [159], hence one could hypothesise there may be differences in chemokine levels. When aiming to parse out chemokine level associations with depression subtypes, another approach may be to parse out associations with specific symptoms associated with depression, or among psychiatric disorders. Such an approach would be in keeping with the Research Domain Criteria (RDoC) approach e.g. negative and positive valence systems, as well as cognitive systems [160]. Finally, in addition to this pilot meta-analysis exploring associations between chemokine levels and MDD diagnosis, future directions should explore the role of chemokines as treatment response biomarkers. For example, in a study by Rethorst et al. [161], in subjects with treatment resistant depression, only subjects with baseline high levels of TNF-α were shown to benefit from the antidepressant effects of aerobic exercise, as compared with those who had low levels of TNF-α.

This study has a number of limitations which should be outlined, and these limitations come from the field being quantitatively assessed. First, only two chemokines could be analysed as there were only two chemokines which had ≥ 3 studies conducted on them, were from unstimulated serum/plasma chemokine markers and had participants free of major medical comorbidities. This may play a factor in our relative lack of significant findings. Secondly, there were limited options to control for covariates. Covariates which should have been controlled for, but which was not possible due to a lack of information from available studies, including body mass index (BMI), smoking states, chronic disease burden, anti-inflammatory drug usage and antidepressant usage. There were a number of studies outlining their antidepressant use, and these varied widely. The wide variation is concerning given various antidepressant classes are likely to influence the immune system in differing ways. For example, a recent meta-analysis of cross-sectional studies on serum inflammatory cytokines reported selective serotonin reuptake inhibitors (SSRIs) as having larger anti-inflammatory effects than other antidepressants [162]. The influence of antidepressant classes on chemokines is very poorly known. Thirdly, subanalysis of mitogen-stimulated chemokines assays or other non-serum/plasma chemokine markers (i.e. genetics, CSF) could not be performed as there were no chemokines with ≥ 3 studies conducted on them.

We have a number of recommendations for the future of this research field. Longitudinal studies in this field are urgently needed to better understand correlation versus causation, and how chemokine levels may change with the fluctuation of depressive symptoms. We believe levels of chemokines in depressed versus non-depressed subjects should be explored in a range of age groups. We speculate the chemokine levels in depressed subjects of varying ages may change depending on the pathophysiology driving depression in various age

groups. For example, in LLD there is a greater burden of vascular-related changes in the brain [12]. Chemokines are known factors involved in cerebral small vessel disease via endothelial dysfunction [163]. More chemokines should be assessed for behavioural effects in *in vivo* models. There are few studies in this field exploring the behavioural outcomes of chemokine-based transgenic modified rodents (e.g. knockout or overexpression). Our group has begun to explore this topic [156, 157]. Given depression is a known risk factor for the development of AD, it is important to better understand the role chemokines play in the development of AD-related pathophysiology (e.g. amyloid and tau neurotoxicity, hippocampal atrophy) [154]. Recent rodent studies have found CX3CR1-deficiency in microglia has been shown to enhance microglial-mediated amyloid clearance [164]; in contrast, CCR2 deficiency is microglia was found to aggravate amyloid deposition [165]. Finally, care must be taken to ensure chemokine ligand and receptors are similar in their biological activity and pharmacology as compared with humans; there is significant variability between species.

3.3.7 Conclusion

This meta-analysis reports significantly heterogeneity in this field among studies. There are higher concentrations of the chemokine MCP-1 in depressed subjects compared with control subjects, and no differences for IL-8. More high quality research and consistent methodologies are needed in this important area of enquiry given the growing evidence accruing from clinical and pre-clinical models.

3.3.8 Conflict of interest declaration

There are no conflicts of interest.

3.3.9 Acknowledgements

This paper has no acknowledgements.

3.4 Genomic predictors of remission to antidepressant treatment in geriatric depression using genome-wide expression analyses: a pilot study

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3.4.1 Statement of authorship

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Overall percentage	40%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 1/2/16

Co-author contribution

By signing the Statement of Authorship, each author certifies that:

I. The candidate's stated contribution to the publication is accurate (as detailed above);

II. Permission is granted for the candidate in include the publication in the thesis; and

III. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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3.4.2 Abstract

Objective: In this first pilot study of genome-wide expression as biomarkers of antidepressant response in LLD, we examined genome-wide transcriptional profiles in a 16-week randomised placebo-controlled trial of combined methylphenidate (MPH) and citalopram (CIT).

Methods: Genome-wide transcriptional profiles were examined in peripheral blood leukocytes sampled at baseline and 16 weeks from 35 older adults with major depression who were randomised to either MPH + CIT, CIT + placebo or MPH + placebo. MPH doses ranged between 10 and 40 mg per day, and CIT doses ranged between 20 and 40 mg per day. Remission was defined as HDRS-24 score of 6 or below. Early remission was achieved in the first 4 weeks of treatment. Differential gene expression analyses tested the hypothesis that observed transcriptional alterations at baseline affected treatment remission to antidepressants.

Results: We analysed gene expression in 24 remitters and 11 non-remitters. At baseline, we found three genes showing higher expression in all remitters versus non-remitters that satisfied the established level of significance: a fold change of two and *p*-value of 0.05 that included HLADRB5; SelenBP1; and LOC 388588. Two gene transcripts showed higher expression in early remitters at baseline compared to non-remitters. The first gene was CA1 carbonic anhydrase gene, on chromosome 8 involved in reversible hydration of CO_2 and respiratory function (fold change 2.54; $p = 0.03$). The second gene was the SNCA-α-synuclein gene, implicated in Parkinson's disease and which binds to DA transporter (fold change 2.1; $p = 0.03$).

Conclusions: Remission to antidepressants in geriatric depression may be associated with a particular gene expression profile in monoaminergic and metabolic pathways and needs to be replicated in a larger sample.

3.4.3 Introduction

It is important to understand the underlying biological mechanisms of treatment resistance with non-remission to medications. Response to psychiatric medications in LLD is highly heterogeneous and complex, however there is a paucity of research into genomic and transcriptional predictors of treatment response, especially in older adults [166]. As a result of the increased burden of disease, accelerated response and remission are particularly important [167]. Typical targets of examination in pharmacogenetic depression studies include candidate genes from the monoaminergic neurotransmitter pathways related to the putative mechanisms of action (i.e. 5-HT, adrenalin, DA), neuroplasticity (e.g. *BDNF, CREB1*) [168, 169], treatment resistance (*ABCB1 (MDR1)*) [170], or immune modulation [171]. The search for a genetic mechanism of treatment response in depression still continues with only a few confirmed results.

MPH is a promising adjunctive therapy in LLD which may be useful in optimising treatment outcomes [172-174]. MPH is a DA reuptake inhibitor, and as a single agent, has been shown to be effective and safe in a few open and controlled studies in the elderly [172, 173, 175-177]. Two large series of medically ill mixed-age patients [178, 179] suggested the value of adjunctive dextroamphetamine (range: 2.5–30 mg) and MPH (range: 5–30 mg/day) in relieving depression with the rapid onset of response within 48 hours. The use of dopaminergic agents like MPH in LLD could be particularly useful in the treatment of LLD given the observed reduction in DA neurotransmission with ageing [172, 173, 177]. Recently, our group conducted the first randomised placebo-controlled trial aimed to test the clinical efficacy of MPH in combination with CIT used to improve antidepressant response in LLD [167]. This study demonstrated a faster and improved response in the combined treatment group compared

to either drug with placebo. The rate of improvement in the CIT + MPH group was significantly higher than that in the CIT + placebo group in the first 4 weeks of the trial.

Peripheral blood gene expression studies have shown to provide a good proxy for what may take place in the brain of psychiatric patients and can assist in predicting response to treatment [180]. In our pilot controlled trial [174] of MPH in 15 older outpatients with major depression, those with DAT VNTR 10/10 genotype had greater cognitive executive dysfunction at baseline, but responded preferentially to MPH added to CIT with a greater reduction in depression severity over time compared to other subjects. Only one prior study has explored gene expression biomarkers of response to antidepressants at a genome-wide level. We are not aware of any studies in late-life populations which explore gene expression biomarkers of response at a genome-wide level [181].

3.4.4 Aim and rationale

The present pilot study examined leukocyte gene transcriptional alterations in remitters versus non-remitters with antidepressant treatment in a subsample of 35 participants from the parent study [167]. Genome-wide transcriptional profiling was carried out in the peripheral blood mononuclear cell (PBMC) samples obtained at baseline and post-intervention. We hypothesised that genome-wide transcriptional analyses would reveal potential predictors of remission in the dopaminergic pathways based on our pilot data.

3.4.5 Methods

Study procedures

From August 2008 to September 2012, 510 individuals were screened according to eligibility criteria by phone yielding 203 individuals for a diagnostic interview. After describing the details of the study to interested and eligible subjects, written informed consent was obtained in accordance with the procedures set by the UCLA Institutional Review Board (IRB).

Inclusion and exclusion criteria

Inclusion criteria were: (a) current episode of unipolar MDD according to DSM-IVTR criteria; (b) HDRS-24 score ≥ 16 [182]; and (c) Mini-Mental State Examination (MMSE) [183] score ≥ 26. Exclusion criteria were: (a) history of any other psychiatric disorders (other than unipolar MDD with or without comorbid anxiety symptoms); (b) severe or acute unstable medical illness, including the presence of either atrial or ventricular arrhythmia, or acute ischaemic changes on the baseline electrocardiogram (ECG); (c) acute suicidal or violent behaviour or history of suicide attempt within the last year; or (d) any other CNS diseases. Patients were free of psychotropic medications for at least 2 weeks before starting the trial.

Primary outcomes

Remission versus non-remission was the primary outcome of the trial. Remission was defined as HDRS-24 score of 6 or below. Early remission was determined by remission by week 4, while delayed response was response at any time point afterwards (between 4 and 16 weeks). We measured comorbid symptoms

of anxiety, apathy, medical and vascular risk factors, health-related quality of life and cognitive performance.

Randomisation procedures

Randomisation was performed using a computer-generated schedule. As there were three groups we used block randomisation to maintain balance over the course of the study with a random mix of block lengths of three and six to help further preserve the blind. Allocation concealment was implemented using sealed, sequentially numbered boxes that were identical in appearance for the three treatment groups. In order to monitor the internal validity of the randomisation and blinding in the trial, we created a guessing scale for the study staff in the first year of the trial and the accuracy of our guessing assignment in two independent trials were 35%.

Intervention procedures

Participants were seen in-person weekly for the first 4 weeks, while MPH dose was titrated for evaluation of safety and detection of accelerated response, and every 2 weeks thereafter for the remainder of 16 weeks. Treatment with both drugs was initiated simultaneously after the baseline assessment in order to track accelerated response. Participants were given a weekly supply of the given study medications prepared and dispensed by the UCLA Pharmacy in matching capsules: CIT 20 mg/day and MPH 2.5 mg (or one cap) twice a day recommended at 9 am and 3 pm, or the matching number of capsules of placebo as a starting dose. We used a 5–40 mg flexible dose of MPH that was increased based on the response and tolerability assessment during each weekly visit in the first 4 weeks of treatment.

The dose range was established in two of our pilot studies that were dedicated to the dose finding and safety evaluation of the optimal MPH dose in older adults [172]. The dose of the MPH was increased at each visit if subjects had the Clinical Global Impression (CGI)-improvement [184] scores of 3 and greater and they showed no serious adverse effects. The increment increase of MPH occurred in the first 4 weeks of the trial by 2.5 mg twice a day every 4 days between days 4 and 28 of treatment, or until subjects were able to achieve CGI score of 1 or 2. After day 28 of MPH titration, subjects remained on the same dose through the end of the trial. If subjects showed minimal improvement with CGI improvement score of 3 or greater by day 28 of treatment, CIT dose was increased to 40 mg and continued to the end of the trial in the majority of subjects, with the exception of 13 subjects, who received another increase in CIT dose to 60 mg at weeks 7–8 of the trial due to insufficient response. The allowed dose adjustment for MPH was decreasing by two pills, to a minimum of 5 mg a day, and decreasing CIT dose to 20 mg. If subjects could not tolerate the minimum allowed dose, they were discontinued from the trial. The use of concomitant rescue medications during the treatment trial was restricted to the use of lorazepam up to 1 mg/day.

Assessment instruments

Subjects were evaluated using validated assessment instruments that included the HDRS, MADRS [185] and CGI [184] to measure depression severity and change over time. We measured anxiety symptoms using the HAM-A Scale [182]. Cerebrovascular Risk Factor Prediction Chart [186] and Cumulative Illness Rating Scale-Geriatrics (CIRS-G) [187] assessed medical comorbidity. Other instruments, and the Medical Outcomes Study Short Form 36-Item Health Survey

(SF-36). The primary outcome measures were administered at all visits. The rest of the clinical measures were administered at baseline and the end of the study by two raters (HL and NSC).

Safety and adherence assessments

Vital signs and weight were measured at baseline and at each visit in addition to a 12-lead ECG performed at baseline, and at weeks 3 and 16, if any cardiac complaints were present. A physical examination was administered at baseline and week 16, or upon early termination. Side effects were assessed at all visits by the *Udvalg for Kliniske Undersogelser* (UKU) Side Effect Rating Scale [188]. Treatment compliance was assessed by employing indirect measures of adherence including questioning of the patients, returned pill count, and drug level measures at weeks 3, 8 and 16. Plasma levels of CIT and metabolite, as well as ritalin and ritalinic acid levels, were obtained.

Leukocyte analysis

Biological genomic analyses were conducted on a randomly selected subsection of the larger clinical trial. RNA samples were QC'd using an Agilent 2200 tapestation and quanted with ribogreen. A range of 100–200 ng of total RNA was used for amplification/labelling using the Ambion total prep 96 kit. Amplified and labelled samples were hybridised to Illumina expression chips according to the standard Illumina protocol [189]. Post-hybridisation washing and staining was done using a SciGene Little Dipper robotic processing platform. Chips were scanned on our Illumina iScan confocal scanner.

Gene expression profiling and analysis

Genome-wide transcriptional profiles were collected from peripheral blood leukocytes sampled at baseline and 16 weeks from 35 older adults with major depression who were randomly selected for testing, and randomised to either MPH + CIT, CIT + placebo, or MPH + placebo. PBMC were isolated by Ficoll density gradient centrifugation of antecubital venipuncture samples drawn between 10 and 11 am at baseline and 16 weeks later following the completion of intervention procedures. Genome-wide transcriptional profiling was carried out as previously described [189]. Remission and non-remission was determined across the treatment groups.

Statistical analysis

All data were entered into the database at the time of their collection and analysed after completion of the trial. Raw cell files were loaded into GeneSpring GX 10.0.2 software (Agilent Technologies, Santa Clara, CA) for analysis. Quantile normalisation was used, and samples were compared with a fold change and T-test analysis. A fold change of greater than two and a *p*-value of less than 0.05 was considered significant. Significant genes were further submitted to Database for Annotation, Visualization and Integrated Discovery (DAVID) analyses, to identify biological meaning. Due to the small number of non-remitters, non-parametric analyses of variance were performed to compare remitters and non-remitters on demographic and clinical variables.

3.4.6 Results

Table 6 presents the baseline demographic and clinical characteristics of the intervention in the 35 intervention completers. Of the 35 study participants, 24 responded to the treatments by meeting the criteria for clinical remission. Thirteen subjects remitted early, within the first 4 weeks of treatment. Remitters and non-remitters did not differ statistically at baseline on demographic measures (sex, education) except for age. Non-remitters were older than remitters (R = 67.2 (5.7) vs. NR = 73.5 (9.3); Wilcoxon statistic = 254.5, $p = 0.05$). Non-remitters also had higher anxiety scores (R = 7.8 vs. NR = 11.2; Wilcoxon statistic = 265.5, $p = 0.02$) and lower MMSE scores than responders (R = 28.8 vs. NR = 27.5; Wilcoxon statistic = 133, $p = 0.02$). Controlling for the group difference in age, the differences for MMSE, HAM-A were no longer significant. Remitters and non-remitters were not significantly different in their drug doses or plasma drug levels (although remitters were borderline different from non-remitters ($p = 0.08$) in their CIT levels).

Table 6: Comparison of clinical and demographic factors at baseline

Variables	Responder (N = 24)		Non-responder (N = 11)		*p*-Value
	Mean (SD)	N (%)	Mean (SD)	N (%)	
Age (years)	67.2 (5.7)		73.5 (9.3)		0.05
Sex (female)		15 (68.6)		4 (31.5)	0.3
Education (years)	15.5 (1.9)		14.3 (4.7)		0.7
Age of first episode (years)	39.6 (21.72)		41.4 (29.3)		0.9
Medical burden 4CIRS • CVRF	 4.9 (3.7) 11.0 (6.2)		 5.5 (3.2) 10.7 (4.6)		 0.6 0.7
Mood • HAM-D (Screen) • HAM-D (16 weeks) • MADRS (screen) • MADRS (16 weeks) • HAM-A*	 18.2 (2.3) 4.5 (4.4) 17.0 (2.4) 7.8 (2.3)		 20.3 (3.6) 12.6 (3.9) 20.1 (4.0)		 0.11 0.0006 0.07

Cognition • MMSE*	28.8 (1.2)		27.5 (1.6)		0.02
Drug doses (ng/mL) • Citalopram • Methylphenidate Drug levels (ng/mL) • Citalopram • Desmethylphenidate • Ritalin	35.00 (20.64) 14.38 (12.45) 91.01 (75.09) 34.20 (34.25) 1.64 (2.28)		16.36 (23.35) 11.82 (9.56) 34.82 (53.95) 13.38 (20.68) 0.56 (0.73)		

CIRS, Cumulative Illness Rating Scale; CVRF, Cerebrovascular Risk Factor Score; HAM-A, Hamilton Anxiety Scale; HAM-D, Hamilton Depression Rating Scale; MADRS, Montgomery-Asberg Depression Rating Scale; MMSE, Mini-Mental State Examination; SD, standard deviation.

*Significance is reduced to $p = 0.07$ for HAM-A and $p = 0.08$ for MMSE, when controlling for age

At baseline, 18 genes had higher expression in the early remitters versus non-remitter group with a *p*-value of less than 0.05 and a fold change of greater than 2 (see figure 4 for full list). When considering biological significance of these 18 genes, only two were considered relevant to the mechanism of action of the utilised antidepressants in this study. These genes included CA1 carbonic anhydrase gene on chromosome 8 involved in reversible hydration of CO_2 and respiratory function (fold change 2.54; $p = 0.03$); SNCA-α-synuclein gene implicated in Parkinson's disease that binds to DA transporter (fold change 2.1; $p = 0.03$). Figure 1 outlines a diagrammatic representation of the fold change in gene expression for each gene. There were no significant correlations between remission and non-remission with final drug doses of CIT and MPH, nor plasma drug levels of CIT, desmethylphenidate and ritalin, or CA1 and SNCA levels. There were no significant relationships between remitters and non-remitters and genomic data at follow-up.

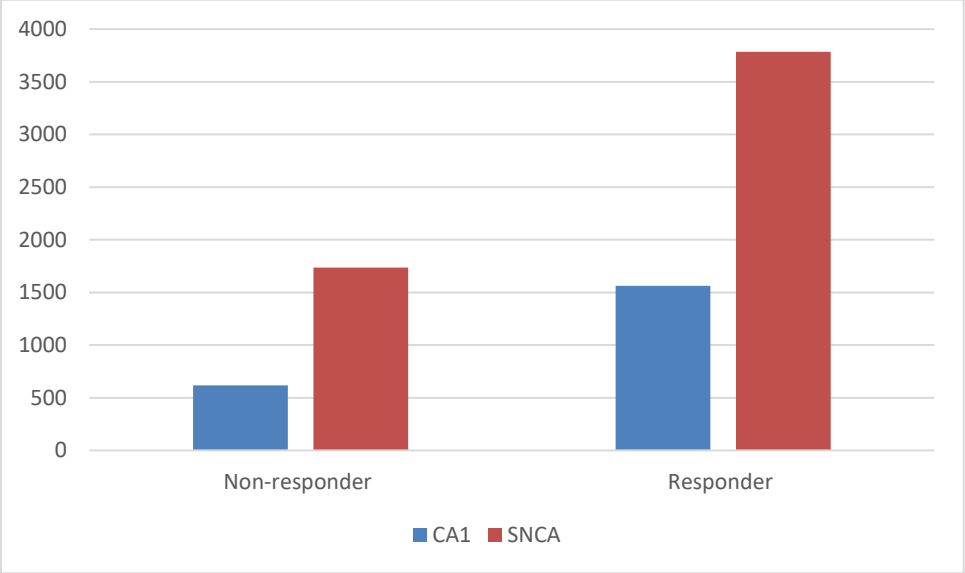

Figure 4: Change in gene expression in early remitters versus non-remitters

NB: Y-axis denote fold change in gene expression. Difference between early remission and non-remission for CA-1 is 0.03; for SNCA is 0.03.

We ran DAVID analyses to look at biologically significant functions among the 18 differentially expressed genes in the early remitter response comparison. A total of 28 Gene Ontology groups were found with a *p*-value of less than 0.1 (see table 5. Of interest, cytosol and protein stabilisation were found significant at *p*-value of 0.015 and 0.025 respectively. Additionally, ion and cellular homeostasis were also significant ($p = 0.005$ and $p = 0.04$ respectively). Finally, immune system development was found to be significant ($p = 0.023$).

Table 7: Gene ontology outputs from DAVID analysis for biologically significant functions relevant early remitters

Gene ontology details	*p*-Value	Genes
002002; haemoglobin metabolic process	3.23E-05	ALAS2, EPB42, AHSP
0050801; ion homeostasis	0.004924	ALAS2, EPB42, SNCA, SLC4A1
0005938; cell cortex	0.005491	EPB42, SNCA, SLC4A1
0048878; chemical homeostasis	0.009184	ALAS2, EPB42, SNCA, SLC4A1
0005833; haemoglobin complex	0.009352	AHSP, HBD
0005829; cytosol	0.014651	AHSP, SNCA, RBM38, SELENBP1, HBD
0030097; haemopoiesis	0.017825	ALAS2, EPB42, AHSP
0048534; haemopoietic or lymphoid organ development	0.021389	ALAS2, EPB42, AHSP
0002520; immune system development	0.023919	ALAS2, EPB42, AHSP
0050821; protein stabilisation	0.024566	AHSP, RBM38
0042592; homeostatic process	0.025726	ALAS2, EPB42, SNCA, SLC4A1
0055072; iron ion homeostasis	0.031483	ALAS2, EPB42
0031647; regulation of protein stability	0.036641	AHSP, RBM38
0030218; erythrocyte differentiation	0.037498	ALAS2, EPB42
0030863; cortical cytoskeleton	0.037694	EPB42, SLC4A1
0006873; cellular ion homeostasis	0.041895	ALAS2, SNCA, SLC4A1
0034101; erythrocyte homeostasis	0.042627	ALAS2, EPB42
0055082; cellular chemical homeostasis	0.043125	ALAS2, SNCA, SLC4A1
0019725; cellular homeostasis	0.062202	ALAS2, SNCA, SLC4A1
0044448; cell cortex part	0.063092	EPB42, SLC4A1
0008092; cytoskeletal protein binding	0.065602	MYL4, SNCA, SLC4A1
0030017; sarcomere	0.074104	MYL4, SLC4A1
0030099; myeloid cell differentiation	0.079478	ALAS2, EPB42
0030016; myofibril	0.083553	MYL4, SLC4A1
0005856; cytoskeleton	0.084634	MYL4, EPB42, SNCA, SLC4A1
0044449; contractile fibre part	0.084999	MYL4, SLC4A1
0048872; homeostasis of number of cells	0.085219	ALAS2, EPB42
0043292; contractile fibre	0.090763	MYL4, SLC4A1

When comparing all remitters (i.e. both early and delayed; N = 24) with non-remitters (N = 11), three genes had a higher expression in remitters versus non-remitters. These genes satisfied a fold change of greater than two and a *p*-value of less than 0.05. These genes included major histocompatibility complex, class II, DR β 5 (HLA-DRB5; fold change 6.53, $p = 0.02$), selenium binding protein 1 (SELENBP1; fold change 2.02, $p = 0.04$) and LOC388588 (fold change 2.39, $p = 0.03$). An additional gene ALAS2, satisfied a fold change of greater than two and a *p*-value of less than 0.1 (see table 6). In this analysis CA1 and SNCA were not significantly affected.

Table 8: Microarray genomic regulation differences between non-remitter and early remitter groups at baseline

Probe ID	Fold change (abs)	Regulation	Symbol	*p*-Value
6370315	11.29394	Down	HLA-DRB5	0.005371
1230376	2.878403	Down	ALAS2	0.040313
7550292	2.587224	Down	SELENBP1	0.019046
2190139	2.538225	Down	CA1	0.02866
2350274	2.511729	Down	AHSP	0.010137
10681	2.421306	Down	LOC100131164	0.023538
2120152	2.399837	Down	SLC4A1	0.017559
5690187	2.360582	Down	EPB42	0.007224
4150563	2.320725	Down	C16orf35	0.017368
6860347	2.301184	Down	FAM46C	0.025754
1450358	2.297963	Down	HBD	0.00744
1510612	2.236673	Down	MYL4	0.034842
5960564	2.180386	Down	SNCA	0.03586
7610615	2.164032	Down	SLC6A10P	0.024972
4180768	2.156951	Down	ALAS2	0.00684
3140750	2.079619	Down	RBM38	0.008701
3840400	2.036645	Down	GMPR	0.043985
3420372	2.005976	Down	RBM38	0.025779

3.4.7 Discussion

Our preliminary report is the first to suggest a unique transcriptional signature in elderly remitters and non-remitters to antidepressant treatment in the monoaminergic and metabolic pathways important for neuroplasticity and brain ageing.

We are not aware of any other study in LLD exploring gene expression biomarkers of antidepressant response at a genome-wide level [181], however a single study investigated a defined number of probesets predicting treatment response. Specifically, in a study of 77 adult outpatients, Mamdani et al. [180] reported differences in baseline peripheral gene expression between remitters and non-remitters in an 8-week CIT treatment trial. A total of 434 probesets displayed significant correlation to change in score (HAM-D 21) and 33 probesets were differentially expressed between eventual remitters and non-remitters. Probesets for SMA- and MAD-related protein 7 (SMAD 7) and sialic acid binding immunoglobulin-like lectin, pseudogene 3 (SIGLECP3) were the most significant differentially expressed genes following FDR correction, and both were down-regulated at baseline in individuals who responded to treatment. Our pilot study does not show significance for these gene expression markers.

In our study, it is important to understand the mechanisms and possible relevance of the genes related to early remission and standard remission. In early remission, the first gene found was CA1, carbonic anhydrase gene, located on chromosome 8. This gene is associated with reversible hydration of CO_2, respiratory function, water and ion transport and pH regulation. One or more of various genetically distinct isoforms is expressed nearly ubiquitously in most living cells [190]. Some isoforms are located in the cytosol, while others express their

activity at the extracellular membrane [190]. Due to the expression of multiple isoforms at different intra- and extracellular locations, it is difficult to discriminate the contribution of these isoforms [190]. Extracellular CA activity was found in hippocampal slices, where the enzyme was implicated in the regulation of excitatory transmission [191]. Functional activity was found again in rodent *in vitro* hippocampal neurons, where the anion exchanger AE3 activity was enhanced by enzymatic activity [192], and for H^+ buffering [193]. The DAVID analysis revealed ion and cellular homeostasis were significantly affected in the responder group, which is consistent with the above details about CA1. Taken together, the exact effect of CA in treatment response to antidepressants is not clear; however it may have a role in modulating excitatory transmission in the brain. The second gene we found correlated to early remission was SNCA-α-synuclein. The function of this gene has been implicated in Parkinson's disease and is associated with DA transporter function [194]. The α-synuclein protein is involved in deposition as insoluble fibrils, proteinase K resistant and is modified by truncation, phosphorylation, oxidation, nitrosylation and ubiquitination [194]. Growing evidence points towards modulation of synaptic plasticity of newborn neurons in olfaction by α-synuclein protein [195]. Similarly, in depression α-synuclein has been shown to decrease olfactory bulb neurogenesis [196-198], whereas treatment with antidepressants resulted in an increased neurogenesis in α-synuclein transgenic mice (expressing human α-synuclein under the oligodendrocyte-specific myelin basic protein promoter (MBP1-hαsyn tg mice)) [199, 200]. In these same transgenic mice, antidepressants might be of interest as anti-inflammatory and α-synuclein reducing agents for Multiple systems atrophy and other related α-synucleinopathies [201]. Multiple systems atrophy is a neurodegenerative disease

characterised by the pathological accumulation of α-synuclein within oligodendroglial cells [201]. This accumulation is accompanied by neuroinflammation, thereby leading to neuronal death [201]. Evidence from other rodent studies suggests a high vulnerability of rat dopaminergic synapses to conversion of transgenic human α-synuclein into insoluble neurotoxic conformers [194]. The DAVID analysis revealed cytosol and protein stabilisation as well as immune system development were significantly affected in the responder group, which is consistent with the above details on SNCA.

Three genes were involved in standard remission (HLA-DRB5, SELENBP1 and LOC388588), however their role in enhanced treatment response is largely unknown. HLA-DRB5 is associated with multiple sclerosis, Parkinson's disease, immunocompetence and histocompatibility [202-204]. We are not aware of any studies exploring this gene in depression. Intuitively, increased HLA-DRB5 expression in remitters suggests immune function is involved in the treatment response. SELENBP1 is involved with selenium metabolism [205] and we are not aware of any studies of this gene in depression. Selenium is implicated in neuroprotection, therefore modulation of SELENBP1 may impact selenium and therefore neuroprotective mechanisms [206]. The role of LOC388588 gene has not been explored in depression and neuroscience, and hence it is unclear what mechanistic role it has in enhancing treatment remission in LLD.

We chose not to correct the correlations between remitters and non-remitters and genomic data for multiple comparisons, as this is the first study of this kind exploring genome-wide data on treatment response in LLD. This study is intended as a hypothesis generating study where it is more important not to miss possibly important findings rather than to prematurely discard potentially useful

observations because of type 2 errors caused by corrections for multiplicity [207]. The statistical analyses used in this study did not control for age, gender or treatment group. This is a limitation which should be addressed in future research as these factors may influence results.

3.4.8 Conclusion

Despite the preliminary nature and small sample, our results are of interest suggesting a unique transcriptional signature of early remission and standard remission in antidepressant treatment with CIT and/or MPH in the monoaminergic and metabolic pathways important for neuroplasticity during brain ageing. Our identified biomarkers of response may be specific to our population and the study drugs, and hence these results will require future replications in a large sample.

3.4.9 Conflict of interest declaration

There are no conflicts of interest.

3.4.10 Acknowledgements

This paper has no acknowledgements.

4.0 The role of neuroimaging in understanding the pathophysiology of late-life depression

Important *in vivo* markers of both structural and functional neuroplasticity come from neuroimaging studies, and these are increasingly being considered for clinical applications [208]. Neuroimaging can allow for the characterisation of cerebral volumes, myelin integrity, WMHs and functional connectivity. These markers allow for some *in vivo* understanding of dysfunctional neuroplasticity processes which are seen in depression, such as reduced neurogenesis, as well as impaired synaptic plasticity and long-term potentiation [60].

LLD studies of neuroimaging are suggested to differ from mid-life depression in a number of ways, hence making the LLD-specific research field essential. For example, the DMN demonstrates less functional connectivity with age [209, 210]. WMH are common in LLD, but rare in mid-life depression [211]. A higher burden of WMHs is associated with greater limbic activation on emotional reactivity tasks [212]. The differences between LLD and mid-life depression may be explained by mechanistic hypotheses. Taylor et al. [12] outlines two key mechanistic constructs to be explored which are relevant to the above-mentioned vascular depression hypothesis: the *disconnection hypothesis* and the *hypoperfusion hypothesis*. The *disconnection hypothesis* by Alexopoulos et al. [213] suggests ischaemia and white matter pathology may disrupt neural connections among regions modulating mood and cognition. In this model, widespread cerebral WMHs may cause focal damage to tracts and circuits. Such focal damage could adversely affect the tract connectivity causing ‘disconnection’ of brain regions. This state is believed to adversely affect the

function of connected regions at rest and during cognitive tasks, and may contribute to circuitry alterations that mediate symptomatology in depression. The *hypoperfusion hypothesis* [12] is suggested given the common vascular dysfunction in LLD [214-218] and the cerebral blood flow reductions that can alter brain function and contribute to depression-related symptomatology. Regional cerebral metabolic activity is tightly correlated with cerebral blood flow, which is regulated by complex interactions between neurons, glia and vasculature [219]. In late-life, vascular disease disorders, such as hypertension, diabetes and atherosclerosis often lead to vascular wall hypertrophy, reduced arterial lumen diameter, arterial stiffness and endothelial cell dysfunction [220, 221].

4.1 Structural neuroimaging in the pathophysiology of late-life depression

A recent study [222] has sought to meta-analyse papers exploring grey matter abnormalities in LLD. In total, 17 regions of interest (ROI) studies were suitable for inclusion into this meta-analysis and included studies examining volumes of the whole brain, orbitofrontal cortex, caudate, hippocampus, putamen and thalamus. Healthy comparison groups were required for inclusion and an average age greater than 60 years. A significant but small ES was found for reductions in hippocampal volume in these studies (Hedges g = 0.31; 15 studies). The meta-analysis also revealed significant volume reductions in the orbitofrontal cortex (Hedges g = 0.42; four studies), putamen (Hedges g = 0.49; four studies) and thalamus (Hedges g = 0.59; three studies). No significant difference was found for whole brain or caudate. This therefore supports dysfunction within the frontal-subcortical and limbic circuitry.

4.2 Functional neuroimaging in the pathophysiology of late-life depression

The most research in functional neuroimaging of LLD surrounds the DMN, with the other commonly studied networks including the affective/frontolimbic network, the cognitive control network (CCN) and the corticostriatal network [223-229]. The DMN is a network of regions showing synchronised activity patterns when the brain is at rest, and connectivity is decreased when the mind is engaged on the external environment [230, 231]. The DMN includes areas in the medial prefrontal cortex (mPFC), the PCC, the precuneus and the medial temporal lobe (MTL) [232, 233]. Evidence suggests involvement of the DMN in self-referential processing, including internal monitoring, autobiographical memory retrieval, future planning, and theory of mind [233-235]. Dysfunction in the DMN may occur due to WMHs and hypoperfusion [223] and may represent an imbalance between control systems involved in negative rumination and preferential internal over external attention, possibly reflecting depressive biases toward internal thoughts at the cost of engagement in the external environment [236, 237]. Self-referential processing dysfunction may lead to negativity bias pronounced with depression [236, 237].

We are aware of three studies in LLD exploring the DMN, two via seed region analysis [226, 238] and one by independent components analysis (ICA) [239]. ICA is a useful methodology as it provides a data-driven approach to defining resting-state networks. The only ICA study in LLD of which we are aware comes from Sexton et al. [239], who explored a cross-sectional, multimodal neuroimaging approach to a mixture of patients with current LLD or past history of LLD. No significant differences in functional connectivity were detected between

the current or past LLD and age-matched healthy control groups in the DMN, anterior DMN, posterior DMN, CCN, or affective/frontolimibic network.

The CCN is a frontoparietal circuit comprising brain areas in the dorsolateral prefrontal cortex (DLPFC), the dorsal anterior cingulate cortex (dACC), and the posterior parietal cortex [240, 241]. The CCN is involved in top-down, attention-dependent executive tasks like decision making, cognitive inhibition, attention allocation, working memory, and task switching [242-244]. Dysfunction of the CCN is thought to be related to difficulty engaging in goal-directed behaviours while ignoring irrelevant, negatively valenced stimuli. Additionally, the CCN influences thought suppression.

The affective/frontolimbic network is a set of interconnected neural structures consisting of the amygdala, the subgenual anterior cingulate cortex (ACC), the hypothalamus, the olfactory cortex (OFC) and the nucleus accumbens (NAcc) [232, 245]. The main functions of affective/frontolimbic network are emotional processing and mediating motivated behaviours. This network is also regulating emotion–mood relationship to visceral functions [245, 246].

Corticostriatal circuits are parallel and segregated loop-shaped neural networks connecting regions in the frontal regions to basal ganglia to thalamus. It has been postulated that each loop mediates specific type of motor, executive control or emotional behaviour [247, 248].

4.3 Tract neuroimaging in the pathophysiology of late-life depression

MRI advancements, such as diffusion tensor imaging (DTI) have more recently been used to quantify subtle changes in White Matter (WM) tract

microstructure and connectivity in the senescent and developing adolescent brain. DTI capitalises on characterisation of water molecule diffusion characteristics as determined by measures, such as fractional anisotropy (FA), radial diffusivity, and axial diffusivity. Furthermore, the use of tractography and diffusion pattern statistical analyses is instrumental in allowing for the spatial definition of individual tracts.

A recent meta-analysis [249] was conducted on FA studies in LLD. In this analysis, nine research studies met inclusion criteria and underwent quantitative review. These studies explored FA in the DLPFC, corpus callosum, cingulum and uncinated fasciculus (UF). Compared with healthy controls, the LLD group showed lower FA in the DLPFC and UF with a large and medium ES respectively. Significant heterogeneity and publication bias were noted. This study again suggests damaged regions in the frontostriatal and limbic networks.

4.4 Molecular neuroimaging in the pathophysiology of late-life depression

As outlined previously, neurotoxicity due to amyloid and tau protein aggregation may represent a pathophysiological cascade which, along with vascular compromise, may predispose individuals to LLD. The 2011 paper by Kumar et al. [13] explored amyloid and tau binding, as per [^{18}F]FDDNP levels, compared between 20 LLD patients and 19 healthy controls. When compared with controls, [^{18}F]FDDNP binding was significantly higher overall and in the PCC and lateral temporal regions in the MDD group. A recent systematic review by Harrington et al. [41] aimed to examine the relationship between Aβ, a key biomarker of AD, and depression in older adults. Studies were also required to include an outcome variable that was a direct measure of Aβ levels in either blood

or CSF samples, or via neuroimaging techniques, such as PET. Nineteen studies were identified, 15 of which found significant differences in Aβ levels between depressed and non-depressed older adults. Five studies used PET neuroimaging as a primary outcome measure, with three observing statistically significant relationships between neuroimaging results and depression status. Of the statistically significant studies, two used [^{18}F]FDDNP binding and one used [^{18}F]florbetapir. The non-significant studies used the Pittsburgh Compound B (PiB) binding compound. Therefore, variations in results may be due to differences in these compounds.

4.5 Altered resting-state functional connectivity in late-life depression: a cross-sectional study

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4.5.1 Statement of authorship

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Overall percentage	30%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 3/1/16

Co-author contribution

By signing the Statement of Authorship, each author certifies that:

I. The candidate's stated contribution to the publication is accurate (as detailed above);

II. Permission is granted for the candidate in include the publication in the thesis; and

III. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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4.5.2 Abstract

Background: Disrupted brain connectivity is implicated in the pathophysiology of LLD. There are few studies in this area using rs-fMRI. In this pilot case-control study, we compare rs-fMRI data between age-matched depressed and non-depressed older adults.

Methods: Older participants (≥ 55 years) with current MDD were recruited to participate in an ongoing study of LLD, and were compared to the age-matched, non-depressed controls. Rs-fMRI data were collected using a 3-Tesla MRI system. In this study, a data-driven approach was chosen and an ICA was performed.

Results: Seventeen subjects with MDD were compared to 31 controls. The depressed group showed increased connectivity in three main networks compared to the controls ($p < 0.05$, corrected), including connectivity between the DMN and the posterior superior temporal sulcus (pSTS). Increased connectivity was also observed within the visual network in the medial, lateral and ventral regions of the occipital lobes, and within the auditory network throughout the right superior temporal cortex.

Conclusion: This data-driven pilot study finds patterns of increased connectivity that may be unique to LLD in the DMN, as well as visual and auditory networks. The functional implications of this aberrant connectivity remain to be determined. These findings should be further explored in larger samples.

4.5.3 Aim and rationale

Our study is the first to apply the ICA methodology in older adults with a current major depressive episode. We used a cross-sectional analysis of high-resolution rs-fMRI data. We hypothesised that LLD would be associated with aberrant connectivity within the DMN, therefore, this network was targeted in our primary analysis. We also performed exploratory analyses of LLD-related differences in functional connectivity in other resting-state networks to determine whether LLD is associated with broader dysfunction.

4.5.4 Methods

Participants

From November 2013 to December 2014, we recruited 17 older adults (≥ 55 years) to participate in the ongoing study of geriatric depression (NCT01902004), and 31 non-depressed age-matched controls. After describing the details of the study to interested and eligible subjects, written informed consent was obtained in accordance with the procedures set by the UCLA IRB.

Depressed subjects

Inclusion criteria were: (a) current episode of unipolar MDD according to DSM-5 criteria; (b) HDRS-24 score ≥ 16; (c) MMSE score ≥ 24; and (d) subjective memory complaints. Exclusion criteria were: (a) history of any other psychiatric disorders (other than unipolar MDD); (b) severe or acute unstable medical illness; (c) acute suicidal, violent behaviour or history of suicide attempt within the last year; or (d) any other CNS diseases. Subjects were free of psychotropic medications for at least 2 weeks before participating in the study.

Non-depressed subjects

Inclusion criteria were: (a) MMSE score ≥ 24; (b) subjective memory complaints; (c) no current, or history of, depression. Exclusion criteria were: (a) history of any psychiatric disorders or dementia; (b) severe or acute unstable medical illness; (c) any other CNS diseases; and (d) no psychotropic medications use.

Clinical measures

Mood evaluation included the HDRS-24; [182], the Hamilton Anxiety Scale (HAS or HAM-A; [250]). Health functioning, medical and vascular comorbidity were collected using the Stroke Risk Factor Prediction Chart [251] and the CIRS-G [187]. Stress coping/resilience was measured by the Connor-Davidson Resilience Scale (CD-RISC) [252].

Image acquisition

Functional resting imaging data were collected with a 3T TIM Trio scanner (Siemens AG, Munich & Berlin, Germany). Participants' heads were positioned comfortably within a 32-channel head coil, and head motion was minimised with firm cushions. We instructed participants to close eyes and stay awake during image acquisition. Resting-state functional images were acquired for 5 minutes and 41 seconds with a multi-band gradient-echo echo-planar imaging (EPI) sequence sensitive to BOLD contrast effects. We acquired 275 contiguous EPI resting-state volumes, and the parameters for functional imaging were repetition time 1.24 seconds, echo time 38.2 milliseconds, flip angle 65°, field of view 21.2 × 21.2 cm^2, acquisition matrix 118 × 118, 1.8 mm^3 iso-voxel size (no gap), 78 slices, and six

bands. We also acquired anatomic images with 3D MPRAGE sequence (acquisition matrix 256 × 256 with 1 mm thick contiguous slices) for co-registration with the functional data.

Image analysis

The rs-fMRI images were pre-processed in FMRIB Software Library (FSL, (www.fmrib.ox.ac.uk/fsl)) for motion correction, high-pass filter (0.01 Hz), image normalisation and 5 mm^3 Gaussian spatial smoothing. Multivariate exploratory linear decomposition into independent components (MELODIC, a tool of FSL) was used to remove significant head motion, scanner, and physiological artefacts using ICA. The processed functional data from all participants were temporally concatenated to form a 4D data set, which was decomposed into group level individual components using ICA. The MELODIC automated dimensionality estimate was used to determine the number and order of the individual components [253]. Each component includes brain structures that share the same temporal pattern of signal after mixture modelling was applied. The dual regression approach was subsequently used to back-reconstruct individual-specific connectivity maps associated with each group level component, which been shown to be an effective and reliable approach to analyses of rs-fMRI data [254]. This approach yielded 36 ICs; 26 of these overlapped grey matter and were considered biologically plausible. For each of these 26 individual components, we compared functional connectivity between the depression and control groups in analyses restricted (i.e. masked) to voxels having 0.5 or higher probability of inclusion in each group level individual component image [253]. One-tailed tests compared depression and control groups in each individual component at a single-voxel threshold of $z > 1.64$, $p < 0.05$, with age and sex serving as nuisance covariates. Although Bonferroni correction is

sometimes suggested to address the inclusion of two one-tailed tests (i.e. depression > controls, depression < controls) as executed per standard procedures in FSL, we chose a more lenient single-voxel threshold ($p < 0.05$) for these exploratory analyses *a priori*, with correction for cluster extent to address the multiple comparisons problem using Random Field Theory at $p < 0.05$, corrected [255].

Statistical analysis of demographic and clinical data

In addition to the above-mentioned imaging analyses, a statistical analysis was performed on the demographic and clinical data obtained from the depressed and non-depressed groups. Data were checked for outliers and normality assumptions. The two study groups were compared on demographic characteristics using T-tests for continuous variables and χ^2-tests for categorical variables. Significance levels were set at 0.05 for demographic, medical and neuropsychological data. Correction for multiple comparisons were not performed as this was an exploratory study [256].

4.5.5 Results

Baseline characteristics

Seventeen depressed older adult and 31 non-depressed older adult participants were included in this analysis. Clinical and demographic characteristics of the sample are presented in table 9. Compared to non-depressed comparators, the depressed group had greater depressive and lower resilience scores.

Table 9: Demographic and clinic characteristics for depression and healthy control subjects

		Non-depressed group (N = 31)	Depressed group (N = 17)	Analysis
Variables		Mean (SD)	Mean (SD)	T-test
Age (years)		67.48 (8.87)	67.28 (6.64)	$T(46) = -0.09$, $p = 0.93$
Education (years)		16.23 (1.56)	16.33 (2.20)	$T(46) = 0.20$, $p = 0.84$
HAM-D		3.32 (3.51)	17.89 (2.89)	$T(46) = 14.91$, $p < 0.01$*
CVRF		7.71 (4.55)	7.50 (3.60)	$T(46) = -0.17$, $p = 0.87$
CIRS total		2.03 (2.07)	2.78 (2.42)	$T(46) = 1.14$, $p = 0.26$
MMSE		28.84 (1.13)	28.28 (1.64)	$T(46) = -1.42$, $p = 0.16$
CD-RISC		75.26 (11.44)	59.11 (16.27)	$T(46) = -4.07$, $p < 0.01$*
		N (%)	N (%)	χ^2 (p)
Sex	Male	14 (45)	5 (29)	
	Female	17 (55)	12 (71)	χ^2 (1) = 0.66, $p = 0.55$
Race	White	23 (74)	13 (76)	
	Other	8 (26)	4 (24)	χ^2 (4) = 2.22, $p = 0.70$
Handedness	Right	25 (81)	14 (82)	
	Left	6 (19)	3 (18)	χ^2 (1) = 0.002, $p = 1.00$

CD-RISC, Connor-Davison Resilience Scale 25; CIRS, Cumulative Illness Rating Scale; CVRF, cardiovascular risk factors; HAM-D, Hamilton Depression Rating Scale; MMSE, Mini-Mental State Examination; SD, standard deviation.
*$p \leq 0.05$.

MRI results

Primary analysis

Default Mode Network

The right pSTS showed increased connectivity with the DMN in depressed versus non-depressed. The DMN recruited the following brain regions: medial and superior frontal gyrus, extending to middle and inferior frontal gyrus, angular gyrus spreading to middle temporal gyrus, and precuneus cortex, right angular gyrus and middle temporal gyrus. See figure 5 for a graphical illustration of these findings.

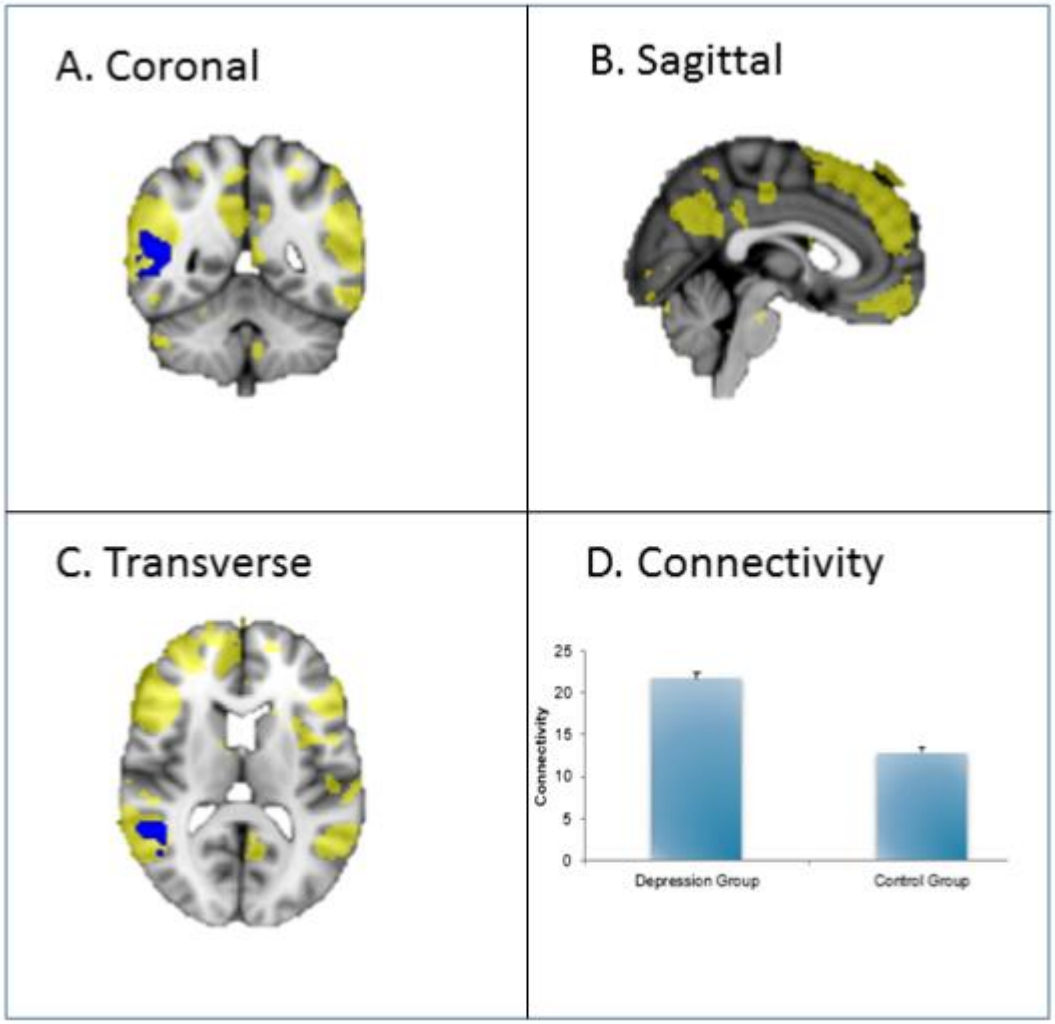

Figure 5: Default Mode Network engagement in a cross-sectional study of late-life depression as compared to control

Legend: This figure shows increased connectivity in the DMN in depression versus control groups ($p < 0.05$, p-corrected). The blue regions indicate higher connectivity in depression versus control group. The yellow region demonstrates the recruited areas of the DMN. See A, B and C. The bar graph (D) indicates mean connectivity for depression and control groups.

Exploratory analyses of resting-state networks

Of the 25 remaining resting-state networks analysed, four networks demonstrated significant differences between groups, including three visual networks overlapping occipital cortex and one auditory network overlapping superior temporal cortex. These findings are discussed below.

Visual networks

Three visual resting-state networks showed significant differences in functional connectivity between depressed and non-depressed subjects, including medial, lateral and ventral visual networks. Within the medial visual network, which overlapped with early visual/occipital cortex, diffuse clusters of increased connectivity were found (see figure 5, column A). The second lateral network primarily overlapped posterior and ventral occipital cortex and inferior temporal cortex, and a cluster in the left occipital fusiform gyrus showed increased connectivity with this network (see figure 5, column B). The third network showed increased connectivity diffusely throughout the bilateral occipital cortex and bilateral lingual gyrus. Other areas recruited were in the intracalcarine cortex (see figure 6, column C).

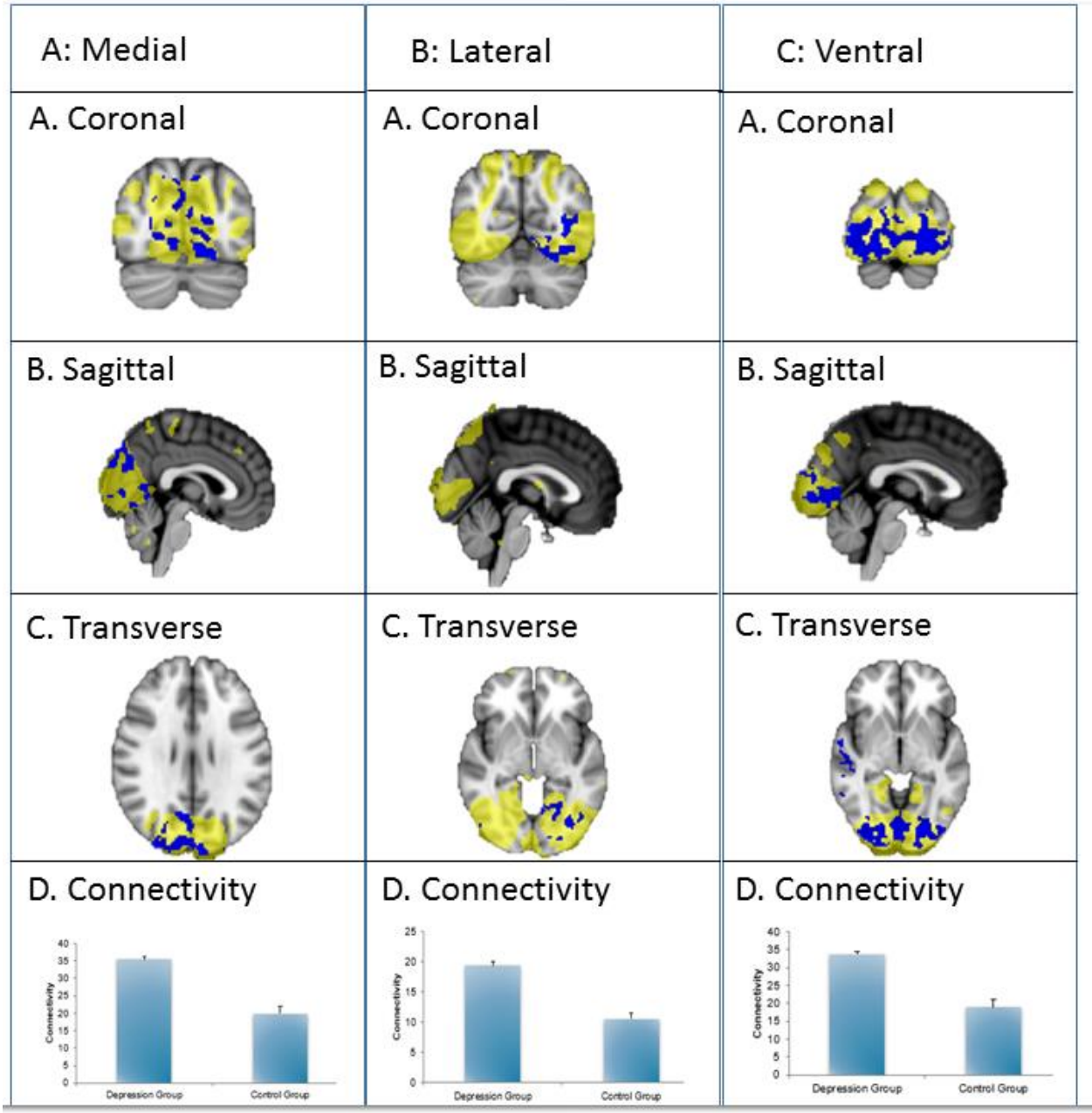

Figure 6: Visual network engagement in a cross-sectional study of late-life depression as compared to control

Legend: This figure shows increased connectivity in the visual networks in depression versus control groups ($p < 0.05$, corrected). The blue regions indicate higher connectivity in depression versus control group. The yellow region demonstrates the recruited areas. See components A, B and C in each column. The bar graph (D) indicates mean connectivity for depression and control groups.

Auditory network

An auditory resting-state network was found engaging bilateral superior temporal cortex. Additionally, multiple clusters of increased connectivity in depressed versus non-depressed groups were found throughout the right middle and superior temporal gyrus, extending to the right temple pole (see figure 6).

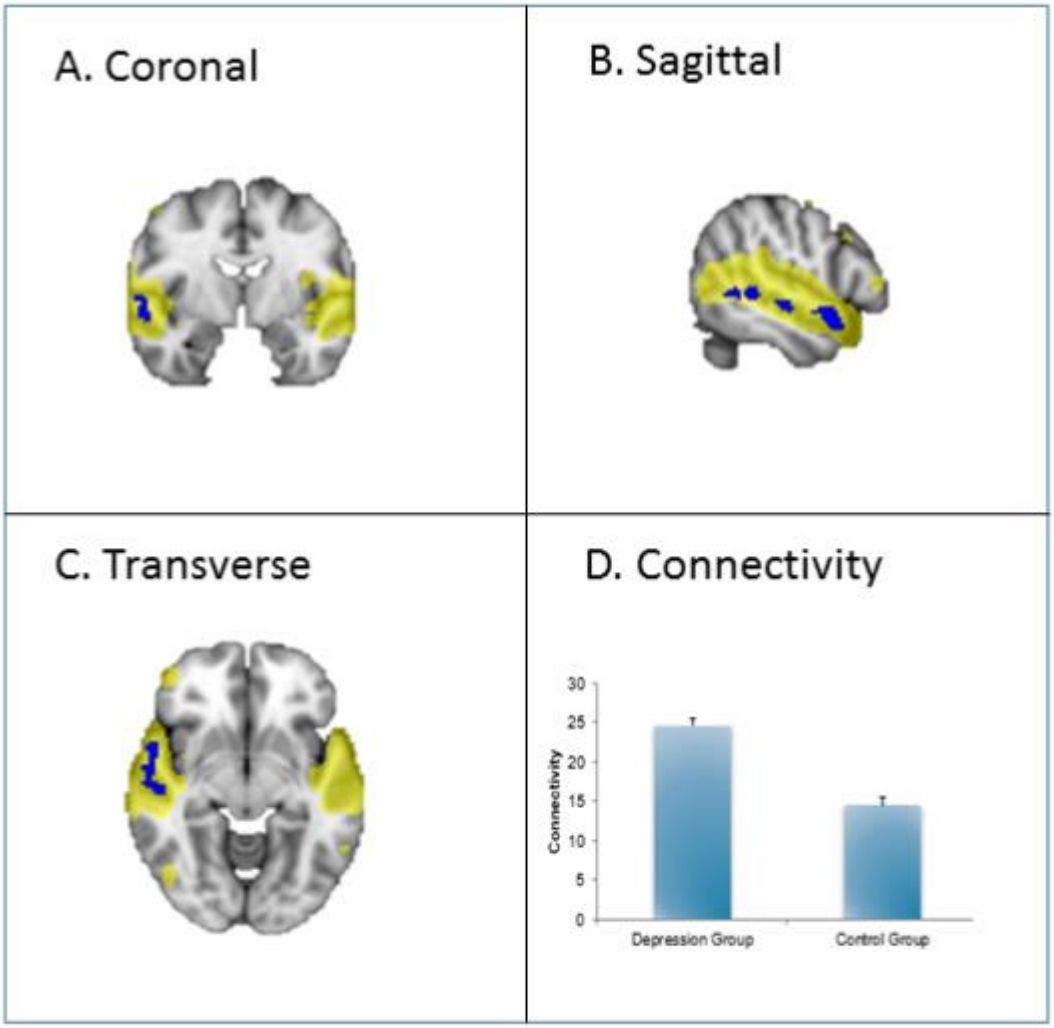

Figure 7: Auditory network engagement in a cross-sectional study of late-life depression as compared to control

Legend: This figure shows increased connectivity in the auditory network in depression versus control groups ($p < 0.05$, corrected). The blue regions indicate higher connectivity in depression versus control group. The yellow region demonstrates the recruited areas. See A, B and C. The bar graph (D) indicates mean connectivity for depression and control groups.

Superior parietal network

Increased connectivity in this network was found bilaterally throughout the superior divisions of the precuneus cortex in the depressed versus non-depressed groups. Recruitment of the network was also found in the superior divisions of the bilateral occipital cortex (see figure 7).

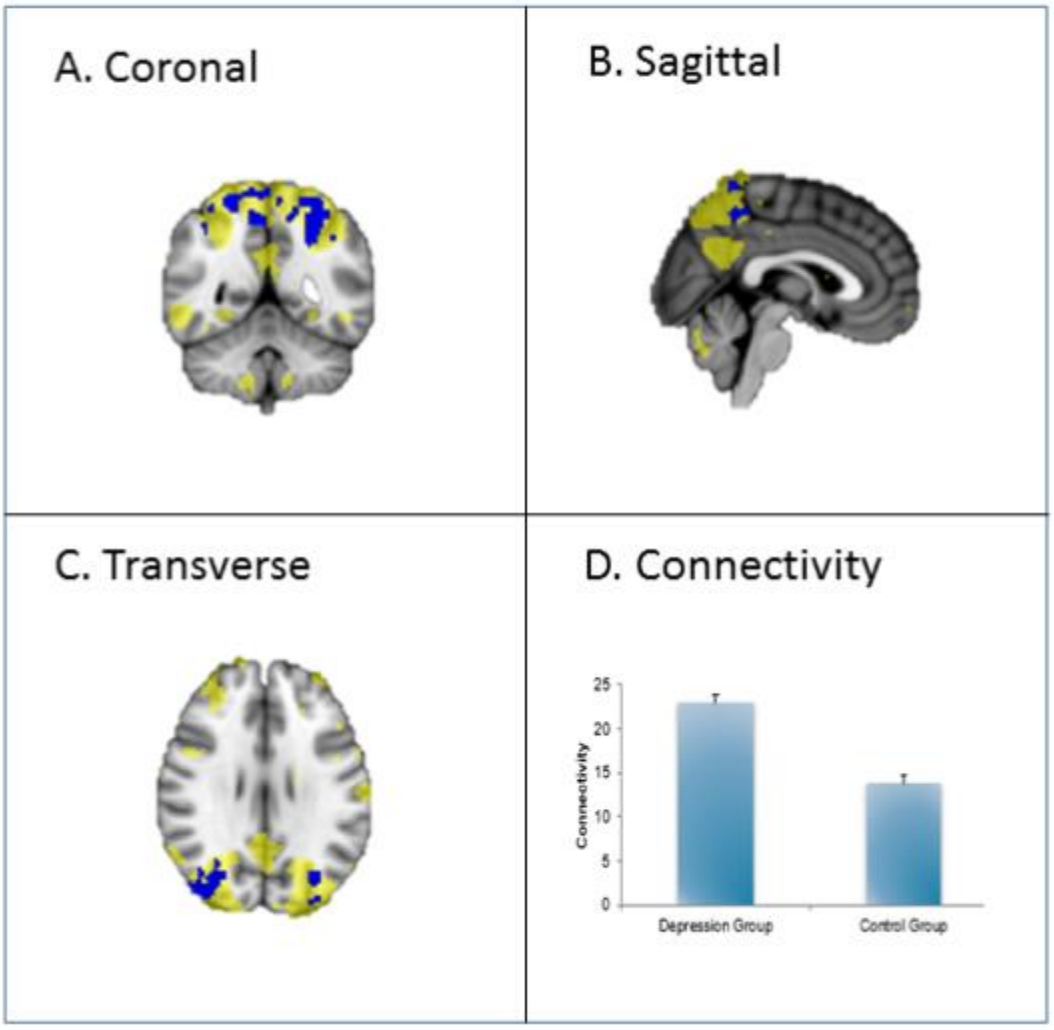

Figure 8: Superior parietal and occipital network engagement in a cross-sectional study of late-life depression as compared to control

Legend: This figure shows increased connectivity in the superior temporal and occipital network in depression versus control groups ($p < 0.05$, corrected). The blue regions indicate higher connectivity in depression versus control group. The yellow region demonstrates the recruited areas. See A, B and C. The bar graph (D) indicates the connectivity for depression and control groups.

Post hoc analyses

No significant differences were found between HAM-D scores and functional connectivity in the above-mentioned brain regions. Further, resilience was not found to significantly correlate with functional connectivity in these brain regions either. Finally, correlations between network connectivity and illness duration in the depressed subjects were not significant.

4.5.6 Discussion

Few studies explore resting-state functional connectivity in LLD populations. Understanding this area is important given the rising burden of disease, the need for more precise diagnostic systems and poor treatment response in LLD. In this study we found increased connectivity in multiple resting-state networks in subjects with LLD as compared with non-depressed older adults – the DMN, as well as visual and auditory networks.

Our study is unique in utilising an ICA analysis of rs-fMRI data and finding aberrant DMN connectivity in an LLD population. Findings in the DMN show increased connectivity within the right pSTS. Previous research associated the pSTS with an array of tasks relevant to social perception including facial processing, motion processing, the integration of audio and visual information, as well as theory of mind (i.e. deciphering the beliefs and perspectives of others) [257]. These types of socially related functions are characteristically impaired in depression [258]. To our knowledge, there are no structural or functional neuroimaging studies exploring the pSTS in either mid-life or LLD, making our results novel. Our results showing hyperconnectivity in the pSTS most likely represent dysfunctional social functioning, however this remains to be tested

empirically. The rs-fMRI component of the multimodal Sexton et al. [239] study, the only study with which we can truly compare our data, utilised data-driven ICA analysis in 36 mixed recovered or current LLD participants with low severity of depressive symptoms compared to 25 age-matched controls. They did not find any functional connectivity differences between groups. The lack of findings of connectivity from this study may be due to relatively low depression severity. The mean HAM-D score in the mixed recovered versus current LLD group was 4.19 (SD 4.77). Our depressed group, by comparison had mean HAM-D scores of 17.89 (2.89). Another study explored the DMN using seed-based approaches to rs-fMRI analysis. Although seed-based versus ICA rs-fMRI analyses are difficult to compare directly, some similarities between these studies emerge. The other study by Alexopoulos et al. [226] explored rs-fMRI DMN data in LLD. In this study they engaged 26 non-MCI older adults, 16 with MDD (mean age 69 ± 5.5) and 10 with no MDD (mean age 68.6 ± 7.0). DMN activity was assessed from a seed placed in the PCC. Hyperconnectivity in the DMN, specifically the left precuneus/medial parietal region, subgenual ACC and lateral parietal regions, distinguished depressed from non-depressed subjects. This again has significant overlap with our study results, suggesting compatible results. In our exploratory analyses, we demonstrated increased connectivity between in the medial and lateral parietal cortices and the superior parietal regions. In the Alexopoulos et al. [226] study, hyperconnectivity within the DMN was positively associated with pessimism. This suggests the DMN may be a useful target which underlies characteristics, like pessimism, which can perpetuate depression and reduce treatment response.

Aberrant connectivity within sensory networks was a prominent feature of this depressed cohort in exploratory analyses, and this finding is novel given few

studies examine sensory networks in depression. Three separate components were found in the visual network region, whereby increased connectivity was seen diffusely throughout the occipital lobes. We believe this visual network hyperconnectivity should be considered in terms of functional and neuropathological underpinnings. From a neuropathological perspective, there are a number of hypotheses for this connectivity dysfunction. The occipital lobes have been implicated on a physiological level whereby those with MDD have reduced muscarinic acetylcholine receptor (M)2 receptor binding in this region [259], reduced FA (marker of white matter integrity) [260] and reduced magnetisation transfer ratios [261]. A recent study by Maller et al. [262] explored the phenomenon of occipital bending in adults with MDD (51 depressed, 48 health controls). This is the first study in adult depression and has not been explored in LLD. Occipital bending is a phenomenon of occipital lobe asymmetry within psychiatric populations. This study found the prevalence of occipital bending is three times higher among depressed adults than non-depressed, though its effects on brain function are unclear and may not be restricted to occipital cortex. It is believed incomplete neural pruning may lead to restricted cranial space for brain growth, or ventricular enlargement may exacerbate natural occipital curvature [262]. From a functional perspective, visual processing deficits may be related to our hyperconnectivity findings and are recognised as a component of LLD [263]. Visual search may be impaired in LLD. It is an established model for studying how manipulation of task attributes affects speed of performance [264]. In a study by Potter et al. [263], visual search performance was compared in 32 LLD and 32 control participants. Data in this study showed specific slowing in the comparison stage of visual search in LLD, rather than in the encoding/response stages. They

also found greater overall slowing in LLD during inefficient versus efficient search. There were no group differences on traditional neuropsychological measures of processing speed. This study therefore highlights the importance of specific analysis of processing speed, and that of the visual network. No study has explored fMRI or rs-fMRI correlations to visual search performance in an LLD sample. Our study also found hyperconnectivity in the auditory networks. There are very few studies conducted on the auditory network or auditory processing in LLD, none using neuroimaging. One study found deficits in pre-attentive auditory processing, specifically mismatch negativity, in LLD, measured using event-related potentials, were associated with poorer semantic fluency and high levels of functional disability [265]. This impairment was localised to the temporal and not frontocentral area. This is likely relevant to the findings of our study, whereby hyperconnectivity may be related to impaired auditory processing.

It may be possible to incorporating our findings together from DMN, auditory and visual networks. Our speculation is auditory and visual processing dysfunction noted in LLD, and via functional hyperconnectivity, is related to increased connectivity between DMN and the pSTS, perhaps causing impaired facial processing, motion processing, and integration of audio and visual information.

Our study did not find any involvement of other major resting-state networks, such as the salience, cognitive control and corticostriatal networks. This is likely due to differences in samples and resting-state analytical techniques between studies. As mentioned previously, ours is the first study exploring LLD versus non-depressed subjects with the ICA technique, therefore there are no

comparisons to our study to date. When considering the salience network, Yuen et al. investigated resting-state connectivity in LLD with right anterior insula seed region [228], and found that LLD subjects (N = 16) exhibited increased connectivity in the bilateral precuenus compared with normal controls (N = 10). In our data, depressed subjects also showed higher connectivity relative to the healthy controls in the bilateral precuneus for salience network, but this effect did not survive cluster-level correction.

This study has a number of limitations. Our study involved a cross-sectional comparison of two relatively small groups that prevents identification of causation, hence future longitudinal assessments are key. Our use of ICA analysis may be seen as a limitation. The data-driven approach using ICA analysis is useful to understanding patterns in the data without a pre-existing model hypothesised. This is as opposed to the model-driven and/or seed region approach which can also be used whereby the analysis is constrained to brain ROIs or hypothesis. Our study did not correct for multiple comparisons which is therefore an area to improve on in future studies.

4.5.7 Conclusion

This pilot study finds increased connectivity in the DMN, visual and auditory networks in LLD versus non-depressed older adults. Our findings of hyperconnectivity in the DMN, pSTS, visual and auditory networks may suggest dysfunction. Aberrant connectivity within the DMN and pSTS may suggest social functioning and self-referential processing impairment. We speculate pSTS hyperconnectivity may represent dysfunction of integration of visual and auditory inputs, leading to visual and auditory impairments. We recommend further large

scale research to understand the neuropsychological correlation to these findings, the relevance of WMHs and occipital bending.

4.5.8 Conflict of interest declaration

There are no conflicts of interest.

4.5.9 Acknowledgements

This paper has no acknowledgements.

4.6 Neural correlates of apathy in late-life depression: a pilot [^{18}F]FDDNP PET study

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4.6.1 Statement of authorship

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Contribution to the paper:	Co-designed research; co-analysed data; primarily wrote the paper.
Overall percentage	35% contribution.
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 4/2/16

Co-author contribution

By signing the Statement of Authorship, each author certifies that:

I. The candidate's stated contribution to the publication is accurate (as detailed above);

II. Permission is granted for the candidate in include the publication in the thesis; and

III. The sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of co-author:	Dr Prabha Siddarth
Contribution to the paper:	Co-designed research; co-analysed data; co-wrote the paper. 15% contribution.
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Contribution to the paper:	Co-designed research; co-analysed data; co-wrote the paper. 5% contribution.
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4.6.2 Abstract

Background: Neurotoxicity associated with amyloid and tau protein aggregation may represent a pathophysiological cascade that, along with vascular compromise, may predispose individuals to late-life depression (LLD). In LLD, apathy is common and leads to worsening of functioning and poorer to antidepressant treatment. Better understanding of the pathophysiological mechanisms of apathy in LLD would facilitate development of more effective diagnostic and treatment approaches. In this cross-sectional pilot study, we performed positron emission tomography (PET) scans after injection of 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)-amino]-2-naphthyl}ethylidene) malononitrile ([^{18}F]FDDNP), an in vivo amyloid and tau neuroimaging, in patients with LLD to explore neural correlates of apathy.

Methods: Sixteen depressed elderly volunteers received clinical assessments and [^{18}F]FDDNP PET scans. The cross-sectional relationship of [^{18}F]FDDNP binding levels with depression (Hamilton Depression Rating Scale (HAM-D)) and apathy (Apathy Evaluation Scale (AES)) were studied using Spearman correlation analyses, due to the relatively small sample size. Age, sex and years of education were partialed out. Significance levels were set at $p \leq 0.05$.

Results: [^{18}F]FDDNP binding in the anterior cingulate cortex (ACC) was negatively associated with the AES total ($r = -0.62$, $p = 0.02$; where low AES score equals greater severity of apathy) – suggesting apathy in LLD is associated with higher amyloid and/or tau levels in the ACC. None of the regional [^{18}F]FDDNP binding levels were significantly associated with HAMD total.

Conclusion: This pilot study suggests that increased apathy in subjects with LLD may be associated with greater amyloid and/or tau burden in certain brain regions.

Future studies in larger samples would elucidate the generalizability of these results, which eventually could lead to improved diagnostic and treatment methods in LLD.

4.6.3 Introduction

Amyloid and tau are key neuropathological hallmarks in the development of Alzheimer's disease (AD) [66, 67], however they are increasingly studied in LLD. Our group has developed and used the 2-(1-{6-[(2-[F18]fluo-roethyl)(methyl)amino]-2-naphthyl}ethylidene)malo-nonitrile ([^{18}F]FDDNP) *in vivo* probe that binds to cerebral aggregates of amyloid-beta plaques and tau neurofibrillary tangles [68]. The [^{18}F]FDDNP biomarker has been found to differentiate individuals with mild cognitive impairment (MCI), AD, and normal cognition, wherein global [^{18}F]FDDNP binding is highest in patients with AD, intermediate in those with MCI, and lowest in normal comparison subjects [68]. Our group is the first to explore this probe in LLD. In a 2011 paper by Kumar et al [13], [^{18}F]FDDNP levels were compared between 20 LLD patients and 19 healthy controls. When compared with controls, [^{18}F]FDDNP binding was significantly higher overall and in the posterior cingulate cortex (PCC) and lateral temporal regions in the MDD group. The authors suggested neurotoxicity due amyloid and tau protein aggregation may represent a pathophysiological cascade which, along with vascular compromise, may predispose individuals to LLD.

Apathy is a common feature of LLD afflicting more than 30% of individuals with LLD [266]. It is defined as a primary motivational impairment resulting in diminished goal-oriented behavior, lack of intellectual interest and flattening of affect [267]. Clinically this leads to poor engagement in treatment, greater disability of functioning [266], a greater burden on caregivers and an increased risk for

functional and possibly cognitive impairment [268, 269]. Understanding biomarkers underpinning the comorbidity of LLD and apathy and therefore important for improving treatment outcomes.

We are aware of two studies examining the neural underpinnings of apathy in LLD subjects with any modality of neuroimaging. The first study [270] comes from our group where 43 patients with MDD and 41 normal comparison subjects were examined with structural magnetic resonance imaging (MRI). Higher degree of apathy was associated with decreased gray matter volumes in the right anterior cingulate cortex (ACC). The ACC is suggested to be a functional intersection of emotion, cognition, drive and motor control, hence highly relevant to apathy. These findings in the ACC can be contextualized to a fronto-limbic network dysfunction which is often noted in LLD. The affective/fronto-limbic network is a set of interconnected neural structures with the main functions of emotional processing and modulating motivated behaviors, as well as regulating emotion–mood relationship to visceral functions [245, 246]. The second study has been conducted by Yuen et al [271] where structural and diffusion tensor imaging (DTI) of the ACC and associated white matter tracts was performed on 45 non-demented elderly subjects with MDD and 43 elderly, psychiatrically healthy comparison individuals. There were no significant differences in white matter fractional anisotropy (FA) between controls, non-apathetic depressed and apathetic depressed. Bilateral dorsal and rostral ACC volumes distinguished controls from apathetic depressed and controls from non-apathetic depressed. There were no other significant differences in ACC volumes from this three-group comparison. While these studies are informative on structural brain changes related to apathy, they do not add to the understanding of the highly relevant amyloid and tau-related pathology relevant to

LLD. We are not aware of any study exploring the relationship between of apathy in LLD and tau and amyloid biomarkers using PET imaging.

4.6.4 Aim and rationale

This study aims to explore the neural correlates of apathy with amyloid and tau PET imaging in a cohort with LLD. We hypothesize that greater severity of apathy will correlate with greater [^{18}F]FDDNP binding in the ACC region based on our previous reports with other imaging modalities [270, 272].

4.6.5 Methods

From December 2013 to December 2014, we recruited 16 older adults (age 55 and older) to participate in the ongoing study of geriatric depression (NCT01902004). After describing the details of the study to interested and eligible subjects, written informed consent was obtained in accordance with the procedures set by the UCLA Institutional Review Board (IRB).

Participants

Inclusion criteria were: 1) current episode of unipolar MDD according to Diagnostic and Statistical Manual (DSM-5) criteria; 2) Hamilton Depression Rating Scale (HAMD-24) score ≥ 16; 3) Mini-Mental State Exam (MMSE) score ≥ 24. Exclusion criteria were: 1) history of any other psychiatric disorders (other than unipolar MDD with or without comorbid anxiety symptoms); 2) severe or acute unstable medical illness; 3) acute suicidal or violent behavior or history of suicide attempt within the last year; or 4) any other central nervous system diseases. Subjects were free of psychotropic medications for at least two weeks before participating in the study.

Mood and apathy measures

Mood evaluation included the Hamilton Rating Scale of Depression 24-item rating scale (HAM-D-24; [182]). Apathy was measured by the self-rated Apathy Evaluation Scale (AES; score range 18-72 [273]); lower AES scores correlate to greater apathy. The AES also measures behavioural subcomponents of apathy including cognitive (i.e., level of goal—directed cognition), emotional (i.e., level of emotional responsivity), behavioral (i.e., goal-directed motor behavior), and other domains (i.e., combined insight and motivation). The AES is a

psychometrically validated instrument in older normal individuals and psychiatric patients {Clarke, 2007 #55}{Marin, 1991 #56}. MMSE was also used to assess cognitive impairment {Folstein, 1975 #57}.

PET neuroimaging methods

The radio-fluorinated imaging probe [^{18}F]FDDNP was prepared at high specific activities (>37 GBq/μmol), as described elsewhere [274]. All brain scans were performed at the University of California, Los Angeles, Ahmanson Biological Imaging Center with the EXACT HR+ tomograph (Siemens Medical Solutions, Inc, Munich, Germany; and CTI Molecular Imaging Inc, Knoxville, Tennessee), with individuals in the supine position and the imaging plane placed parallel to the orbito-meatal line. After the injection of the positron emission tomographic tracer (320–410 MBq) as a bolus via the indwelling venous catheter, the consecutive dynamic scans via PET were performed for as long as 2 hours. All scans via PET were decay corrected and reconstructed using filtered back-projection (Hann filter, 5.5-mm full width at half maximum) with scatter correction and measured attenuation correction. The resulting images contained 63 contiguous sections with a plane-to-plane separation of 2.42 mm.

Image data were analyzed and regions of interest (ROIs) determined, with investigators masked to clinical findings. Quantification of the data regarding [^{18}F]FDDNP binding was performed with the Logan graphic method, with the cerebellum as the reference region for time points between 30 and 125 minutes [275, 276]. Similar results were obtained when analyses were performed in intervals of between 30 and 60 minutes. The slope of the linear portion of the Logan plot is the relative distribution volume (DVR), which is equal to the distribution volume of the tracer in an ROI divided by the distribution volume of the tracer in

the reference region. Early frame [^{18}F]FDDNP images via PET (sum of 0–5 minutes) were oriented in anterior commissure–posterior commissure orientation by rigid co-registration with the SPM2 software package (The MathWorks, Inc, Natick, Massachusetts) to the template for PET provided in the package. The parameters determined in this step were used to orient the [^{18}F]FDDNP DVR images in the same, co-registered orientation.

A set of ROIs was drawn bilaterally on the frontal, PA, PC, anterior cingulate, mesial temporal, and lateral temporal lobe areas and the cerebellum on each co-registered early frame [^{18}F]FDDNP image via PET separately using the ROI set outlined previously [13]. The resulting ROI sets were imported in their corresponding [^{18}F]FDDNP DVR images and DVR values were extracted. Drawing of ROIs and extraction of DVR values were performed using the AMIDE Medical Image Data Examiner software package [275]. Each regional DVR or binding value was expressed as the mean of the left and right regions, and global DVR values were calculated as means of the values for all these regions. Rules for ROI drawing were based on the identification of gyral and sulcal landmarks with respect to the atlas of Talairach and Tournoux [277].

Brain MRI was obtained for co-registration were obtained for all study participants using a 3T scanner (Siemens Medical Solutions, Inc),. For each of these individuals, coronal sections that were 1.6-mm thick were obtained (repetition time, 20 milliseconds; echo time, 6 milliseconds; field of vision, 22 cm; 256×256 matrix; number of excitations, 1.5; and flip angle, 45°). Axial sections 3-mm thick also were obtained (repetition time, 4000 milliseconds; echo time, 14/112 milliseconds; field of vision, 24 cm; 256×256 matrix; and number of excitations, 1). All MRI results were examined for space-occupying and other focal lesions, including

stroke. Patients described in this study were free of overt neuroanatomical abnormalities.

The [^{18}F]FDDNP DVR parametric images of 16 patients with MDD with available T1-weighted MRI results were co-registered to the T1-weighted MRI results using the transformation parameters determined during the co-registration of [^{18}F]FDDNP images, summed for the first 5 minutes after injection, to the T1-weighted MRI results using statistical parametric mapping (SPM) software. The T1-weighted MRI results and co-registered images via PET were further transformed into the common space using SPM software. The ROIs were drawn on the normalized T1-weighted MRI results bilaterally on the superior and middle frontal gyri on the frontal lobe; the middle temporal gyrus in the lateral temporal lobe; the hippocampus proper, the entorhinal cortex, and the parahippocampal gyrus in the medial temporal lobe; the inferior lobule in the PA lobe; the anterior cingulate gyrus; and the PC gyrus.

The ROI sets were used to extract the DVR values from co-registered [^{18}F]FDDNP parametric images. The DVR values for each brain region are given as the means of the left and right hemisphere DVR values. We imported positron emission tomographic–drawn ROIs into co-registered MRI results and found good matching of ROIs with gray matter areas on MRI results.

Data analysis

Data were checked for outliers and descriptive statistics were obtained. The relationship of [^{18}F]FDDNP DVR binding levels with depression levels (HAMD total) and apathy (AES total) were studied using Spearman correlation analyses, due to the relatively small sample size. Age, sex and years of education were

partialed out. As this is an exploratory study to examine how regional [^{18}F]FDDNP binding is related to the outcome measures in depressed individuals, we did not correct for multiple comparisons and significance levels were set at 0.05.

4.6.6 Results

Study participants ranged in age from 63 to 83 years (mean 72.8 years with a standard deviation (SD) of 6.8 years). The sample was well educated (mean 15.8 years, SD 2.3 years) and depression scores were indicative of moderate depression (mean HAMD 17.5, SD 2.2, range 16 – 23). See table 10 for further details.

Table 10: Clinical and demographic characteristics at baseline

Variables		Mean (SD)
Age (years)		72.8 (6.8)
Education (years)		15.8 (2.3)
HAM-D		17.5 (2.2)
MMSE		28.0 (2.0)
AES		32.5 (11.0)
		N (%)
Sex	Male	8 (50%)
	Female	8 (50%)

AES, Apathy Evaluation Scale; HAM-D, Hamilton Depression Rating Scale; MMSE, Mini-Mental State Examination; SD, standard deviation.

[^{18}F]FDDNP binding in the ACC was negatively associated with the AES total ($r = -0.62$, $p = 0.02$; where low AES score equals greater severity of apathy; see figure 9) – suggesting apathy in LLD is associated with higher amyloid and/or tau levels in the ACC. None of the regional [^{18}F]FDDNP binding levels were significantly associated with HAMD total.

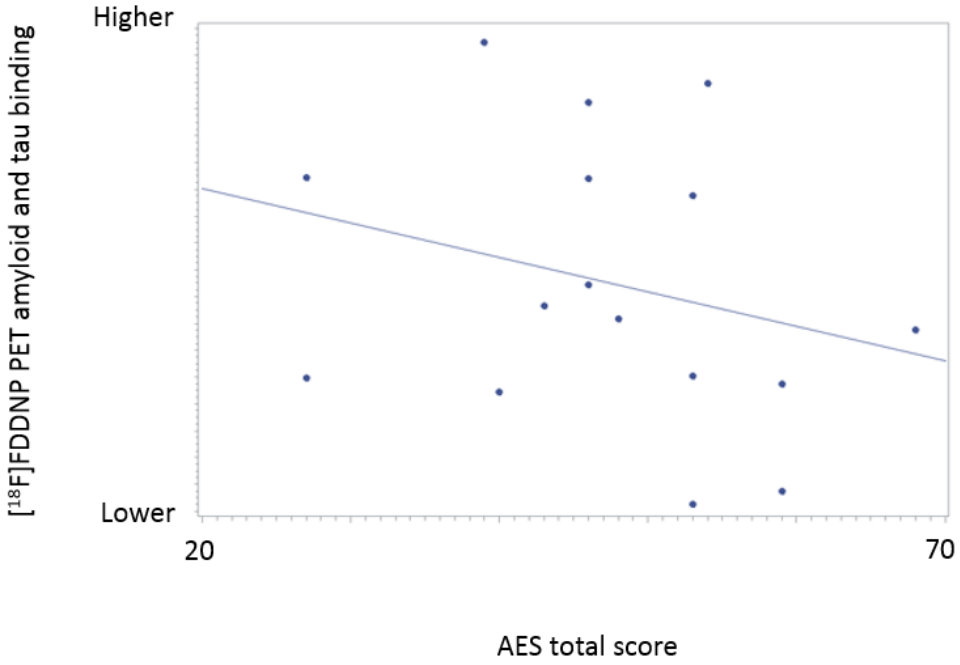

Figure 9: Associations between amyloid and tau binding in the anterior cingulate cortex and apathy

This figure shows associations between amyloid and tau binding in the ACC from the [^{18}F]FDDNP PET ligand and apathy scores as assessed by AES. [^{18}F]FDDNP binding in the ACC was negatively associated with the AES total ($r = -0.62$, $p = 0.02$).

4.6.7 Discussion

Our pilot study in an LLD population is the first to report the associations of the severity of apathy with [^{18}F]FDDNP binding in the ACC. This research is important given apathy in LLD is associated with poorer functioning and reduced antidepressant response.

Anterior cingulate cortex involvement in late-life depression

The ACC is a key part of the frontolimbic networks involved in LLD. The ACC is divided into dorsal, perigenual ACC regions. The rostral and subgenual control cognitive and emotional processes, respectively [278-281]. More specifically, the perigenual ACC assesses the salience of emotional input and regulates emotional responses [279, 282]. The dorsal ACC controls aspects of executive function (conflict detection, cognitive inhibition, and conflict resolution) [283, 284]. The ACC is suggested to be a functional intersection of emotion, cognition, drive and motor control, hence highly relevant to apathy. The dysfunction in the frontolimbic region may be due to the *disconnection hypothesis* and/or the *hypoperfusion hypothesis*. The *disconnection hypothesis* [213] suggests ischemia and white matter pathology may disrupt neural connections among regions modulating mood and cognition. In this model, widespread cerebral white matter hyperintensity (WMH) severity is less relevant to LLD than is focal damage to tracts and circuits. The *hypoperfusion hypothesis* [12] is suggested given vascular dysfunction is common in LLD [214-218] and cerebral blood flow reductions can alter brain function, contributing to depression-related symptomatology.

Several MRI-based neuroimaging studies have been conducted exploring the effect of apathy in LLD. As mentioned previously, the first study [270] found the severity of apathy was associated with decreased gray matter volumes in the right ACC. This is consistent with our [^{18}F]FDDNP results with increased amyloid and tau binding in the ACC. The second study found [271] no significant differences in white matter FA between controls, non-apathetic depressed and apathetic depressed. Bilateral dorsal and rostral ACC volumes distinguished control from apathetic depressed and control from non-apathetic depressed. There were no other significant differences in ACC volumes from this three-group comparison. The lack of ACC findings from this study may be due to small sample size.

While we are not aware of any amyloid or tau PET studies exploring binding in relation to apathy in subjects with LLD, there are 2 studies exploring associations between amyloid and tau PET binding and depressive symptoms. One is by Kumar et al. [13] who compared [^{18}F]FDDNP binding in 20 LLD patients vs. 19 healthy controls. Binding was significantly higher overall and in the PCC and lateral temporal regions in the MDD group. The second study is from Lavretsky et al. [58] explored [^{18}F]FDDNP binding in 23 MCI patients and 20 cognitively normal; depressed subjects were excluded, but depression scores were measured. The MCI and comparison subjects did not differ by the depression scores. In the MCI group, depression scores correlated with lateral temporal binding. In the comparison group, depression scores correlated with medial temporal binding.

A recent systematic review by Harrington et al [41] aimed to examine the relationship between Aβ, a key biomarker of AD, and depression in older adults. Studies were also required to include an outcome variable that was a direct measure

of Aβ levels in either blood or cerebrospinal fluid (CSF) samples, or via neuroimaging techniques such as PET. Nineteen studies were identified, 15 of which found significant differences in Aβ levels between depressed and non-depressed older adults. Five studies used PET neuroimaging as a primary outcome measure, with three observing statistically significant relationships between neuroimaging results and depression status. Of the statistically significant studies, 2 used [^{18}F]FDDNP binding (mentioned above) and 1 used [^{18}F]florbetapir. The non-significant studies used the PiB binding compound. Therefore, variations in results may be due to differences in these compounds.

Comparing neuroimaging and clinical data on apathy and LLD as risk factors for progression of cognitive decline

Data suggests that 1 in 10 cases of dementia world-wide can be attributed to depression [34]. There is some variation in the literature exploring the role of depression as a risk factor for dementia [41]. This may be partly due to the heterogeneity of depression phenotypes studied, including vascular depression, melancholic and atypical. One way to enhance the research is by investigating commonly shared clinical and neural features between depression and dementia.

Apathy comorbid with LLD may increase rates of cognitive decline, however data is conflicting from clinical studies. Prospective cohort study [285] of 397 subjects explored the effect of apathy on progression from MCI to AD. The presence of symptoms of apathy without symptoms of depressive affect increased the risk of progression while apathy in the context of depressive affect did not increase the risk of progression. This study utilized the GDS-15 to measure depressive symptoms, which is a significant limitation. A small, 2-year prospective

cohort study [286] of 124 MCI patients found rates of conversion to dementia were highest in apathetic individuals (60%). However, depression and apathy appeared to reduce the rates of conversion from MCI to dementia with rates of conversion higher for MCI normal (24%) than for MCI depressed and apathetic (19%) and MCI depressed (7.9%). These findings may be due to a small sample size.

To help discern the role of apathy in LLD in affecting rates of cognitive decline, the [^{18}F]FDDNP amyloid and tau marker has been explored in AD patients with apathy. Apathy in Alzheimer's disease was found to correlate with neurofibrillary tangle density in the ACC [287] and reduced grey latter volume in the ACC [288]. *In vivo* studies with PET markers of amyloid or tau do not find the ACC implicated in the progression of cognitive impairment. Findings from the [^{18}F]FDDNP study [68] differentiating individuals with MCI, AD, and normal cognition did not analyze ACC binding values and found global values of [^{18}F]FDDNP binding (average of the values for the temporal, parietal, posterior cingulate and frontal regions) were lower in the control group than in the group with MCI ($P<0.001$), and values in the MCI group were lower than in the group with AD ($P<0.001$). Other functional neuroimaging modalities have found hypometabolism in the ACC associated with apathy in AD [289-291], however other data is conflicting [292].

Our data showing apathy in LLD is correlated with increased amyloid and tau binding in the ACC may suggest apathy and LLD together increase the rate of cognitive decline. This, however, must be carefully explore in a larger population with more robust neuropsychological analyses.

Limitations

The findings our pilot study must be considered in the context of a number of limitations. Our cohort was of 16 individuals with LLD, hence making a small sample size. There was an absence of a control group in this study, which means only within-group, not between-group, analyses were possible. It is therefore difficult to distinguish between whether apathy is a symptom of depression or a preclinical symptom of late-life depression. This study didn't include comprehensive neuropsychological assessments which are significant limitation and area for improvement in future studies. Additionally, CT and MRI findings were not available in this cohort, meaning the presence of white matter hyperintensities and lacunar infarcts, vascular disease markers, were not able to be determined. Finally, the ligand used in this study binds to both amyloid and tau proteins, which means further studies are required to understand if the associations between apathy in LLD and binding are related to amyloid, tau or both proteins. We and others are not aware of any tau PET studies in LLD {Harada, 2016 #58}.

4.6.8 Conclusion

This pilot study suggests that increased apathy in subjects with LLD may be associated with greater amyloid and/or tau burden in certain brain regions. Future prospective studies in larger samples should elucidate the generalizability of these results and correlate with cognitive assessment, which eventually could lead to improved diagnostic and treatment methods in LLD.

4.6.8 Conflict of interest declaration

There are no conflicts of interest.

4.6.10 Acknowledgements

This paper has no acknowledgements.

5.0 Innovations in multimodal approaches for exploring the pathophysiology of late-life depression

The majority of past studies exploring the aetiology, pathophysiology and diagnosis of LLD have explored a relatively narrow set of characteristics often in isolation. These characteristics include clinical, socio-demographic, neuropsychological, structural brain imaging, functional brain imaging, molecular brain imaging, genomics or proteomics. The four studies outlined above all provide novel findings which will help with furthering the field. This often isolated analysis has yielded useful findings, and continues to provide novel findings. However, this approach is limited given the neurobiological mechanisms of LLD are complex and likely to involve interplay of changes in brain structure, brain function, neurochemistry and neuropathology [11-14]. The multimodal approach to LLD takes into account a broader spectrum of measures, in a more integrated view, with the aim of gaining a more complete and accurate understanding of brain functioning. However, novel methods are required to further progress this field, such as machine learning.

5.1 Role of machine learning in multimodal research analyses in psychiatry

A significant amount of scientific research producing new knowledge is conducted based on exploring associations in clinical practice or research settings, and hypotheses about pathophysiology which lead to novel treatment approaches that address the underlying mechanism [293]. The first approach is by nature unpredictable and relies on ‘creativity’ in the context of unbiased observations [293]. The latter is limited by incrementalism and can only proceed with new

understandings of molecular biology [293]. However, machine learning is another approach which may accelerate discovery and allow for new targets for traditional hypothesis testing research.

Machine learning, a type of artificial intelligence, involves 'the development of computer systems that automatically improve with experience and follow the fundamental laws that govern learning processes'. Machine learning involves systems which 'learn' from observation, experience, and become more efficient and effective over time. It does not require *a priori* hypotheses, generates unexpected patterns and structures, and incorporates disparate data types [293, 294]. Machine learning allows characterisation at the level of the individual [294]. Additionally, as inherent multivariate approaches, machine learning methods are sensitive to spatially distributed and minor effects in the brain otherwise undetectable using traditional univariate methods which focus on gross differences at group level [293, 294]. Taken together, it offers great opportunities for exploratory analyses of large and heterogeneous datasets.

There are a number of types of machine learning – supervised, semi-supervised and unsupervised. Supervised learning is performed when all data is labelled, and data is used to train the machine. Semi-supervised learning is performed when there is unlabelled data long with labelled data. Unsupervised learning is performed when all of the data is unlabelled, and here the data is clustered into different classes.

5.1.1 Example studies using machine learning methods and multimodal approaches in LLD

Below are example studies using machine learning methods to further elucidate the pathophysiology of LLD. The first study utilises only peripheral biomarkers, the second only neuroimaging biomarkers, and the third uses both peripheral and neuroimaging biomarkers. Together these studies provide helpful examples of methods which may be used to further this field.

Peripheral biomarkers

Plasma biomarkers of depressive symptoms were recently explored in older adults by Arnold et al. [295]. An unbiased analysis of 146 plasma protein biomarkers in relation to number of depressive symptoms from 556 participants of the Alzheimer's Disease Neuroimaging Initiative was conducted at baseline and 12-month assessments. The markers most highly associated with depressive symptoms included hepatocyte growth factor, insulin polypeptides pregnancy-associated plasma protein-A and vascular endothelial growth factor. Other major factors did not attenuated the significance of these findings, i.e. past history of psychiatric illness, antidepressant use, apolipoprotein E genotypes, BMI, serum glucose and CSF Aβ levels. Finally, machine learning techniques with Random Forests found good accuracy (−80%) for these biomarkers in classifying groups with and without depressive symptoms.

Neuroimaging markers

Another study comes from Patel et al. [227] where researchers aimed to estimate accurate prediction models for LLD diagnosis and treatment response using multiple machine learning methods with inputs from multimodal imaging and

non-imaging whole brain and network-based features. Structural imaging, DTI and rs-fMRI were used. The aim with a broader spectrum of measures was to gain to more complete and accurate understanding of underlying brain mechanisms associated with LLD. A small sample of LLD patients (N = 33) and older non-depressed individuals (N = 35) were recruited. Treatment was heterogeneous including either SSRIs or serotonin noradrenaline reuptake inhibitors (SNRIs). A machine learning method for diagnosis, called alternating decision trees, included measures of age, MMSE score and structural imaging (e.g. whole brain atrophy and global WMH burden). This model provided 87.27% accuracy for LLD diagnosis. A similar approach was used to develop a model for treatment response and this model included measures of structural and functional connectivity. This model found 89.47% accuracy for predicting treatment response.

Combined peripheral and neuroimaging biomarkers

A recent pilot study by Diniz et al. [11] explored how plasma biosignatures and brain pathology relate to persistent cognitive impairment in LLD. The authors used a convergence of various investigative modalities to explore this topic given the biological mechanisms underlying cognitive impairment in LLD are complex and involved abnormalities in multiple pathways. They outline that current knowledge on the pathophysiology of LLD if fragmented and require an integrated view. They used a data-driven approach including comprehensive proteomic analysis (multiplex immunoassay including 242 proteins), along with measures of structural brain abnormalities (grey matter atrophy and WMH volume via MRI), and brain Aβ deposition (PiB-PET). The goal was to uncover novel biological interplays to inform subsequent hypothesis-driven research studies. They analysed

data from a small study of 80 older adults with remitted major depression (36 with LLD + MCI and 44 with normal cognitive (LLD + NC)) function. To predict the classification of LLD + MCI and LLD + NC, the researchers used support vector machines (with linear kernel), a form of supervised learning. In this study, cognitive impairment in LLD seemed to be related to greater cerebrovascular disease along with abnormalities in 24 proteins related mainly to immune-inflammatory control (e.g. CCL13, MCP-4, CXCL11), cell survival, intracellular signalling, protein and lipid homeostasis and clotting processes. Individuals with LLD + MCI, however had greater WMH burden compared to LLD + NC, but no difference in GM volume or Aβ binding. The protein most related to cognitive impairment and brain pathology in LLD was the cytokine IL-12 P40, involved in adaptive cell-mediated immunity.

6.0 Summary and conclusions

A number of points are clear with LLD. With the context of an ageing global population, the prevalence is projected to rise. Diagnosis is hampered by being based on subjective assessments of behavioural symptoms and signs, and hence suboptimal diagnostic reliability. There is currently a lack of biomarkers to support diagnosis. Issues with poor diagnosis are compounded by the significant rates of treatment resistance to antidepressants. Better understanding the pathophysiology of LLD is hoped to spawn novel biomarkers for diagnosis, and this may in turn allow for more precise and effective treatments. To date, however understanding the pathophysiology of LLD has occurred via more singular investigative methods. However, given the pathophysiological mechanisms of LLD are complex and involve abnormalities in multiple neurobiological systems and pathways, multimodal approaches are now being explored, as well as machine learning methods. The goal of this thesis is to understand how multimodal analyses of the pathophysiology of LLD may be a more integrated and effective method.

Research into the pathophysiology of depression has historically encompassed many interrelated systems including the HPA axis, neurotransmitter systems, neurotrophins and neuroplastic processes and neuroinflammation. There are, however new systems being explored, which show promise for further unravelling the complexities of LLD. Chemokines are explored given their demonstrated roles in NSC development, neuroplasticity and neurotransmitter-like effects. *In vivo* neuroimaging has allowed for better understanding of the vascular hypothesis of LLD, which suggests cerebrovascular disease predisposes and certain symptoms. Further, *in vivo* PET-based amyloid and tau imaging have demonstrated

suggested neurotoxicity due amyloid and tau protein aggregation may represent a pathophysiological cascade which may predispose individuals to LLD.

In this thesis, a meta-analysis of clinical studies was used to explore correlations between chemokines concentrations in MDD subjects versus controls. This yielded findings to suggest that out of all chemokines studied, there are higher concentrations of the chemokine MCP-1 in depressed subjects compared with control subjects, and no differences for IL-8. More high quality research and consistent methodologies are urgently needed. This area of inquiry is relevant to the second study using functional neuroimaging, given chemokines are thought to play a role in endothelial dysfunction and cerebral small vessel disease. It is also relevant to the third study using amyloid and tau binding molecular neuroimaging, given chemokines are suspected to play a role in amyloid clearance via glial cells. The second study utilised rs-fMRI analyses in LLD patients versus controls. The findings from this study suggested patterns of increased connectivity that may be unique to LLD in the DMN, as well as visual and auditory networks. The third study was conducted in an LLD population and used [^{18}F]FDDNP PET ligand to explore associations between amyloid and tau binding and apathy, an important and under investigated symptom which can co-occur with LLD. The pilot study found increased apathy may be associated with greater amyloid and tau burden in certain brain regions. The fourth study examined genome-wide transcriptional profiles in a 16-week randomised placebo-controlled trial of combined MPH and CIT. Remission to antidepressants in geriatric depression may be associated with a particular gene expression profile in monoaminergic and metabolic pathways.

Taken together, these studies provide rationale and direction for further integrating investigations into the pathophysiology of LLD, as well as exploring

chemokines, genomics and amyloid and tau deposition. Machine learning methods which allow for data-driven approaches involving multimodal biological analyses may support further development.

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8.0 Other academic outputs during PhD period

Peer-reviewed publications

Primary papers

Vaswani N, Wang W, Eyre H, Joyce C. Mapping the non-clinical career transitions of Australian doctors using the MABEL study. Asia Pacific Journal of Health Management. Vol. 10, No. 2, 2015: 33-41

C Joyce, Eyre H, W Wang, C Laurence. Australian doctors' non-clinical activities: results from the MABEL survey of doctors. The Australian Health Review. doi: 10.1071/AH14223

Book chapters

Eyre H, Baune BT, Lavretsky H, chapter 'Exploring the effects of complementary, alternative and integrative therapies in late-life psychiatry through the mechanisms of aging'. Eds Lavretsky H, Sajatovic M, Reynolds III C, title 'Complementary, Alternative and Integrative Interventions in Mental Health and Aging'. Submitted 2014. ISBN: 9780199380862

Eyre H, Baune BT, Lavretsky H, chapter 'Complementary, alternative and integrative therapies in late life psychiatry'. For the Section on Geriatric Psychiatry (Ed D Jeste). For the Comprehensive Textbook of Psychiatry, Eds Sadock, Sadock and Ruiz. Submitted December 2014.

Eyre H, Lavretsky H, Baune BT, chapter 'Pharmacological treatment of depression-CVD comorbid patients'. Eds Baune BT, Tully P, title 'Cardiovascular Diseases and Depression - Treatment and Prevention in Psychocardiology'. Submitted.

Forbes M, Eyre H, Wong CX, Baune BT, chapter 'Screening for depression in patients with coronary heart disease'. Eds Baune BT, Tully P, title 'Cardiovascular Diseases and Depression - Treatment and Prevention in Psychocardiology'. Submitted.

Abbott R, Eyre H, Lavretsky H, chapter 'Qi gong and tai chi for psychiatric practice'. Complementary and Integrative Treatments for Psychiatric Practice. American Psychiatric Association Publishing. Planned for submission December 2015.

Singh A, Eyre H, Callaly E, Berk M, chapter 'Bipolar disorder and early intervention: current techniques, challenges and

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Review papers

Eyre H, Singh A, Reynolds C (2016) Tech Giants Enter Mental Health. Accepted in World Psychiatry (IF 15)

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