Exploring the relationship between circulating inflammatory markers and the brain.

Rajeev Krishnadas
MBBS, MD, DipNB, MRCPsych

A thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

Institute of Health and Wellbeing

College of Medical, Veterinary and Life Sciences

University of Glasgow

June - 2014

Abstract

Over the last decade, there has been a burgeoning interest in clinical research that link psychiatric illnesses - particularly major depressive disorder - to inflammatory processes. Most of the evidence that link inflammation to major depressive disorder in humans come from three observations - a) findings from at least 3 meta-analytic studies suggest that major depressive disorder is associated with elevated circulating inflammatory biomarkers; b) inflammatory illnesses - both central (brain) and peripheral (e.g. arthritis) - are associated with greater rates of major depressive disorder than the general population and c) patients treated with cytokines - both experimentally as well as therapeutically (for cancer or hepatitis) are at greater risk of developing a major depressive illness. As a corollary, anecdotal and experimental evidence suggest that anti-inflammatory medications may have some antidepressant effects. In fact, a number of preclinical studies have provided clues towards potential mechanistic pathways through which inflammatory processes may directly have an effect on the brain, causing changes that may contribute to the aetiopathogenesis of major depressive disorder.

The aim of the project was to explore the relationship between circulating (peripheral blood) inflammatory markers and brain structure in humans using state of the art magnetic resonance imaging (MRI) and single photon emission tomography (SPECT). I explored this relationship in two datasets. Firstly, in a series of cross sectional observational analyses on the PSOBID study sample (http://www.gcph.co.uk/work_themes/additional_work/psobid), I examined the association between circulating inflammatory markers and cortical thickness (MRI - surface based morphometry) in a group of neurologically healthy adult males. I found that circulating inflammatory markers explained significant variance in cortical thickness. Greater inflammatory marker levels were associated with cortical thinning across the cortical mantle. Using mediation analysis, I found that greater circulating inflammatory markers mediated the association between neighbourhood-level deprivation (a high risk condition for major mental illnesses) and cortical thinning. I then used complex network analysis using graph theory to show that greater inflammation mediated the

association between neighbourhood-level deprivation and poorer network structural properties of cortical thickness covariance networks. I also showed that greater inflammatory markers mediated the association between neighbourhood deprivation and smaller volumes pertaining to the limbic stress network. Next, in an experimental study, I examined the association between circulating inflammatory markers and serotonin transporters in the midbrain of patients with psoriasis/psoriatic arthritis using SPECT. I found that greater inflammatory marker levels were associated with greater serotonin transporter levels in the midbrain. I also showed that administration of an anti-inflammatory medication (anti-TNF-α agent) was associated with a reduction in the serotonin transporter levels.

These findings provide some evidence to suggest that circulating inflammatory markers account for significant differences in cortical thickness and subcortical volumes in the human brain. I have shown that circulating inflammatory markers may mediate the association between high risk conditions - like neighbourhood deprivation and inflammatory medical conditions - and brain changes that may underlie the pathophysiology of major depressive disorder. Future work will focus on cementing the precise role of inflammation in depressive illness, through sophisticated animal models and clinical neuroscience. This may result in potential biomarkers that may facilitate diagnosis, help predict prognosis and aid the development of beneficial treatments for what remains a significantly disabling psychiatric illness.

Table of Contents

List of Tables

List of Figures

Acknowledgements

First and foremost, I am deeply indebted to my supervisory team Dr Jonathan Cavanagh and Prof Iain McInnes for their patience, continuous advice and valuable support over the period of my thesis.

I would like to thank all the participants in the studies that are presented in this thesis. Without their cooperation, this work would have been impossible. I would like to thank Prof Sally-Ann Cooper for the support throughout the period. I would like to thank Prof Eve Johnstone and Prof Angus McKay for general feedback throughout the project. Prof Kate O'Donnell and Mrs Margaret Ashton for putting up with late submissions and other delays.

I am most grateful to Dr John McLean and Dr Jongrae Kim, for their support and advice regarding MRI image analysis and graph theoretical analysis. I would like to thank Dr Sally Pimlott, Dr Alice Nicol, Dr David Brennan, Dr James Patterson and Prof Donald Hadley, for their support with SPECT and MRI image acquisition, analysis and interpretation. I would like to thank Dr Emilie Combet Aspray for helping with the ELISAs. I am very grateful to the Glasgow Centre for Population health and in particular, the PSOBID team for giving me the opportunity to use the PSOBID cohort. In particular I would like to thank Dr David Batty, Dr Jennifer McLean, Prof Keith Millar, Prof Carol Tannahill, Dr Kevin Deans, Dr Alex McConnachie and Prof Chris Packard for their continuous support.

I would like to thank the Rheumatology team, particularly Nurse May Gallagher and Dr Hilary Wilson. In addition, I would like to thank Prof David Burden, Dr Joyce Lehman and Nurse Anne Thorat for help with recruitment of participants for the Etanercept study. In particular I would like to thank Dr Navesh Puri, Dr Eugene Wong and Dr Seonaid Cleare for helping with the recruitment and scans.

Dr Lena Palaniyappan was a constant inspiration throughout the project and someone I looked up to when in doubt. Dr Filippo Queirazza showed me that hard work and perseverance pays off. Dr Sameer Jauhar taught me to be critical about even the most basic of assumptions. Prof Chris Williams was my mentor throughout this period. Dr David Brown and Dr Susan Miller supported me as a higher trainee in the West of Scotland training scheme. Dr Martin Livingston and

Dr Alison Blair were most supportive as clinical supervisors. They gave me the opportunity to flexibly train in General Adult Psychiatry during the most crucial phases of this project.

I would like to thank the Dr Mortimer and Theresa Sackler foundation for part funding my PhD project. I would also like to thank Glasgow University for part funding my PhD. I would like to thank Pfizer for funding the Etanercept project.

Lastly, but most importantly, I would like to thank my wife, Dr Sindhu Prabhakaran and my children Shiva Das and Maya Das, for all the missed evenings and weekends, and without whose support, patience and tolerance, I would never have been able to complete this thesis. I would also like to thank my brother Dr Ranjit Krishnadas and parents Drs Mary and PS Krishnadas for their continuous support.

Author's Declaration

This thesis has been composed and documents the original work carried out by Rajeev Krishnadas.

This thesis has not been previously submitted in any form to any institution or University. However, sections of the work described herein have been presented and published elsewhere by the author as listed below.

Rajeev Krishnadas

January 2014

Publications

Krishnadas R, Cavanagh J. Depression: an inflammatory illness? Journal of Neurolology Neurosurgery and Psychiatry. 2012 May;83(5):495-502

Krishnadas R, et al. Socioeconomic deprivation and cortical morphology: psychological, social, and biological determinants of ill health study. Psychosomatic Medicine 2013 Sep;75(7):616-23

Krishnadas R, et al. Cardio-metabolic risk factors and cortical thickness in a neurologically healthy male population: Results from the psychological, social and biological determinants of ill health (pSoBid) study. Neuroimage Clinical. 2013 Apr 22;2:646-57

Krishnadas R, et al. The envirome and the connectome: exploring the structural noise in the human brain associated with socioeconomic deprivation. Frontiers in Human Neuroscience 7:722.

Krishnadas R, Cavanagh j. Sustained remission of rheumatoid arthritis with a specific serotonin reuptake inhibitor antidepressant: a case report and review of the literature. Journal of Medical Case Reports, 5, 112.

Abbreviations

3HK - 3 Hydroxy kynurenine

5-HT - Serotonin

5-HTTLPR - serotonin-transporter-linked polymorphic region

ACC - anterior cingulate cortex

ADAM - N,N-dimethyl-2-(2-amino-4-[(18)F]fluorophenylthio)benzylamine

AMPA - α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANCOVA – Analysis of covariance

Apo - Apolipoprotien

ANS - autonomic nervous system

BBB - Blood brain barrier

BDI - Becks Depression Inventory

BDNF - Brain derived neurotrophic factor

Beta CIT / [^{123}I] -beta-CIT - 2beta-carbomethoxy-3beta-(4-iodophenyl)tropane

BMI - body mass index

CIMT - carotid intima-media thickness

CNS - Central nervous system

CRP - high sensitivity C-reactive protein

CT – cortical thickness

DAT - dopamine transporter

ELISA - enzyme linked immunosorbent assay

FACIT-F -The Functional Assessment of Chronic Illness Therapy - fatigue scale

FDR - False discovery rate

FS - FreeSurfer

GHQ - General health questionnaire

GIT - Gastrointestinal tract

GLM – general linear model

GM - Gray matter

GR - glucocorticoid receptors

HDL - High-density lipoprotein

HPA - hypothalamus pituitary axis

ICAM - Intercellular Adhesion Molecule-1

ICV – Intracranial volume

IDO - Indolamine deoxygenase

IFN α - Interferon alpha

IFN γ -Interferon gamma

IL1β - Interleukin -1 beta

IL6 - Interleukin-6

Kyn - Kynurenine

LD - least deprived

LDL - low-density lipoprotein

MD - most deprived

MDD- Major depressive disorder

MZIENT - 2β-carbomethoxy-3β-(3′-((Z)-2-iodoethenyl)phenyl)nortropane

MRI - Magnetic resonance imaging

NMDA - N-methyl-D-aspartate

NNT - Number needed to treat

NO - nitric oxide

OR - odds ratio

PCA - Principal components analysis

Ps - Psoriasis

PsA - Psoriatic arthritis

PSoBiD - Psychological, social and biological determinants of ill health

QDEC - Query, Design, Estimate, Contrast

QUIN -Quinolinic acid

RA - Rheumatoid arthritis

ROI - region of interest

SA – surface area

SAM - sympatho-adreno-medullary axis

SED - Socio-economic deprivation

SERT - Serotonin transporter

SES – Socioeconomic status

SIMD - Scottish Index of Multiple Deprivation

SPECT - Single photon emission tomography

SSRI - Selective serotonin reuptake inhibitors

TAG - Triglycerides

TCA - tricyclic antidepressants

TNF-α - Tumour necrosis factor

tPA -Tissue plasminogen activator

vWF - von Willebrand factor

Chapter 1 – Review of Literature

1.1 Introduction

Mental, neurological and substance use disorders account for a significant proportion of the global burden of disease, surpassing that of cardiovascular disease and cancer. Major depressive disorder (MDD) is the third leading cause of global disease burden (Collins et al., 2011).Research over the last few decades have increased our insight into the pathophysiology of MDD. A number of biological findings have been replicated and have proven fruitful in terms of research outcomes. However, our understanding of what is essentially a highly heterogeneous disorder remains incomplete. Despite major research funding over the last twenty years, bringing the above research findings together and translating those into effective treatments have been largely ineffective (Krishnan and Nestler, 2010).

Major depressive disorder is a heterogenous condition and is characterised by a number of emotional, cognitive and physical symptoms. The core symptoms of major depression are “depressed mood” and “anhedonia”. The associated symptoms include changes in sleep, appetite, weight loss, psychomotor retardation/agitation and cognitive symptoms. The DSM IV and ICD 10 criteria for a depressive episode are shown in Table 1-1(Gruenberg et al., 2005).

Table 1-1: DSM IV and ICD 10 criteria for Major depressive disorder

	DSM IV	ICD 10
Clinical Significance	Symptoms cause clinically significant stress or impairment in social, occupational or other important areas of functioning.	Some difficulty in continuing with ordinary work and social activities, but will probably not cease to function completely in mild depressive episode; considerable difficulty in continuing with social, work or domestic activities in moderate depressive episode; considerable distress or agitation, and unlikely to continue with social, work, or domestic activities, except to a very limited extent in severe depressive episode.
Duration of symptoms	Most of day, nearly every day for at least 2 weeks.	A duration of at least 2 weeks is usually required for diagnosis for depressive episodes of all three grades of severity.
Severity	Five or more of following symptoms; at least one symptom is either depressed mood or loss of interest or pleasure: (1) Depressed mood (2) Loss of interest (3) significant weight loss or gain or decrease or increase in appetite (4) Insomnia or hypersomnia (5) Psychomotor agitation or retardation (6) Fatigue or loss of energy (7) Feelings of worthlessness or excessive or inappropriate guilt (8) Diminished ability to think or concentrate, or indecisiveness (9) Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or suicide attempt or a specific plan	Depressed mood, loss of interest and enjoyment, and reduced energy leading to increased fatigability and diminished activity in typical depressive episodes; other common symptoms are: (1) Reduced concentration and attention (2) Reduced self-esteem and self-confidence (3) ideas of guilt and unworthiness (even in mild type of episode) (4) Bleak and pessimistic views of the future (5) Ideas or acts of self-harm or suicide (6) Disturbed sleep (7) Diminished appetite Typical examples of "somatic" symptoms are: loss of interest or pleasure in activities that are normally enjoyable; lack of emotional reactivity to normally pleasurable surroundings and events; waking in the morning 2 h or more before the usual time; depression worse in the morning; objective evidence of definite psychomotor retardation or agitation; marked loss of appetite; weight loss; marked loss of libido.

Over the last two decades, there has been a burgeoning interest in preclinical and clinical research linking psychiatric illnesses to inflammatory processes (and vice versa). Most of this has arisen from an attempt to link these illnesses – particularly MDD - with "stress" biology, and have raised the possibility of an "initial common pathway" whereby immune/inflammatory and stress biomarkers combine to cause changes in brain structure and function (Raison et al., 2006). Most of the evidence that links inflammation and major depressive disorder come from 3 observations(Capuron and Miller, 2011).

1. MDD (even in the absence of medical illness) is associated with raised inflammatory markers.

2. Inflammatory medical illnesses – both CNS and peripheral – are associated with greater rates of major depression.

3. Patients treated with cytokines for various illnesses are at increased risk of developing major depressive illness

In this chapter, I intend to discuss each of the above observations. I then go on to discuss the possible mechanisms involved in the aetio-pathogenesis of MDD, in the context of inflammation. Most of what is presented in this chapter is published in *Krishnadas R, Cavanagh J. Depression: an inflammatory illness? J Neurol Neurosurg Psychiatry. 2012 May;83(5):495-502. (Reused with permission)*

1.2 MDD is associated with increased inflammatory marker

Inflammation has been linked to MDD in a number of ways (Capuron and Miller, 2011, Raison et al., 2006, Raison and Miller, 2011). More robust findings including:

1) Mean value for inflammatory mediators/ markers is higher in MDD than normal, non-depressed subjects.

2) Approximately one-third of people with MDD have higher levels of inflammatory markers, compared to the normal, non-depressed population.

3) These increases are more modest than in autoimmune or infectious disease, e.g. 2-3 times higher than healthy controls. However, as Raison and Miller point out, small physiological differences can have profound consequences over time, especially if they change in a consistent direction(Raison and Miller, 2011). Similar findings have been found in cardiovascular disease, stroke and diabetes.

Alterations in serum and CSF concentrations of a number of inflammatory markers, including cytokines, chemokines and acute phase reactant proteins, have been found in patients with MDD, and exist in the absence of co-morbid medical illness. The most replicated findings pertain to raised CRP and proinflammatory cytokines - TNF-α and IL6, confirmed by at least 3 recent meta-analyses of cross sectional studies(Dowlati et al., 2010, Liu et al., 2012a, Howren et al., 2009). All three meta-analyses found significant heterogeneity among the included studies; however, there was no evidence of publication bias.

More recent of the three meta-analyses, by Liu et al found that TNF-α and IL6 are higher in patients with MDD compared to controls (Liu et al., 2012a). In addition, IL1B also was found to significantly different in

European patients. The standardized mean differences from the study are shown in

Table 1-2. The results suggest the difference in cytokine level between MDD and healthy controls was of a moderate to large effect size.

Interestingly, there is some evidence to say that the increase in inflammatory markers is corrected to an extent in response to antidepressant treatment. A meta-analysis of 22 antidepressant treatment studies found that, IL1β and IL 6 levels (but not TNF-α) decreased in response to therapy, along with a reduction in depressive symptoms (Hannestad et al., 2011). This response was found to be specific for selective serotonin reuptake inhibitors (SSRIs), the most common first line pharmacological treatments for MDD. These findings propose the possibility that inflammatory cytokines contribute to depressive symptoms, and that antidepressants may block the effect of inflammatory cytokines in the brain.

Table 1-2: Results of sensitivity analyses based on quality of studies for TNFα, IL6 and IL1β.

	TNF- α				IL-6				IL-1β			
	SMD	I^2	n	p	SMD	I^2	n	p	SMD	I^2	n	p
All studies	0.56	89.7	15	0.01	0.68	63.89	18	< 0.001	-0.52	95.13	10	0.22
High quality studies	0.99	92.17	8	0.01	0.791	72.31	9	< 0.001	-1.30	142.08	4	0.34

I^2 denote heterogeneity. SMD - standardised mean difference. n - number of studies. High quality studies were based on an arbitrary cut off score of >7 on the Newcastle Ottawa Scale (a scale to tease out high quality studies). Reused with permission from Liu Y, Ho RC, Mak A. Interleukin (IL)-6, and tumour necrosis factor alpha (TNF-α) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. Journal of Affective Disorder. 2012 Aug;139(3):230-9

The results of the above three meta-analyses show only a correlation between inflammatory markers and depression, and does not necessarily show a direction of relationship (or causation) between the two . More recently, longitudinal studies have shown an association between circulatory inflammatory markers and development of depression. Valkanova et al undertook a systematic review of longitudinal studies to investigate whether raised circulating inflammatory was suggestive of the development of subsequent depressive symptoms (Valkanova et al., 2013). They examined the relationship between CRP and IL6 on the subsequent development of depressive symptoms. They found a significant association between CRP and depressive symptoms at follow up (adjusted r=0.05). The relationship at best was thought to be modest (yet significant).

Despite these findings it is still hard to justify describing MDD as a primary "inflammatory" illness, because inflammation is neither necessary nor sufficient to be the sole cause of MDD. This is complicated by the fact that immune/ inflammatory disruption have been found in a number of other psychiatric conditions including schizophrenia and PTSD(Miller et al., 2011, Spitzer et al., 2010). It is therefore more likely that inflammation and its mediators may play a more subtle role, as part of a generalised physiological response, or may act as a trigger to a cascade of events that ultimately lead to the depressive phenotype. Raison and Miller in this context have described a "super-network", with immune response element amplification (IREA)(Raison and Miller, 2011). This includes multiple mechanisms through which inflammation may act, in precipitating the depression phenotype. These include -

- Insensitivity to glucocorticoid inhibitory feedback

  Proinflammatory cytokines including TNF-α, induce glucocorticoid resistance. Cytokines induce functional inhibition by preventing the cortisol-glucocorticoid receptor complex entry into the nucleus and also preventing its binding to the DNA (Pace et al., 2007, Pace and Miller, 2009). This in turn leads to altered expression of glucocorticoid receptors in the cells. This phenomenon has been documented in major depressive disorder in the form of an impaired dexamethasone suppression test(Evans and Nemeroff, 1987).

- Impaired parasympathetic signalling

  Preclinical studies in rodents suggest that inducing a peripheral inflammatory state often produce a sickness behaviour that is dependent on vagal afferents(Ek et al., 1998). Visceral terminals of the vagal afferent nerves express cytokine binding sites(Goehler et al., 1997). Binding of pro-inflammatory cytokines to these sites, result in activation of brain structures implicated in interoception and homeostasis, and results in sickness behaviour(Wan et al., 1994). In addition, damage to the vagal afferents, result in an attenuation of this sickness behaviour(Bret-Dibat et al., 1995).

- Increased subgenual anterior cingulate cortex (SgACC) activity

  The ACC ventral to the genu of the corpus callosum has been implicated in neuroimaging studies of major depressive disorder(Drevets et al., 2008). Mayberg demonstrated that provoking transient sadness in healthy volunteers increased subgenual cingulate blood flow(Mayberg, 1997, Mayberg et al., 1999). In depressed patients, this increase in blood flow was normalised on treatment with antidepressants. Grecius et al, using resting state fMRI found that during resting state, depressed patients, the SgACC showed greater connectivity with the default mode network(Greicius et al., 2007). Post mortem studies in suicidal patients with severe depression have shown an increase in microglial quinolinic acid in the SgACC, as an evidence for immune modulated glutamatergic neurotransmission(see below)(Steiner et al., 2011). Studies that induce inflammation in humans have shown an increased activation of SgACC that directly correlated with negative mood symptoms(Harrison et al., 2009a). Magnetic resonance spectroscopy studies have shown an increase in glutamate metabolites in the SgACC in those receiving interferon treatment(Haroon et al., 2013).

- Reduced hippocampal volume.

  In health, proinflammatory cytokines like TNF-α are known to regulate synaptic plasticity by influencing the expression of AMPA glutamatergic

receptors (see below)(McAfoose and Baune, 2009). However, at higher concentrations, proinflammatory cytokines induce glutamate mediated excitotoxicity. In addition, inflammation also plays a role in adult hippocampal neurogenesis. Firstly, Ekdahl et al showed that intrahippocampal administration of LPS reduced neurogenesis in the adult hippocampus (Ekdahl et al., 2003). Monje et al, showed that this effect was prevented by indomethacin, a non-steroidal anti-inflammatory drug(Monje et al., 2003). In addition, Belarbi et al, showed that treatment with LPS also prevents the integration of new neurons into behaviourally relevant network(Belarbi et al., 2012). In humans, Marsland et al have shown that circulating interleukin-6, covaries inversely with hippocampal grey matter volume in middle aged adults even after controlling for a number of confounding factors(Marsland et al., 2008).

The concept of IREA also resonates with the concept of “allostatic load” described by McEwen(McEwen, 1998). Allostasis has been described as the process of adaptation to acute stress, involving the activation of both the Sympathetic Adreno-medullary (SAM) and Hypothalamic – Pituitary – adrenal (HPA) axes in order to restore homeostasis when faced with a challenge. “Allostatic load” refers to the price the body pays for being forced to adapt to adverse situations – i.e. the wear and tear that the body experiences as a result of activation of the above systems. This wear and tear, represents either the ‘excess’ or the ‘inefficient’ operation of the above systems and can occur due to one of four mechanisms – repeated stress leading to repeated activations of these systems over time, failure of the systems to adapt to multiple stimuli, failure to shut down after being activated once, or the other extreme, where the systems do not get activated at all (e.g. in autoimmune diseases). It is not surprising that, in this context, inflammation plays a key role in the process of allostasis. A number of studies have shown a significant association between allostatic load (measured using a composite score of inflammatory and metabolic markers) and medical health (for e.g. cardiovascular disease) and mental ill health (like major depression) (Juster et al., 2010). If indeed, inflammatory processes are important in the aetio-pathogenesis of MDD, a number of important questions still remain un-answered.

- Are the suggested biomarkers important in the aetio-pathogenesis of MDD or are they merely an epiphenomenon associated with MDD?

- Do peripheral markers of inflammation in humans correlate with brain markers in what is essentially an illness of the central nervous system?

- Is there CNS inflammation in MDD?

- Does correcting peripheral inflammation also correct central inflammation?

- If inflammatory markers are involved in the pathogenesis of major depression, does a dose response relationship exist between these markers and brain function?

- How stable are these markers and what is the normal variability associated with the measures? Further studies are required to elucidate these processes.

1.3 High risk of MDD is associated with greater inflammation

In this section, I will explore the literature pertaining to the relationship between inflammation and mental health/brain in the context of socioeconomic status (SES) and medical illness. While the primary aim of this dissertation is not to explore the relationship between SES / medical illness and inflammation, in this section, I would like to present evidence that suggest SES and medical illnesses are risk factor for both raised inflammatory markers and mental illness. While the relationship between medical illnesses, particularly those that involve autoimmune processes and inflammation are clearly obvious, the relationship between SES and inflammation are less clear. I will review data from both cross sectional and longitudinal studies that illustrate associations and potential causal links between SES/medical illnesses, inflammation and mental health. My review will concentrate primarily on major depressive disorder, as this is the most prevalent mental illness that has been associated with SES and greater inflammation. However, I will occasionally dip into evidence from other psychiatric conditions.

1.4 SES and mental illness

1.4.1 What is SES

Socio-economic status (SES) refers to a multidimensional construct that is usually measured using a number of economic (e.g. income) and noneconomic (e.g. education) indicators (Hackman et al., 2010). SES can be measured at an individual/household or at a neighbourhood level. At an individual level, it may be measured using a number of markers that take into account a person's SES as a child or as an adult. However, individual level explanations for poor health do not capture important disease determinants (Diez Roux and Mair, 2010). Neighbourhood level deprivation has been associated with poor health outcomes due to segregation based on ethnicity and individual level SES. This can lead to inequalities in resource distribution, which can further worsen the segregation. These segregated neighbourhoods have physical (e.g. access to food, quality of housing) and social (e.g. violence, cohesion) attributes that are contributors of health outcomes. However, Individual level characteristics may modify the relationship between neighbourhood and health outcomes (Stafford and Marmot, 2003).

1.4.2 Lower Socioeconomic status is associated with greater risk of mental illnesses.

A number of studies have shown an association between socioeconomic disadvantage and mental illness. While most of the studies have shown an association between lower SES and greater prevalence of mental illnesses, some studies have also shown an association between SES and incident mental illnesses. While greater prevalence of mental illness does not necessarily show a causal relationship, greater incidence of mental illness suggests that there may be a causal link between the two.

In a cross sectional analysis of the National Comorbidity Survey, Kessler et al found that rates of all disorders increased with lower SES as measured using education status or income (Kessler et al., 1994). They found that the significantly greater risk involved with low SES were consistently larger in predicting 12-month than lifetime prevalence (Kessler et al., 2005). They

suggest that this meant that socioeconomic status was associated not only with onset but also with course of disorder. The cross sectional nature of the data prevented them from commenting on whether this was due to causal influence or to drift.

Pulkki-Raback et al in a large cross sectional study of nationally representative sample of 4561 men and women aged 30–65 years in Finland – a high income country - found that low income (but not education or occupation status) was associated with an increased risk of depressive disorders [OR = 1.73] and anxiety disorders [OR=1.56] (Pulkki-Raback et al., 2012).

In a longitudinal analysis of two waves of data collected in the New Haven epidemiologic catchment area study, Bruce et al found that poverty status at the first wave of the study, predicted an increased risk of development of any psychiatric disorders (OR = 1.92) – including major depressive disorder (OR=2.5)(Bruce et al., 1991). de Graaf et al using data from the Netherlands Mental Health Survey and Incidence Study, a prospective epidemiologic study of 7,076 adults age 18–64 years who were interviewed with the Composite International Diagnostic Interview, found that lower educational level was associated with an increased risk of anxiety disorders (OR = 2.32) (de Graaf et al., 2002). In addition, lower educational level (OR =2.69), and unemployment (OR = 1.73) were associated with comorbid anxiety and mood disorders but not with pure mood disorders.

Lorant et al conducted a meta-analysis of 51 prevalence studies, five incidence studies and four persistence studies to examine the relationship between SES and psychiatric morbidity (Lorant et al., 2003). They found that those from the lowest SES, had a higher odds of being depressed (OR-1.81) compared to those from the highest SES. They were also at greater risk of developing a new episode of depression (OR = 1.24) and also persisting depression (OR=2.06).

1.4.3 Social causation vs Social selection theories

Whether socioeconomic status is a cause or consequence of mental illness has long been a topic of debate. In this context, there are two main theories that have dominated the research that have explored the relationship between SES

and mental illnesses. The social causation theory suggests that conditions of life associated with low socioeconomic status markedly increase the risk of mental disorders. In other words, a person with a low SES is likely to develop a mental illness due to various adverse situations that he/she goes through. On the other hand, the social selection hypothesis suggests that SES per se do not increase the risk of developing mental illnesses. However, once a person develops a mental illnesses, it impairs his/her attainment of social and occupational status that may in turn, drive the person down the social ladder (social drift).

Classic studies by Kessler etc, have suggested the former explanation for the relationship between SES and mental health (Kessler et al., 2005, Kessler et al., 1994). In fact, recent longitudinal studies do suggest that these mechanisms are particularly relevant for some conditions, but may not be for other conditions. For example, the Dunedin study found no support for either causation or selection processes suggesting that SES and depression have little influence on each other before the age of 21(Miech et al., 1999). In that study, depression at age 15 was not overrepresented among lower SES families, it did not influence subsequent educational attainment, and increases in depression between ages 15 and 21 were not significantly overrepresented among study members with low educational attainment. However, the study population examined there consisted of adolescents and was not followed up to adulthood. Some of the life stressors that have been consistently linked to major depression – like divorce, or loss of jobs - may not have occurred at this age group, and hence the negative findings.

More recently, Skapinakis et al in a longitudinal study of 2406 individuals in the general population found that financial difficulties at the baseline were independently associated with depression at 18 months follow up (Skapinakis et al., 2006). Another longitudinal study, using the Canadian national population health survey cohort, followed up 9589 patients over 6 years. They found that low education level and financial strain were associated with greater risk of MDE in those who worked (Wang et al., 2010).

A seminal study published in 2005 by Christopher Hudson, who used structural equation modeling to show that SES does indeed exhibit a causal role in this relationship, presents the best evidence for social causation (Hudson, 2005).

Hudson used a statewide epidemiological data to show an association between lower SES and presence of mental illness. In addition, he showed that greater economic stress (but not family disintegration) played a mediating role in this relationship. Hudson found no evidence for either a geographic drift or individual socioeconomic drift in this population.

1.4.4 Stressful life events may account for the link between SES, greater inflammation and mental health.

Hatch et al (2007) conducted a systematic review of articles published between 1967 and 2005 to examine the predictors of stressful and traumatic life events (Hatch and Dohrenwend, 2007). They searched the following databases: Health and Psychological Instruments, MEDLINE, Psychinfo, Pubmed, and Socio- logical abstracts using the following search terms "life events, stressful life events, life change, life stress, traumatic events, and/or stress". They found that both traumatic and other stressful evens are reported at higher rates in the lower socioeconomic status groups. They suggest that this higher rates could be attributed to the limited opportunities and resources in lower SES that may expose the groups to greater negative events. However, they also conclude that the lower SES also may be associated with recall biases related to higher levels of distress in those living in low SES environments.

In addition, neighbourhood level deprivation has been associated with greater stressful life events. Steptoe et al, in a study of 419 residents from 18 high SES neighbourhoods and 235 from 19 low SES neighbourhoods, found that neighbourhood problems were more common in those who lived in the low SES neighbourhoods and was associated with poor self rated physical and mental health (Steptoe and Feldman, 2001). They suggest that residential neighbourhood problems can constitute a source of chronic stress that may increase the risk of poor health. However, it is likely that individual level SES may moderate the effects of neighbourhood deprivation. There is now evidence that although both individual level and neighbourhood deprivation increase the risk of poor mental health, the effect of neighbourhood deprivation was more marked in poorer individuals(Stafford and Marmot, 2003).

More recently, prospective studies have shown an association between SES and mental health. In particular, some reports have shown that life stressors determine the association between SES and mental health. Businelle et al conducted a mediation analysis on data from two waves of the National Epidemiologic survey on alcohol and related conditions study (Businelle et al., 2013). Controlling for mental health during wave 1, they examined the relationship between socioeconomic variables during wave 1 and mental health 3 years later – during wave 2. They also measured stressor exposure during wave 1. They found that SES during wave 1 predicted changes in mental health ratings in wave 2. More interestingly they found that the number of life stressors mediated the relationship between socioeconomic status and mental health.

Prospective studies in children have also shown that cumulative life risks across childhood, mediate the relationship between poverty and chronic physiological stress. For example, Evans and Kim showed that the relationship between the time spent in poverty between birth and age 9 was linked to elevated allostatic load – a marker of chronic physiological stress (measured as a composite measure of resting diastolic and systolic blood pressure; overnight epinephrine, norepinephrine, and cortisol; and BMI)- at age 17 (Evans and Kim, 2012). More importantly they showed that a cumulative risk exposure measured at age 13 (measured as exposure to physical risk factors and psychosocial risk factors) mediated the relationship between the exposure to poverty and elevated physiological risk. Evans and Schamberg also showed in the above population that the cumulative physiological stress (allostatic load) mediated the relationship between exposure to poverty in childhood and working memory in adulthood (Evans and Schamberg, 2009).

Similar to greater life stressors, a number of cross sectional and longitudinal studies have shown an association between lower SES and greater circulatory inflammatory markers. Petersen et al found that both individual level and neighbourhood level SES was associated with greater inflammatory markers – CRP and Il6 in adults aged 30 to 54 years (Petersen et al., 2008). Koster et al examined the association between SES and inflammatory markers in adults aged between 70 and 79 (Koster et al., 2006). They examined SES using education, income, and ownership of financial assets. They found that low SES was associated with greater serum levels of interleukin-6, C-reactive protein, and

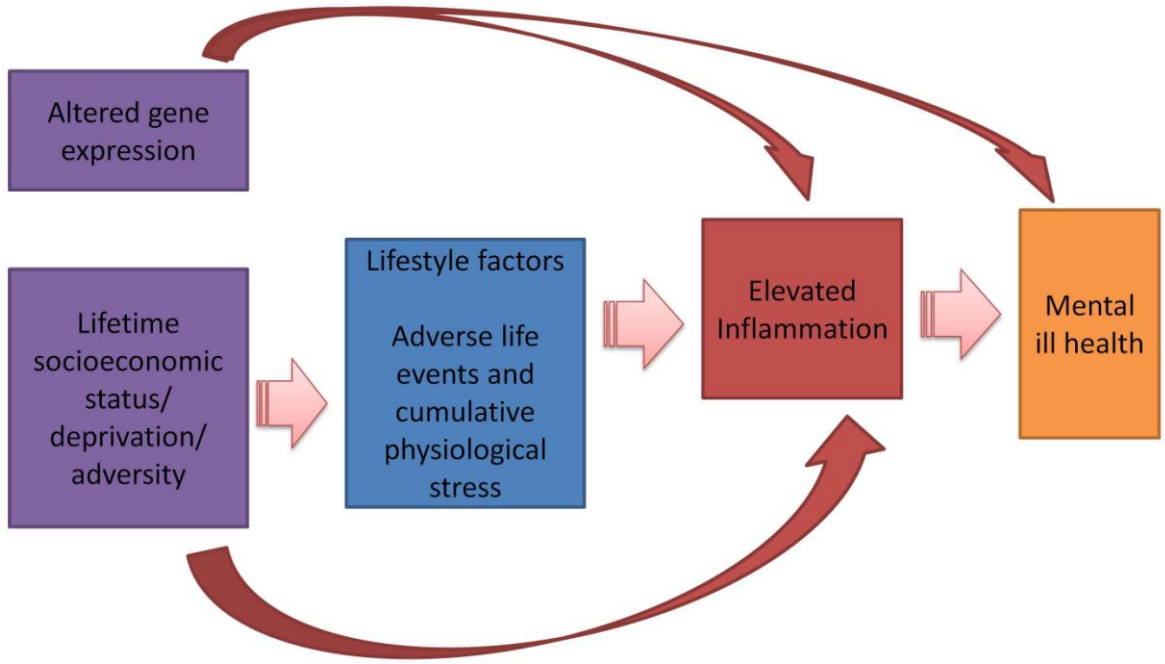
tumour necrosis factor-alpha, which was partly explained by greater smoking, drinking and obesity in the low SES group.

More recently, prospective cohort studies have shown an association between life course socioeconomic status and raised inflammation. Stringhini et al examining the a prospective occupational cohort of adults from the British Whitehall II study found that cumulative exposure to low SES from childhood to middle age was associated with greater inflammatory markers – CRP and IL6 levels after adjustment for sex, age and ethnicity (Stringhini et al., 2013). They also found cumulative low SES to be associated with greater risk of type 2 diabetes.

Caroll et al examined the association between SES across distinct periods of childhood (averaged across 2 year periods between age 1 and 18) and levels of IL 6 in adulthood (Carroll et al., 2011). They found that lower SES during early childhood (years 1 and 2) was associated with greater levels of IL 6 in adulthood. They suggest that early environment may program immune phenotypes that contribute to disease risk. SES differences in gene regulation of response to stress can be reflective of environmental/dietary exposures occurring over the life course or be a direct consequence of developmental programming in early life.

Stringhini et al proposed that elevated inflammation that result from altered gene expression and unhealthy lifestyle might mediate the association between SES and type-2 diabetes (Stringhini et al., 2013). Taking a similar approach, it could be argued that as autonomic nervous system (ANS) and the HPA axis are thought to play an important role in regulating circulating inflammatory markers, SES could impact on peripheral immune/ inflammatory markers through these stress-mediated pathways (HPA/ANS). The inflammatory and stress related pathways together have an impact on the brain (through signaling pathways mentioned earlier), thereby making it vulnerable to mental ill health (Figure 1-1).

Figure 1-1: A simplified pathway through which socioeconomic status may affect mental health.



Socioeconomic deprivation has been shown to be associated with greater risk of mental ill health. This relationship may be mediated through elevated inflammation that results from altered gene expression, unhealthy lifestyles, dysregulation of HPA and ANS axis as a result of adverse life events/ stressors. *(This diagram is modified from Stringhini S, Batty GD, Bovet P, Shipley MJ, Marmot MG, et al. (2013) Association of Life-course Socioeconomic Status with Chronic Inflammation and Type 2 Diabetes Risk: The Whitehall II Prospective Cohort Study. PLoS Med 10(7): e1001479.)*

1.5 Depression in the context of inflammatory medical conditions

One possible way of improving our understanding of the relationship between inflammation and MDD is the study of MDD co-morbid with physical (medical) illness. This allows us to explore how a known inflammatory patho-physiological process might impact on the brain. MDD occurs at a 5-10 times higher rate in those with medical illness, and worsens prognosis and disability. This is particularly true when the medical illness is associated with an autoimmune process. MDD is by far the most common psychiatric manifestation of Multiple Sclerosis, with a lifetime prevalence of around 50% and rates of suicide as high as 15% (Lo Fermo et al.). This association is also seen in peripheral (as opposed to only CNS) inflammation, in diseases such as Psoriasis, Rheumatoid Arthritis (RA) and inflammatory bowel diseases. Conservative estimates of rates of MDD are between 13% and 30% in these patients (Krishnadas et al., 2011, Dickens et al., 2002, Graff et al., 2009). This is also true in cases of medical illness with "low grade" inflammation, eg cancer, stroke, coronary artery disease and epilepsy that are not traditionally considered to have a primary inflammatory aetio-pathology. Acute brain ischemia is associated with an inflammatory response that contributes to ischemic damage (Pascoe et al., 2011). Conservative estimates suggest that around 30% of people who survive a stroke experience clinical MDD (Hackett and Anderson, 2005). Similarly, epilepsy is associated with significantly high rates of MDD. Pro-inflammatory cytokine mediated changes in glutamatergic neurotransmission are thought to be relevant in the etio-pathogenesis of seizure and epilepsy(Vezzani et al., 2008a, Vezzani et al., 2011). Similar rates of MDD are seen in cancer and cardiovascular illness (Celano and Huffman, 2011). In the context of medical illness, inflammation may trigger a major depressive episode in vulnerable individuals. Inflammation, in this context may act as a precipitating and perpetuating factor in predisposed individuals. Karg et al in a recent meta-analysis found that those with a short allele (SS) functional polymorphisms (5-HTTLPR), of the promoter region of the serotonin transporter gene, (predisposition) were more likely to develop a MDD

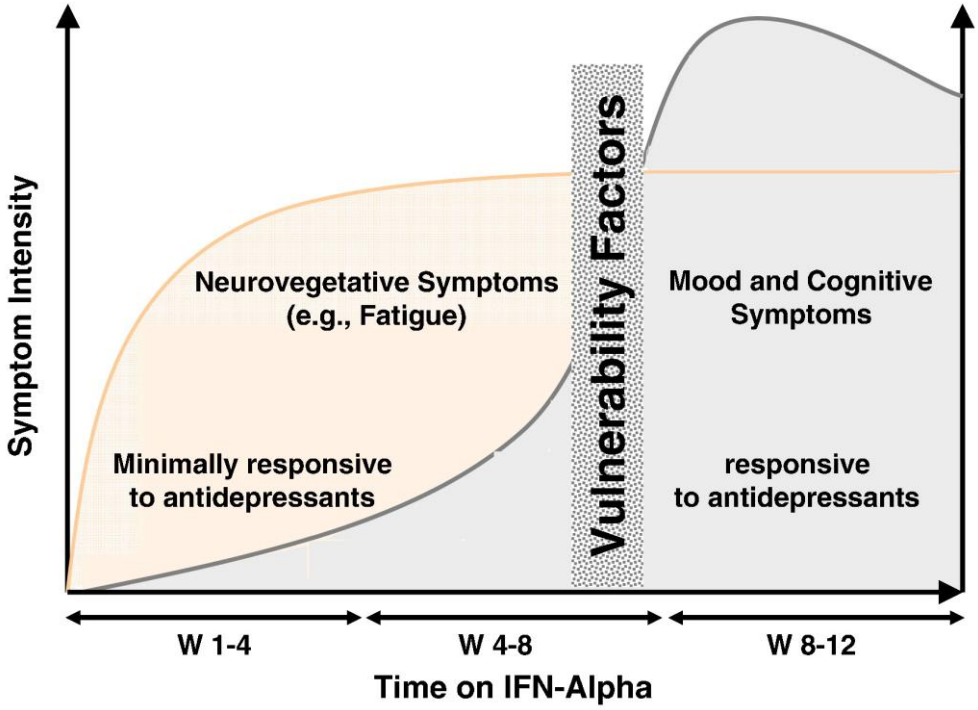
episode in the presence of specific medical condition (stressor)(Karg et al., 2011).

1.5.1 Cytokine therapy induces depressive symptoms

Cytokines such as IFN-α (interferon alpha) and IL-2 (interleukin 2) are used as immunotherapy for the treatment of chronic hepatitis C and cancer (Myint et al., 2009, Capuron et al., 2002). There is good evidence to suggest that those who undergo these treatments are more prone to develop MDD. Sockalingam et al recently reviewed 9 prospective studies that used clinician-rated measures to detect major depressive disorder in patients treated with interferon-α for hepatitis C (Sockalingam et al.). They found that the prevalence of IFNα-induced depression was in the range of 10% to 40% with more rigorous studies suggesting a prevalence approximating 20% to 30%. How these cytokines induce depressive changes, is a matter of much debate. Interferon-α-induced depressive symptoms are associated with changes in cytokine levels in the serum. Capuron and Miller proposed the timeline for the development of depressive symptoms during treatment with IFN alpha (Capuron and Miller, 2011). They suggest that within the first 4 weeks of treatment, people develop neurovegetative factors (similar to sickness behaviour) . However, in people who are vulnerable, core mood/ cognitive symptoms start to appear within 8 to 12 weeks (Figure 1-2). The temporal relationship between the onset of inflammation, the onset of physical and emotional symptoms may however depend on the type of inflammatory stimulus, the instruments used to measure the symptoms and the presence of other comorbidities. For example, Wright et al have shown that injection with Typhoid vaccine in human adults can induce negative mood within hours of injection(Wright et al., 2005). They measured used the profile of mood states (POMS) scale, an instrument that can detect subtle fluctuations in mood.

.

Figure 1-2: Temporal evolution of the neuropsychiatric symptoms induced by chronic interferon-alpha therapy.



Interferon (IFN)-alpha therapy induces two types of behavioural symptoms with differential time course and responsiveness to antidepressants. The neurovegetative symptoms (e.g., fatigue, anergia and psychomotor slowing) develop rapidly (as soon as week 1 [W1]) in almost every individuals exposed to cytokines and persist during the duration of IFN-alpha therapy. These symptoms are minimally responsive to antidepressant treatment. In contrast, the mood and cognitive symptoms (e.g., depressed mood, anxiety, irritability, memory and attentional disturbance) develop in vulnerable patients at later stages of IFN-alpha therapy (between weeks 8 and 12) and are highly responsive to antidepressant medication. Reused with permission from Capuron and Miller 2011 Immune system to brain signaling: Neuropsychopharmacological implications Pharmacology and therapeutics. Volume 130, Issue 2, May 2011, Pages 226–238

A number of studies have therefore examined if there are certain biomarkers associated with depressive changes, that may predict who will go on to develop depression when treated with these cytokines. IFN-α treatment may directly or indirectly affect neurotransmitter systems in the CNS. A reduction in peripheral serotonin level has also been demonstrated in hepatitis C patients during IFN-α treatment (Schafer et al., 2010). Several studies have demonstrated positive correlation between increased depression symptoms during IFN-α therapy and metabolites of Indolamine deoxygenase enzyme (see below) in blood and CSF of patients with hepatitis C (Raison et al., 2010). Another area of much interest

has been, trying to predict who will develop MDD in response to these treatments. Interestingly the short allele of 5HTTLPR has been associated with increased risk of depressive symptoms during IFN-α therapy(Bull et al., 2009). However, the findings are not consistent. A more recent study of hepatitis C patients demonstrated that those with 'high transcription' serotonin genotype (LL) showed greater depressive symptoms during IFN-α therapy compared to those with the short allele (SS) (Pierucci-Lagha et al., 2010). Other studies have implicated polymorphisms in other genes (e.g. IL6) that may confer greater risk or protection from developing MDD in response to cytokine treatments (Bull et al., 2009).

Lotrich et al in a prospective study of 124 euthymic people undergoing IFN alpha, assessed if serum BDNF and the Val66Met BDNF polymorphism and the SERT ss/ll promoter region polymorphism predicted the depressive symptoms during treatment (Lotrich et al., 2009). Lower pre treatment BDNF was associated with higher depressive symptoms during IFN alpha treatment. However the Met allele was only associated with increased MADRS scores but not BDI or HADS. Met allele was also associated with suicidal ideation and sadness and worthlessness (cognitive / mood symptoms) but not the neurovegetative symptoms. Interestingly, the SERT ss polymorphism was associated with neurovegetative symptoms, but not cognitive symptoms. IFN therapy also reduced the BDNF levels in serum. However, this was not associated with the development of depression. They suggest that while decrease in BDNF was not associated with a worsening of depressive symptoms, the fact that those who developed depressive symptoms had lower pre treatment BDNF, suggest that greater BDNF levels prior to treatment may protect against developing mood symptoms

1.6 How may pro-inflammatory cytokines cause MDD?

Pro-inflammatory cytokines may provoke changes in brain structure and function, leading to the development of MDD. The mechanisms by which these peripheral inflammatory responses signal the brain is unclear. Cytokines can directly modulate pathways implicated in the aetiology and treatment of

depression. Suggested mechanisms include the effect of cytokines on the HPA axis, on neurotransmission; and direct action on hippocampal neurogenesis.

1.6.1 Cytokines exist in the brain, and may therefore exert an effect on the brain

Traditionally the brain was considered an “immune –privileged” organ. However, it is now known that the brain is indeed susceptible to immune-mediated insult. Cytokines are large proteins – proteins, peptides or glycopeptides – that form part of a large family of cell signalling molecules. They are formed and released as a “cascade”, where induction of one of the molecules can trigger the activation of a number of molecules. In the brain, cytokines are produced by neurons, microglia and astrocytes. Cytokines in the brain are “gliotransmitters” that act on a number of receptors, and are thought to be key in a number of brain functions. They may be activated in a number of ways as shown Capuron and Miller in Figure 1-3. Where the primary focus of immune activity is the brain (eg post-stroke depression, MS), it is thought that cytokines are produced in the brain itself. Additionally, we now know that peripheral cytokines can signal the brain through at-least 5 mechanisms(Capuron and Miller, 2011).

a. Passage of cytokines through “leaky” regions in the blood brain barrier (BBB) – e.g. the circumventricular organ - Humoral pathway

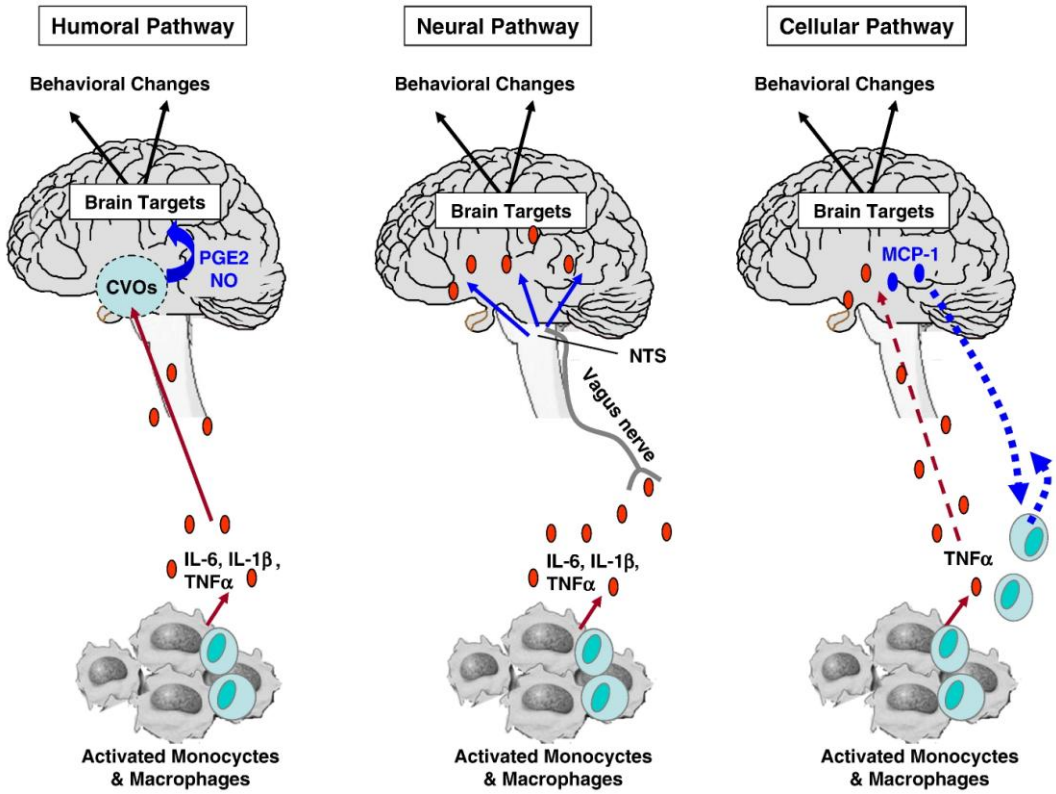
b. Active transport of cytokines across BBB

c. Transmission of signals along the afferent vagal pathway - neural pathway

d. Entry of activated monocytes from periphery into the brain – chemokines are increasingly seen as having a role here.

e. Second messenger signals from endothelial lining of the BBB, which in turn, leads to an excess production of cytokines by glia.

Figure 1-3: Communication pathways from the periphery to the brain.



Different pathways by which cytokine signals access the brain have been identified. A) Humoral pathway: Pro-inflammatory cytokines released by activated monocytes and macrophages access the brain through leaky regions of the blood-brain barrier such as the choroid plexus and circumventricular organs (CVOs). Within the brain parenchyma, the activation of endothelial cells is responsible for the subsequent release of second messengers (e.g., prostaglandins [PGE2] and nitric oxide [NO]) that act on specific brain targets. B) Neural pathway: Pro-inflammatory cytokines released by activated monocytes and macrophages stimulate primary afferent nerve fibres in the vagus nerve. Sensory afferents of the vagus nerve relay information to brain areas through activation of the nucleus of the tractus solitarius (NTS) and area postrema. C) Cellular pathway: A cellular pathway has been recently described by which pro-inflammatory cytokines, notably TNF-α , are able to stimulate microglia to produce monocytes chemoattractant protein-1 (MCP- α), which in turn is responsible for the recruitment of monocytes into the brain. Reused with permission from Capuron and Miller 2011 Immune system to brain signalling: Neuropsychopharmacological implications Pharmacology and therapeutics. Volume 130, Issue 2, May 2011, Pages 226–238.

1.6.2 Action on neurotransmitters

1.6.2.1 Monoamine neurotransmission

Serotonin (5-hydroxytryptamine, 5-HT), a monoamine neurotransmitter, has been implicated in many normal behaviours, including sleep, appetite, sexual behaviour(Canli and Lesch, 2007). It has also been implicated in cognitive and emotional function. More importantly, it is implicated in the mono-amine theory of depression (Schildkraut, 1965). In short, this hypothesis suggests that depressive states are caused due to a decrease in monoamine neurotransmitters – particularly serotonin in the synaptic cleft. Serotonin that is released into the synaptic cleft is either metabolised or actively transported back into the neuron by a protein in the neuronal cell membrane(Maximino, 2012). This protein is called the Serotonin transporter / reuptake transporter (SERT). The gene that codes for this protien (SERT) is situated on chromosome 17. This gene transcription is regulated by a polymorphic promoter region – (5HTTLPR) (Canli and Lesch, 2007).

Serotonin levels in the brain are controlled by a number of factors including rate of synthesis, rate of release into the synaptic cleft, rate of enzymatic breakdown, rate of diffusion through the extracellular space and active reuptake (Hornung, 2010, Maximino, 2012). In the brain, SERT seems to be the predominant mechanism controlling the strength and duration of serotonergic neurotransmission. Therefore it is likely that SERT availability is directly related to serotonergic neurotransmission. In humans, SERT has been clearly identified in a number of brain regions, including the brain stem, hypothalamus, occipital and frontal cortices (Hornung, 2010). In the neurons, SERT is located in the presynaptic terminus – i.e. the axonal nerve endings. SERT plays a key role in scavenging serotonin transporter from synaptic cleft back to the axonal nerve ending. Surprisingly SERT is found in neuronal cells and non-neuronal cells. In humans, SERT has been found in glia, platelets, the GIT, monocytes and the placental syncitioblast.

A number of studies have shown that the immune system may have prominent effects on SERT in cell systems and in adult animals (Baganz and Blakely, 2013). For example, as early as 1995, Ramamoorthy et al showed that interleukin-1ß

(IL-1ß) stimulates the activity of SERT in human JAR choriocarcinoma cells(Ramamoorthy et al., 1995). The stimulation was associated with an increase in the steady state levels of transporter mRNA and transporter density. They also showed that this increase in SERT mRNA was blocked by actinomycin D – a transcription inhibitor, thus suggesting that the increase in mRNA may due to an increased transcription of the SERT DNA. They in addition showed that this regulation may be independent of the cAMP pathway. However, there also seems to be cell surface SERT trafficking independent mechanisms through which inflammation may affect serotonin neurotransmission. Zhu et al. (2006) showed, that both IL-1ß and TNF-α cause rapid activation of SERT in raphe neuron derived RN46A cells (Zhu et al., 2006). They also found that this increase in activity was p38 MAPK dependent. Given p38MAPK has an important role in sustaining SERT expression at the plasma membrane immune factors that operate via this signalling mechanism could have marked effects on serotonergic neurotransmission.

SSRI antidepressants have been reported to be effective in inducing and sustaining remission of inflammation in patients with rheumatoid arthritis (Krishnadas and Cavanagh, 2011). There seems to be a bidirectional relationship between serotonergic systems and inflammation. A key site of action of antidepressants is the serotonin transporter (SERT), which regulates serotonergic neurotransmission. There is increasing data in animal and humans to suggest that inflammation is associated with neuronal SERT activity. Cavanagh et al in a small pilot study of 6 subjects, have previously shown that treatment with TNF-α blockade agent adalimumab led to a decrease in serotonin transporter binding by up to 20%, using [123I] Beta CIT – SPECT (Cavanagh et al., 2010).

There is evidence that proinflammatory cytokines including TNF-α induce glial indoleamine dioxygenase (IDO)(Maes et al., 2011). This activates the kynurenine pathway, thus channelling the available dietary tryptophan (the substrate for serotonin synthesis) to form Kynurenine (Kyn), 3 Hydroxy kynurenine (3HK) and Quinolinic acid (QUIN), rather than the serotonin (5HT). In addition to decreasing serotonin availability in the neuron, the accumulating 3HK and QUIN- both NMDA receptor agonists, contribute to excitotoxicity and calcium mediated cell death (Christmas et al., 2011).

Conversely, serotonergic systems have been found to significantly impact on inflammatory pathways. Descending spinal serotonergic pathways have been implicated in the physiology of pain modulation. Zhao et al showed that knockout mice that lacked these descending serotonin pathways in the brain exhibited enhanced inflammatory pain (but normal visceral and thermal pain) compared with their littermate control mice. They showed that the analgesic effects of SSRI antidepressants were absent in this strain of mice, suggesting that serotonergic pathways play an important role in modulating inflammatory pain, compared with mechanistic pain (Zhao et al., 2007).

Recent findings suggest that antidepressants have anti-inflammatory and analgesic properties. O'Brien et al showed that CRP levels dropped following treatment with antidepressant (O'Brien et al., 2006). Piletz et al found that raised proinflammatory biomarkers in patients with major depressive disorder showed a decrease in response to treatment with venlafaxine (a serotonin and nor-epinephrine reuptake inhibitor, exhibiting serotonin reuptake inhibition at lower doses, and norepinephrine reuptake inhibition at higher doses) at the serotonergic (lower) dose range rather than the norepinephrine (higher) dose range, suggesting that sertonergic pathways mediate anti-inflammatory response to antidepressants (Piletz et al., 2009).

Recent experimental data show that peripheral activation of 5-HT2A receptors in primary aortic smooth muscle cells leads to an extremely potent inhibition of TNF-α mediated inflammation, another possible mechanism of action of SSRIs in mediating the anti-inflammatory action (Yu et al., 2008). SSRIs including escitalopram are thought to increase extracellular serotonin concentrations at these receptors. However, SSRIs are thought to down-regulate 5HT2A in the long run. Surprisingly, blockade of 5HT2A receptors also has the same effect, i.e. down-regulation. However, it is possible that down-regulation of these receptors decreased with age, suggests that SSRI antidepressants may have a potential role in treating inflammatory conditions, at least in the older population. In spite of the good evidence for the use of tricyclic antidepressants and venlafaxine in the treatment of neuropathic pain (NNT = 3), data regarding the use of SSRI in neuropathic pain is inconclusive (Saarto and Wiffen, 2007). Evidence for the use of antidepressants in inflammatory conditions is even less promising, largely due to the lack of good quality data. Richards et al reviewed the available evidence

for the efficacy of antidepressants in pain in patients with RA, and found no conclusive evidence (Richards et al., 2011). They reviewed eight randomised controlled trials looking at tricyclic antidepressants and two trials evaluated a SSRI as a comparator. The quality of the studies included was poor, and there was insufficient data for a number needed to treat to be calculated for the primary outcome measure of pain. They conclude that there is currently insufficient evidence to support the routine prescription of antidepressants as analgesics in patients with RA and that the use of these agents may be associated with greater adverse events. Similarly, Micocka-Walus in a review of 12 non randomised controlled studies of antidepressants in inflammatory bowel disease, found no conclusive evidence of efficacy of antidepressants on disease prognosis in IBD (Mikocka-Walus et al., 2006). They found that although there was some benefit in the use of antidepressants in IBD, the quality of data available to reach a conclusion was not good enough. The authors of the both the above reviews, propose that better conducted prospective studies are required to address this issue.

More recently, Thorsland et al showed a relationship between SERT availability and disease severity, chronic stress and depression in patients psoriasis (Thorslund et al., 2013). They used biopsies from involved and non involved skin from the back of 20 patients with chronic plaque psoriasis. SERT levels in the skin were assessed using immunohistochemistry. They showed a positive correlation between number of SERT positive dendritic cells in patients with psoriasis and PASI scores. There was a negative correlation between the number of SERT positive cells and salivary cortisol levels. They suggest that the relationship between chronic stress (as measured using cortisol) and disease activity in psoriasis may be mediated by local SERT expression.

Another monoamine that has been implicated in major depression is dopamine, particularly in symptoms associated with anhedonia and sickness behaviour. As with serotonin, proinflammatory cytokines influence the synthesis and reuptake of dopamine (Moron et al., 2003, Wu et al., 2007).

1.6.2.2 Glutamate neurotransmission

Glutamate induced excitotoxicity - excess activation of neuronal glutamate receptors that ultimately leads to cell death - has been implicated in mediating neuronal death in many disorders, including stroke and neurodegenerative disorders. Glutamate induced excitotoxicity has also been implicated in psychiatric disorders like depression (Lee et al., 2002). Excessive accumulation of intracellular calcium is thought to be the major step that leads to neuronal cell death. The type of receptor that has been most implicated in glutamate excitotoxicity is the NMDA subtype. It is thought that overstimulation of these receptors would lead to an overload of calcium, and in turn, neuronal death. Other receptors, like the AMPA and Kaininate receptors, are also thought to play a role in excitotoxicity, as their ion channels are partially permeable to calcium (Mark et al., 2001). Inflammatory processes have been found to be associated with an increase in glutamate-induced neurotoxicity. Pro-inflammatory cytokines are thought to mediate this process (Vezzani et al., 2008b). Mechanisms by which these inflammatory mediators cause an increase in glutamate neurotransmission include

a. Up-regulation and augmentation of glutamatergic pathway

   It has been recognized that hippocampal neurons exposed to IL1ß and TNF-α intensify the excitotoxic neuronal damage induced through NMDA and AMPA receptors(Bernardino et al., 2005, Pickering et al., 2005). Action of IL1 ß on the glutamatergic system is thought to be through its action on the IL1 R1 receptors. These receptors co-localise with NMDA receptors on hippocampal neurons. NMDA receptors consist of two subunits NR1 and NR2. NR2 subunits have further isoforms (Mark et al., 2001). It is proposed that IL1 ß induces phosphorylation of the NR2B isoform, which leads to an upregulation of NMDA receptor function. This leads to an increase in calcium influx into the neuron, and consequent cell death (Viviani et al., 2003).

b. Increased release and reuptake inhibition of glutamate

   IL1 ß has also been found to inhibit the reuptake of glutamate by the glial cells. This is thought to be mediated through the action of these

proinflammatory cytokines on the expression of glutamate transporter. This malfunction of the transporter leads to an increase in extracellular glutamate and further NMDA mediated excitotoxicity. In addition to this, IL1β has been found to activate NO synthase which leads to an increase in production of NO and hence an increase in glutamate release (Hu et al., 2000).

c. Action on AMPA receptors

TNF-α has been shown to influence the trafficking of AMPA glutamate receptors in inflammatory conditions. Normally AMPA receptors have 4 subunits GluR 1-4. The presence of TNF-α leads to a production of AMPA receptors lacking the GluR2 subunit. This receptor conformation is said to facilitate calcium influx into the neuron. This predisposes the neuron to glutamate induced excitotoxicity (Stellwagen et al., 2005).

d. Activation of kynurenine pathway

The impact of the kynurenine pathway on excitotoxicity was described earlier. This activation of kynurenine pathway by pro inflammatory cytokines thus channels the available tryptophan to form Kynurenine (Kyn), 3 Hydroxy kynurenine (3HK) and Quinolinic acid (QUIN). 3HK and QUIN are NMDA receptor agonists. High concentrations of these compounds are thought to contribute to excitotoxicity and calcium mediated cell death (Maes, 2008).

1.6.3 Action on Hypothalamus- Pituitary – Adrenal (HPA) axis - HPA axis over-activity and glucocorticoid resistance

HPA axis abnormalities have been reported in MDD (Pariante, 2003). Over-activity of this system has been attributed to glucocorticoid receptor (GR) resistance, secondary to either reduced expression of GR or decreased functionality of GR. There is some evidence that proinflammatory cytokines including TNF-α, induce glucocorticoid resistance through the above mechanisms. Functional inhibition is induced by preventing the entry of the cortisol-GR receptor complex into the nucleus (by inducing Jun aminoterminal kinase – JNK), and also by preventing the binding of the complex to the DNA (by inducing nuclear factor kB – NFkB) (Pace et al., 2007, Pace and Miller, 2009).

This in turn leads to altered expression of GR in the cells. Change in expression and functionality of the system can be measured in vitro, pre and post treatment, with TNF-α blockers. Recent work by Anacker et al links glucocorticoid mechanisms to neurogenesis (a process thought to be key in mediating the action of antidepressants). They suggest that an activation of the GR is necessary for the antidepressant-induced modulation of neurogenesis in humans (Anacker et al., 2011).

1.6.4 Cytokine mediated neurogenesis and neuronal loss

Neurogenesis in the hippocampus of the adult brain is thought to contribute to memory and learning. While, a pathogenic role for reduced hippocampal neurogenesis in depression is unclear, there is now considerable experimental evidence that the generation of new neurons in the dentate gyrus of the hippocampus is enhanced by antidepressant treatment (Sahay and Hen, 2007). There is a body of evidence to suggest that inflammation induces a decrease in neurogenesis. As mentioned above, this decrease may in part be due to HPA axis abnormalities, which, when corrected, restores neurogenesis. Cytokine-mediated regulation of hippocampal neurogenesis in experimental animals is indicated by several pieces of evidence:-

• Monje et al reported that neuroinflammation inhibits neurogenesis and that inflammatory blockage with an NSAID restores neurogenesis (Monje et al., 2003).

• in exploring a potential mechanism underlying depression induced by IFN a, treatment, Kaneko et al found that IFN a suppressed neurogenesis in the dentate, and that IL1 ß played an essential role in that suppression (Kaneko et al., 2006).

• stress-induced reduction in hippocampal neurogenesis is attenuated by blockade of the IL1-beta receptor (Koo and Duman, 2008).

• Mice deficient in TNF-α receptor-1 (responsible for neuronal damage) exhibit enhanced hippocampal neurogenesis (Iosif et al., 2006).

There is some data to suggest that inflammatory cytokines may affect the expression of trophic and growth factors. However the results are inconsistent (Saha et al., 2006). In spite of the above studies, there is still a dearth of data on the involvement of inflammatory mediators in decreasing neurogenesis. There are very few studies that have looked at the effect of anti-inflammatory drugs on neurogenesis and depression.

1.6.5 Cytokine-induced inflammation modulates sickness behaviour and neuronal activation

Systemic inflammation is known to elicit symptoms in healthy mammals, collectively called “sickness behaviour”(Capuron and Miller, 2011). This consist of behavioural changes, including disturbance in sleep, appetite, psychomotor slowing, memory impairment and behaviour that are thought to be very similar to biological symptoms of depression in humans(Dantzer et al., 2008, Kelley et al., 2003, Konsman et al., 2002). Interferon therapy is associated with sickness-like symptoms, including lack of sleep, loss of appetite, weight loss, fatigue, usually occurred during the first 2-4 weeks of the therapy and can be early indicators for depression, which usually develops 1-3 month after the treatment (Capuron et al., 2002, Capuron and Miller, 2011). Capuron et al found that in spite of considerable overlap in symptom between cytokine-induced depression and idiopathic depression in medically healthy subjects, psychomotor symptoms were much greater in the cytokine induced MDD group, while cognitive distortions were greater in those with idiopathic MDD (Capuron et al., 2009). A common clinical experience is people receiving flu or typhoid vaccination developing symptoms of fatigue, psychomotor slowing and depressed mood. Clinical studies have shown that these changes are significantly associated with increased IL-6 levels (Wright et al., 2006, Wright et al., 2005). On fMRI, these people have also been shown to activate brain regions thought important in modulating mood. Areas that have been shown to be activated include the insula, an area thought to be important in body representation and subjective emotional experience, and substantia nigra that correlated with measures of fatigue and psychomotor slowing – findings that are consistent with the clinical findings of Capuron et al described above (Brydon et al., 2008, Harrison et al., 2009b).

1.6.6 Cytokine-mediated cognitive dysfunction

Neurocognitive function reflects functional integrity of neuronal structures. Patients suffering from MDD show a number of cognitive deficits during periods of illness – particularly in the domains of attention, memory and executive function. In patients who have recurrent episodes of MDD, deficits on performance on tests of executive function have been shown to persist into periods of recovery, suggesting that these deficits may be trait markers of the illness (Smith et al., 2006). Cognitive dysfunction has been shown to correlate positively with the presence of proinflammatory cytokines and other inflammatory mediators in various illnesses (Wilson et al., 2002). There is considerable evidence to show that proinflammatory cytokines play an important role in cellular mechanisms underlying cognition including synaptic plasticity (Albensi and Mattson, 2000). IL1B and TNF-α play an important role in long term potentiation and depression. It is postulated that while normal levels of cytokines are essential for the consolidation and integration of AMPAs on the neuron, in excess, they tend to have a detrimental effect. In addition, TNF-α seems to play an important role in synaptic scaling (homeostatic plasticity) in hippocampal neurons(McAfoose and Baune, 2009). Any imbalance in the normal milieu of TNF-α is said to affect long term synaptic plasticity and hence cognition. In this respect, measures of neuro-cognition before and after treatment with TNF-α antagonists may provide us with some clue to the effect of the treatment on neuronal (structural/functional) integrity in various circuits/ parts of the brain involved in these tasks (McAfoose and Baune, 2009). Recent reports show that TNF-α antagonist etanercept administered intrathecally has been used in the treatment of patients with Alzheimer's disease (Tobinick, 2009). Currently, there is one randomised controlled study underway, looking at the effects of sub-cutaneous etanercept in the treatment of alzheimer's disease. (Clinicaltrials.gov - NCT01068353)

Chapter 2. Problem statement and motivation for the project

In the first chapter, I have summarised some evidence that may link inflammatory processes with mental illnesses, particularly Major depressive disorder. I have explored this in the context of major depressive disorder (a primary psychiatric condition), medical illnesses and socioeconomic deprivation – the latter two being conditions that are high risk factors for the development of MDD. However the relationship between circulating inflammatory markers and the brain is far from clear, especially in humans. Considering the theoretical evidence behind the relationship between inflammation and the brain, it is reasonable and worthwhile to explore the relationship between peripheral circulating inflammatory markers and the brain.

In the following chapters, I intend to describe methods and findings of a series of experiments that were conducted to answer one question (among the number of unanswered questions)

Is there a relationship between circulating inflammatory markers in peripheral blood and the brain structure?

In order to address this, I have done a series of analyses on both observational and interventional (experimental) data. Firstly, I present observational data from a cohort of neurologically healthy individuals who have high levels of peripheral inflammatory markers in the context of neighbourhood deprivation(Velupillai et al., 2008). This cohort was derived from the larger PSOBID study, the details of which are available online. http://www.gcph.co.uk/work_themes/additional_work/psobid

Secondly, I present data from an experimental study, exploring the effect of TNF-α Blockade treatment with Etanercept on the serotonin transporters in a cohort of patients with a peripheral (non-CNS) inflammatory pathology – Psoriasis/ Psoriatic arthritis.

In order to measure the brain substrates, I have used two modes of imaging techniques – structural MRI and SPECT (to measure serotonin transporter).

2.1 Aims of the thesis

To explore the relationship between circulating inflammatory markers and the brain

2.2 Objectives

To use observational and experimental neuroimaging data to explore the relationship between circulating inflammatory markers and the brain.

2.3 Overview of the following chapters

1. In Chapter 3, I present the findings of an observational study that explores the relationship between circulating inflammatory and metabolic risk factors and cortical thickness in a cohort of neurologically healthy adult males. In this chapter, I conduct a correlation analysis of circulating inflammatory and metabolic markers with cortical thickness across the whole cerebral cortex. Here I show that inflammatory markers and indeed other metabolic markers may contribute to significant variance in the thickness across the cortical mantle. These findings were recently published as **Krishnadas *R, et al. Cardio-metabolic risk factors and cortical thickness in a neurologically healthy male population: Results from the psychological, social and biological determinants of ill health (pSoBid) study. Neuroimage Clinical. 2013 Apr 22;2:646-57 (Krishnadas et al., 2013b). The materials in the chapter are presented with permission from the publishers.***

2. In Chapter 4, I explore the above relationship further, by demonstrating that a composite index of circulating inflammatory markers mediate the relationship between neighbourhood deprivation and cortical thickness. Using surface based morphometry, I initially show that neighbourhood deprivation is associated with differences in cortical thickness and surface area in regions of interest that have been implicated in key cognitive functions in the past. I then

used mediation analysis to show that the relationship between SES and cortical thickness pertaining to the language regions of the brain, are at least in part mediated by greater inflammatory markers. These findings were recently published as ***Krishnadas R, et al. Socioeconomic deprivation and cortical morphology: psychological, social, and biological determinants of ill health study. Psychosomatic Med. 2013 Sep;75(7):616-23 (Krishnadas et al., 2013c). The materials in the chapter are presented with permission from the publishers.***

3. In chapter 5, I use complex network analysis using graph theory to show that network structural properties covary with neighbourhood deprivation. Specifically neighbourhood deprivation is associated with differences in modularity and overlapping modularity - a novel marker called grey nodes. I also show that greater circulating inflammatory markers at least in part mediate the difference in structural properties. The findings were recently published as ***Krishnadas R, et al. The envirome and the connectome: exploring the structural noise in the human brain associated with socioeconomic deprivation. Frontiers in Human Neurosciences. 7:722. doi: 10.3389/fnhum.2013.00722 (Krishnadas et al., 2013a). The materials in the chapter are presented with permission from the publishers.***

4. In chapter 6, I explore the relationship between neighbourhood level deprivation and cortical and subcortical regions that constitute the stress circuits. These included the hippocampus, amygdala and other cortical structures like perigenual anterior cingulate cortex (pgACC) and orbitofrontal cortex (OFC). Once again, I demonstrate that inflammatory pathways mediate the relationship between neighbourhood deprivation and cortical and subcortical stress circuits.

5. In chapter 7, I present the findings of an experimental study that explores the relationship between circulating inflammatory markers and SERT in a cohort of patients with Psoriasis and psoriatic arthritis. Here, I also explore if TNF-α blockade therapy using Etanercept would be associated with a reduction in inflammation, and if this reduction in inflammation is associated with changes in SERT binding using SPECT (Beta CIT). I have also explored if this is associated with behavioural changes.

6. Finally, in chapter 8, I have summarised the finding from the thesis. I have detailed the limitations of the studies presented above, and provided a brief update on human research relevant to this area.

Chapter 3 - Relationship between cardio-metabolic and inflammatory risk factors and cortical thickness in a neurologically healthy male population

In this chapter, I will explore the relationship between circulating cardio-metabolic risk markers - including the classic risk factors like circulating lipid levels and novel "emerging" risk factors like circulating inflammatory markers and the brain. The aim of the chapter is to explore the variance in cortical thickness across the mantle, that is attributable to circulating inflammatory and metabolic risk markers. Here, I used T1-weighted MRI to create models of the cortex for calculation of regional cortical thickness in 40 adult males (average age = 50.96 years). I examined the relationship between the above mentioned risk markers and cortical thickness across the whole brain, using general linear models. I also explored the relationship with other covariates of interest. The findings of this chapter have been published in *Krishnadas R, et al. Cardio-metabolic risk factors and cortical thickness in a neurologically healthy male population: Results from the psychological, social and biological determinants of ill health (pSoBid) study. Neuroimage Clinical 2013 Apr 22;2:646-57.*

3.1 Introduction

3.1.1 Classic cardio-metabolic risk factors and the brain

Epidemiological studies have demonstrated a link between increasing body mass index (BMI) – a commonly used index to define obesity, elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and triglycerides (TG) and cerebrovascular and cardiovascular disease (Denke et al., 1994, Krauss et al., 1998). While the above mentioned risk factors are thought to drive the development and progression of atherosclerosis, greater high-density lipoprotein (HDL) is thought to be anti-atherogenic and therefore protective against these diseases(Toth, 2004). These “classic” cardiovascular risk factors have also been associated with poor physical, mental health and neuro-cognitive function (Elias et al., 2005a, Elias et al., 2005b). Most studies that have examined the relationship between these risk factors and the brain in healthy adults have generally found an inverse association between the risk factors and brain

volume. Gunstad et al in a large sample of 201 healthy individuals using voxel based morphometry (VBM) analysis, found that obese (BMI > 30) individuals had significantly smaller whole brain and total grey matter (GM) volume compared to normal and overweight individuals (Gunstad et al., 2008). Raji et al using tensor-based morphometry (TBM) in 94 elderly subjects found that obese subjects with a high BMI (BMI > 30) showed atrophy in the frontal lobes, anterior cingulate gyrus, hippocampus, and thalamus compared with individuals with a normal BMI (18.5-25)(Raji et al., 2010). Studies that have examined the relationship between cholesterol and grey matter are fewer and have shown inconsistent results. For example, Ward et al in a sample of 183 individuals using VBM analysis did not find any association between non-HDL cholesterol and grey matter volume (Ward et al., 2010). They however found a significant positive correlation between HDL cholesterol and regional GM volumes pertaining to bilateral temporal and occipital regions. More recent studies have suggested that the relationship between these risk factors and brain morphology may be more complex, particularly in relation to cortical thickness (CT) - a robust measure that has been validated against histological analysis (Fischl et al., 2008, Rosas et al., 2002).

3.1.2 Emerging risk factors (inflammatory markers) and the brain

In recent years, novel biomarkers associated with inflammation and endothelial dysfunctions have 'emerged' as potential independent cardiovascular disease risk factors(Umemura et al., 2011, Wersching et al., 2010). These include high-sensitivity C-reactive protein (hsCRP), an acute phase reactant protein, Interleukin-6 (IL-6), a pro-inflammatory cytokine and circulating forms of adhesion molecules like intercellular adhesion molecule (ICAM)-1, that are induced by inflammation and play a role in promoting atherosclerosis (Luc et al., 2003, Danesh et al., 2008). These inflammatory markers have been shown to be associated with poor cognitive functioning in the healthy population and in disease (Phillips et al., 2011, Wright et al., 2006). Prospective studies have shown such factors to predict cognitive decline in initially healthy elderly subjects over follow-ups of between one and ten years (Yaffe et al., 2003, Teunissen et al., 2003). Greater inflammatory markers have also been shown in several psychiatric illnesses like depression, and schizophrenia (Dowlati et al.,

2010, Miller et al., 2011). Although the precise role of these factors in neurocognitive function is not clear, there is evidence to suggest that inflammation may play a role in the aetiopathogenesis of mental and cognitive disorders(Krishnadas and Cavanagh, 2012). Other markers of haemostasis and endothelial function have also been found to be independent predictors of coronary heart disease and ischemic stroke. These include von Willebrand factor (vWF) and Fibrinogen, which play an important role in platelet adhesion and aggregation, and tissue Plasminogen activator (tPA) – which plays an important role in endogenous fibrinolysis(Wannamethee et al., 2012, Smith et al., 2005). They have been found to be significant risk factors for vascular dementia and cognitive impairment in older adults (Quinn et al., 2011, Gallacher et al., 2010).

3.1.3 The rationale and the aims of the study

The potential aetiological links between cardio-metabolic risk factors and brain structure and function need further exploration as possible explanations for the relationship between the burden of physical and mental ill health. A key question here is, is there a relationship between vascular health and neural health? Are cardiovascular risk markers potential endophenotypes for neuronal and hence psychiatric illnesses? However, whether variance in risk markers in the healthy adult population explains inter-individual differences in grey matter correlates of neurocognitive function is not fully clear.

The aim of this study was to explore the relationship between various circulating blood markers of inflammation and cardio-metabolic risk and cortical thickness (CT).

Here, I specifically examined if “classic risk factors” - including blood lipid fractions, carotid intima-media thickness (CIMT) and BMI, and “emerging” risk factors - including hs C-reactive protein (CRP); inerleukin-6, (IL6); fibrinogen; tissue plasminogen activator (tPA) antigen and markers of endothelial dysfunction - Intercellular Adhesion Molecule (ICAM) and von Willebrand Factor (vWF) - could potentially explain inter-individual variance in cortical thickness (Helfand et al., 2009).

3.2 Materials and methods

3.2.1 Participants

Participants were recruited as part of a larger study (Psychological, social and biological determinants of ill health (PSoBiD) (http://www.gcph.co.uk/work_programmes/psobid)(Knox et al., 2012, McGuinness et al., 2012, Shiels et al., 2011, Packard et al., 2011). Details of the design of PSoBiD have been described elsewhere (Deans et al., 2009). Briefly, election of participants was based on the Scottish Index of Multiple Deprivation 2004 (SIMD), which ranks small areas on the basis of multiple deprivation indicators across six domains, namely: income; employment; health; education, skills, and training; geographic access and telecommunications; and housing. Sampling was stratified to achieve an approximately equal distribution of the 666 participants across males and females and age groups (35–44, 45–54 and 55–64 years) within the most (bottom 5% of SIMD score) and least deprived areas (top 20% of SIMD score). Participants could opt-in for the neuroimaging component of the study. From a total of 327 male participants, 140 volunteered, and 42 (21 from least deprived and 21 from most deprived areas) of these were randomly selected. Individuals were excluded if they had a history suggestive of neurological or serious psychiatric illness. Participants with a history of cerebrovascular disease were excluded from the study. They were also excluded if they had a history of head injury or any contraindication for MRI. In addition, a Consultant Neuroradiologist examined all the scans. None of the participants included in the present study showed significant pathology on the scans. This chapter presents the results from the 40 individuals who had the complete data on the biomarkers. All participants in the MRI study were white Scottish males, who spoke English.

3.2.2 Blood biochemical analysis

This has been previously described in Deans et al (Deans et al., 2009). The below is an excerpt taken from the document from Deans et al, that is relevant for the analysis presented in this chapter. It should be noted that the coefficient of variations are for the whole sample of 666 participants from the original PSOBID

cohort. Ten to twelve hour fasting morning blood samples were collected, separated and frozen at -80^{o}C within 1 hour of venepuncture, except for samples for high sensitivity C-reactive protein (CRP), which were analysed on fresh plasma. High sensitivity C-reactive protein (CRP) was measured by an immunoturbidimetric assay (Roche Diagnostics Ltd., Burgess Hill, United Kingdom; sensitivity = 0.1 mg/L) and had a coefficient of variation (CV) of less than 3%. Interleukin-6 (IL-6) (sensitivity = 0.11 pg/mL) and Intercellular Adhesion Molecule-1 (ICAM) (sensitivity = 0.254 ng/mL) were measured by sandwich ELISA (R&D Systems Europe Ltd., Abingdon, United Kingdom). The between batch CV for IL-6 was 8.3% at a concentration of 2.84pg/mL and 10.0% at 5.38pg/ml. The between batch CV for sICAM-1 was 5.5% at an analyte concentration of 190ng/ml and 8.1% at 240ng/ml. Fibrinogen was measured on an automated coagulometer (MDA-180, Organon Teknika, Cambridge, United Kingdom) with a between batch CV of 3.7% at a fibrinogen concentration of 2.89g/l. von-Willebrand factor (vWF) was measured using an in-house enzyme linked immunosorbent assay (ELISA), employing rabbit anti-human polyclonal antibodies (DAKO UK Ltd, Ely, UK) and had a between batch CV of 3.4% at 128IU/dl. Tissue plasminogen activator antigen (tPA) was measured by ELISA (Hyphen, Neuville-sur-Oise, France; sensitivity = 0.5 ng/mL) with a CV 6.5% at an analyte concentration of 4.42ng/ml. Total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoproteins (Apo) A1 and B were analysed on fresh plasma. Their concentrations were determined by enzymatic colorimetric assays on a Roche Hitachi 917 analyser (Roche Diagnostics Ltd, Burgess Hill, UK). Lipid fractions were measured using ultracentrifugation and precipitation methods. All the lipid analysis had a between batch CV of less than 3%.The details of the biomarkers are shown in Table 3-1.Table 3-1: The demographic and biomarker details of the participants in the study.

Table 3-1: The demographic and biomarker details of the participants in the study.

	Reference	Mean	Std. Deviation
Age (years)	-	50.96	8.28
Alcohol units per week	-	17.68	16.51
Number prescribed Statins	-	7	
Number of smokers		19	
CIMT (mm)		.70	.13
BMI (Kg/m^2)		27.93	4.5
Total Cholesterol (mmol/L)	<5.0	5.41	.94
Triglycerides (mmol/L)	0.77 – 1.7	2.01	1.70
VLDL (mmol/L)	0.9 -1.8	1.06	.65
LDL (mmol/L)	<3.0	3.10	.80
HDL (mmol/L)	>1.0	1.24	.29
APO-A1 (g/L)	1.0 – 2.2	1.40	.25
APO-B (g/L)	0.6 – 1.3	.99	.21
hsCRP (mg/l)	<1.0	2.14	2.5
ICAM (ng/ml)		269.90	72.31
IL6 (pg/ml)	0 - 14	2.58	4.03
Fibrinogen (g/l)		3.06	.79
tPA (ng/ml)	3.5 – 7.2	4.42	1.85

VLDL - Very Low Density Lipoprotein; LDL – Low density lipoprotein; HDL – High density lipoprotein; APO – apoprotein; BMI – Body mass index; CIMT – Carotid intima-media thickness; ICAM – Intercellular adhesion molecules; IL6 - Interleukin 6; tPA – tissue Plasminogen activator; vWF – von Willebrand factor; hsCRP – C reactive protein;

3.2.3 Dimension reduction of lipid fractions

In order to reduce the number of dependent variables and to derive factors representing broad domains within the markers that are statistically independent, I subjected the raw scores from 6 measures of lipid fraction including HDL, LDL, VLDL, Trigycerides, Apo A1, Apo B, to Principal components analysis (PCA). The statistical independence of the factors meant that the relationship of each factor to cortical thickness was likely to estimate the specific association that each factor had on CT. Varimax rotation was used and the minimum eigenvalue for extraction was set to one. The analysis revealed a set of 3 orthogonal factors that explained a total of 95.7% of the variance (Table 3-2). The first factor called "TAG" explained 38.4% of the variance with high loadings from triglycerides and VLDL. The second factor was called an "LDL" factor, which explained 29.79% of the variance, with high loadings from LDL and APO-B. The third factor was called an "HDL" factor and explained 27.5% of the variance with high loadings from HDL and Apo A1. Factor scores were extracted using the regression method. These factors were used as independent variables in the GLM model described below. A similar approach was attempted with the "emerging" factors. However, the Bartlett's test of sphericity suggested that the correlation matrix was an identity matrix, which implied that the factor model was inappropriate. Therefore an exploratory analysis of individual risk factors was performed separately.

Table 3-2: The result of the PCA – the rotated factor matrix and factor loadings.

	Component		
	TAG factor	LDL factor	HDL factor
Triglycerides	**.975**	.013	.012
VLDL	**.955**	.045	-.054
LDL	-.196	**.963**	.015
APOB	.293	**.932**	-.088
HDL	-.337	-.103	**.920**
APOA1	.240	.025	**.958**

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. VLDL - Very Low Density Lipoprotein; LDL – Low density lipoprotein; HDL – High density lipoprotein; APO – apoprotein; BMI – Body mass index.

3.2.4 Carotid intima-media thickness (CIMT) measurement

Carotid ultrasound scans were performed on an ACUSON Sequoia 512 Ultrasound System with an L7 5-12 MHz linear array broadband transducer (Siemens Medical Solutions, Erlangen, Germany). The details of the scan acquisitions have been published in detail previously (Deans et al., 2009). Below is an excerpt taken from Deans et al (Deans et al., 2009). Briefly, the same research nurse, who was trained in ultrasound techniques at the Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands, performed majority of the scans. As pre-specified in the study protocol, research nurses performing ultrasonography were required to complete a minimum of 10 paired replicate volunteer scans before scanning participants. The mean absolute difference for the mean common carotid artery IMT measurement between nurses was 0.0542mm, which was well within the predefined performance requirement for sonographer certification, with the requirement having been determined by the Department of Vascular Medicine, Academic Medical Centre, Amsterdam, to be a mean absolute difference of <0.15mm, and was also within the 88 more

stringent requirements suggested by the American Society of Echocardiography, who suggest a certification requirement of a mean absolute difference of <0.055mm (Stein et al., 2008). Further paired replicate scans of volunteers were performed at intervals throughout the study to demonstrate continued fulfilment of the quality criteria. Both left and right common carotid arteries were assessed. Intima-media thickness was measured on the far wall of each arterial segment, averaged along a 1 cm length or as much as could be read. The average of left and right maximum common carotid IMT was computed (van der Meer et al., 2004).

3.2.5 MRI acquisition

All MRI examinations were performed using GE Medical systems, 3T Signa Excite HD system (Milwaukee, USA) with an eight-channel phased array (receive only) head coil. An axial 3D T1-weighted IR-FSPGR was acquired with the following imaging parameters: TR = 6.8ms; TE = 1.5ms, Inversion Preparation time = 500ms; Flip angle=12°; FOV = 26cm; Phase FOV= 70%; matrix: 320 x 320; Bandwidth 31.25 kHz; number of slices = 160; Slab thickness = 1mm. The acquisition time for this scan was 8min 54s.

3.2.6 Cortical thickness (CT) measurements and analysis

I used the method similar to that of Leritz et al (2011), who examined the association between cerebrovascular risk factors to CT in a normative sample of community dwelling adults (Leritz et al., 2011). Surface extraction, cortical parcellation and thickness computation was performed with the FreeSurfer image analysis suite.

The pre-processing was carried out according to documentation, which has been validated and the description is available at (http://surfer.nmr.mgh.harvard.edu/)(Fischl and Dale, 2000b, Fischl et al., 1999a, Dale et al., 1999). This is described below, as given in the website (http://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferMethodsCitation).

Briefly, this includes motion correction and averaging (Reuter et al., 2010) of multiple volumetric T1 weighted images (when more than one is available),

removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Segonne et al., 2004), automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles)(Fischl et al., 2002, Fischl et al., 2004a) intensity normalization (Sled et al., 1998), tessellation of the gray matter white matter boundary, automated topology correction (Fischl et al., 2001, Segonne et al., 2007), and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class.

In more detail,(as given in https://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferAnalysisPipelineOverview) first, the volume is registered with the Talairach (Talairach J and P, 1988) atlas (this is an affine registration). This allows FreeSurfer to compute seed points in later stages. The B1 bias field is estimated by measuring the variation in the white matter intensity. The main body of the white matter is used to estimate the field across the entire volume. Likely white matter points are chosen based on their locations in Talairach space as well as on their intensity and the local neighbourhood intensities. The intensity at each voxel is then divided by the estimated bias field at that location in order to remove the effect of the bias field. The skull is stripped using a deformable template model. Voxels are then classified as white matter or something other than white matter based on intensity and neighbour constraints. Cutting planes are chosen to separate the hemispheres from each other as well as to remove the cerebellum and brain stem. The location of the cutting planes is based on the expected Talairach location of the corpus callosum and pons, as well as several rules-based algorithms that encode the expected shape of these structures. An initial surface is then generated for each hemisphere by tiling the outside of the white matter mass for that hemisphere. This initial surface is then refined to follow the intensity gradients between the white and gray matter (this is referred to as the white surface). The white matter surface is tessellated by assigning 2 triangles to the square face of each surface voxel. This process yields approximately 160 000 vertices per hemisphere.

The white surface is then nudged to follow the intensity gradients between the gray matter and CSF (this is the pial surface). The distance between the white and the pial gives us the thickness at each location of cortex. Cross-subject registration of hemispheric cortical surfaces was performed by projecting them onto the spherical representations. The maps produced are not restricted to the voxel resolution of the original images and are thus capable of detecting sub-millimetre differences between groups. Procedures for the measurement of CT have been validated against histological analysis and manual measurements. In addition, FreeSurfer morphometric procedures have demonstrated good test-retest reliability across scanner manufacturers and across field strengths and across various sequence parameters.

Once the cortical models are complete, a number of deformable procedures can be performed for in further data processing and analysis including surface inflation (Fischl et al., 1999a), registration to a spherical atlas which utilized individual cortical folding patterns to match cortical geometry across subjects (Fischl et al., 1999b), parcellation of the cerebral cortex into units based on gyral and sulcal structure (Desikan et al., 2006b, Destrieux et al., 2010, Fischl et al., 2004b), and creation of a variety of surface based data including maps of curvature and sulcal depth. This method uses both intensity and continuity information from the entire three dimensional MR volume in segmentation and deformation procedures to produce representations of cortical thickness, calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl and Dale, 2000a). The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The maps produced are not restricted to the voxel resolution of the original data thus are capable of detecting submillimeter differences between groups. Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003, Salat et al., 2004). Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (Han et al., 2006, Reuter et al., 2012).

3.2.7 Statistical analysis

The procedure previously described by Leritz et al was adopted (Leritz et al., 2011). Statistical comparisons of global data and surface maps were generated by computing a general linear model (GLM) of the effects of each risk factor variable (independent variable) on thickness (dependent variable) at each vertex in the cortical mantle, using the Query, Design, Estimate, Contrast (QDEC) interface of FreeSurfer. QDEC is a single-binary application included in the FreeSurfer distribution that is used to perform group averaging and inference on the cortical morphometric data produced by the FreeSurfer processing stream. (http://surfer.nmr.mgh.harvard.edu/fswiki/Qdec)

In case of the lipid fractions, the factors extracted from PCA were entered as independent variables of interest. For BMI, CIMT and the 'emerging' factors, individual risk factors were entered as independent variables of interest. Maps were created using statistical thresholds of p=0.05 and were smoothed to a full width half maximum (FWHM) level of 20mm. Since this analysis involved performing a GLM analysis at 160000 vertices, these maps were corrected for multiple comparisons by means of a cluster-wise procedure using the Monte Carlo Null-Z simulation method adapted for cortical surface analysis and incorporated into the QDEC processing stream. For these analyses, a total of 10,000 iterations of simulation were performed for each comparison, using a threshold of p=0.05. The simulation cluster analysis was run for thickness analyses with each independent variable separately (Hagler et al., 2006, Leritz et al., 2011). There is considerable debate on the distinction between exploratory and confirmatory analysis and whether exploratory analysis should be corrected for multiple testing. Considering the fact that previous results have shown inconsistent relationships and that some of the biomarkers I tested were novel, I considered the present analysis to be predominantly exploratory in nature. However, rather than use conservative family wise error tests at this level, in order to adjust for exploring the relationship between 11 different risk factors on CT, I took an approach of exploring the strength of the significance of the relationship. Therefore, in the previous step, if the clusters survived the Monte Carlo simulation at a threshold of 1.3 ($p<0.05$), I repeated the analysis at thresholds of 2.0 and 2.3 corresponding to p values of 0.01 and 0.005. I report

the clusters that survived all 3 levels (Bender and Lange, 2001). Similar technique was also employed by Leritz et al (Leritz et al., 2011).

3.2.8 Covariates in the model

In order to improve the variance explained by the predictor of interest, I added as nuisance covariates, those variables that showed significant relationship with mean whole brain CT (dependent variable).

There was a significant negative correlation between age and mean whole brain CT (rho = -0.57; $p<0.001$). The association between age and CT was widespread bilaterally. I therefore used age as a nuisance covariate in the model for all analyses. Alcohol use was examined next. A unit of alcohol here is defined as equivalent to 10ml or 8g of pure alcohol. I found no association between number of alcohol units consumed per week and mean CT (rho=0.06; p=0.68). Alcohol use was therefore not included as a nuisance covariate in further analyses. Similarly socioeconomic status as measured using the SIMD did not explain any significant variance in mean CT (Mann-Whitney U = 182.0; p=0.3).

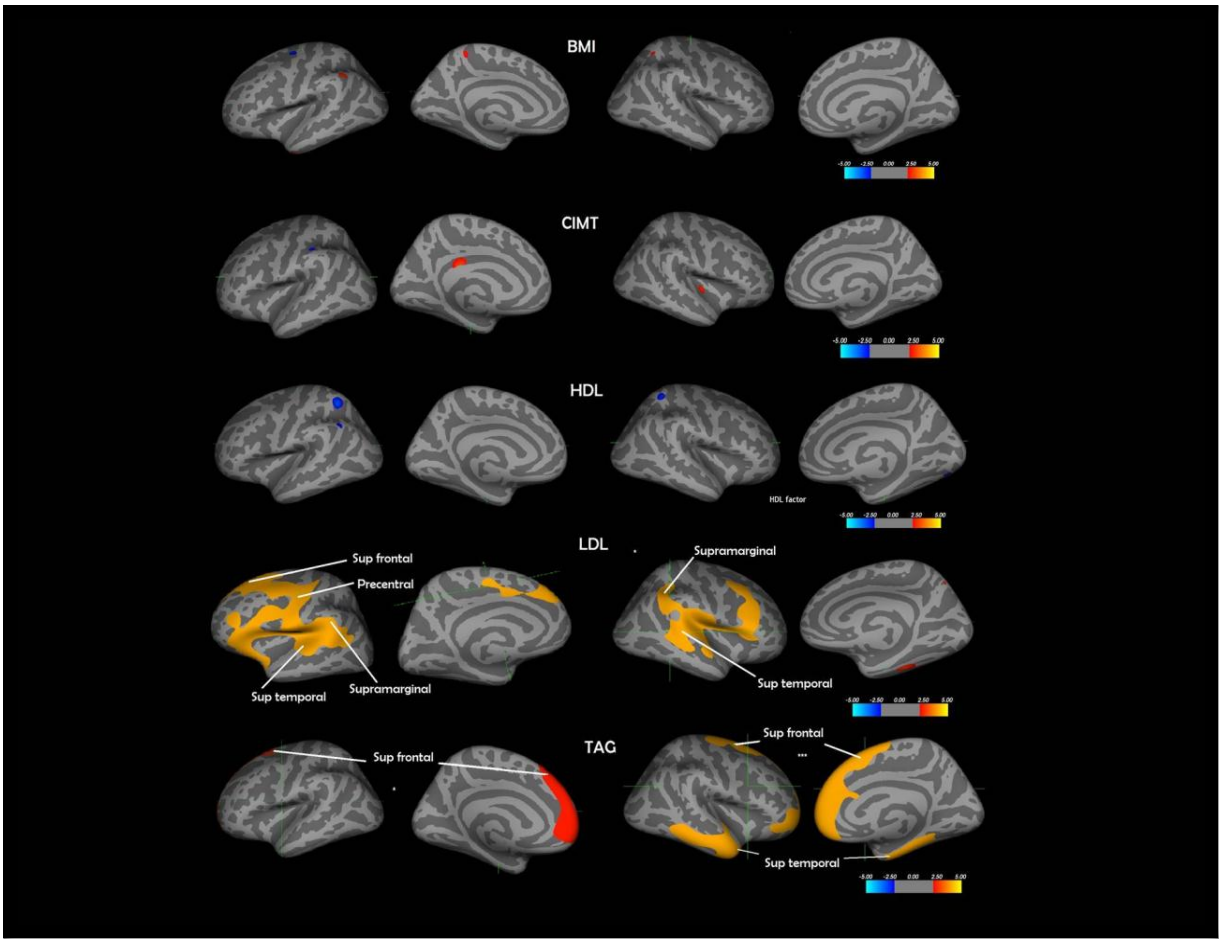
Two other variables - education status and smoking status - were found to have a significant association with the mean whole brain CT. There was a significant positive correlation between number of years spent in full time education (FTE) and mean whole brain CT (rho=0.36; p=0.02). Regionally, greater FTE was associated with greater CT in the left perisylvian regions. I also found significant association between smoking status and mean whole brain CT (t=4.17; p=0.001). The relationship between smoking status and CT was spread across the cortex bilaterally. However, I also found that in the present sample, FTE showed a positive correlation with the predictor variables LDL factor (r= 0.24; p = 0.17) and a significant negative correlation with CRP (rho = -0.48; p=0.002) and ICAM (r=-0.45; p=0.004) levels. Smoking status was associated with greater ICAM levels (t=-1.9;p=0.06) and lower LDL factor (t=1.9; p=0.05). I therefore did not include FTE or smoking status in the initial analysis, but I conducted a post hoc exploratory analysis of the relationship between LDL factor, ICAM, CRP, smoking status, FTE and CT. All covariates were examined at $p<0.05$ (cluster-wise corrected).

3.3 Results

3.3.1 Classic risk factors

The cluster-wise cortical analysis showed a number of regions of significant association (Figure 3-1). Only those for LDL and the TAG factors remained significant after multiple comparison correction. The regions corresponding to those that survived multiple testing are labelled with an asterisk in figure 1 (c and d). Both LDL and TAG factors showed a significant positive association with CT (warm colours). The clusters that survived for the LDL factor were located in the inferior and middle frontal, precentral gyrus, inferior post central, supra-marginal and superior temporal cortex on the left hemisphere, and supramarginal, inferior post central and posterior superior temporal gyri on the right. TAG was associated with medial frontal on the left and medial frontal, middle temporal and parahippocampal gyri on the right. Although a few clusters showed up on initial analysis with HDL factor, CIMT and BMI, none of them survived multiple testing corrections. Table 3-3 shows the details of the significant clusters that were associated with each risk factor across various thresholds. Figure 3.3 shows the scatter plots of individual predictors plotted against CT pertaining to the most significant voxel that survived multiple testing corrections.

Figure 3-1: The relationship between classic risk factors and cortical thickness.

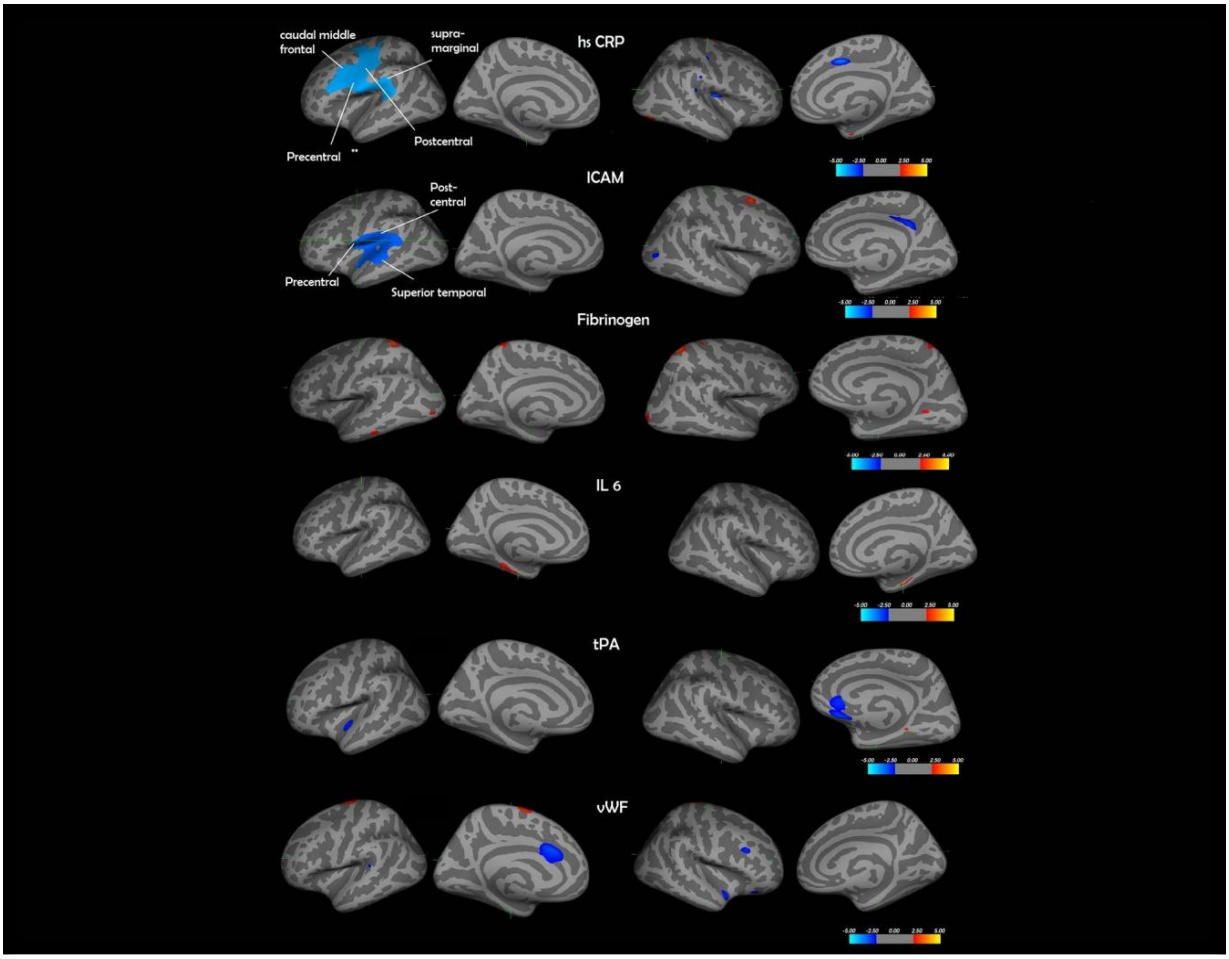


a) BMI – Body mass index; b) CIMT – Carotid intima-media thickness; c) HDL factor – HDL and Apo A1; d) LDL factor – LDL and Apo B; e) TAG factor – Triglycerides and VLDL – * Only the relationship between LDL factor and cortical thickness and TAG factor levels and cortical thickness survived multiple correction using the Monte Carlo Null-Z simulation technique. The regions that survived are labelled with * for p<0.05; ** if p<0.01 and * if p<0.005. The cortical surface is inflated, and the dark grey areas represent sulci, and light grey represents gyri.**

3.3.2 Emerging risk factors

Table 3-1 shows the details of emerging risk factors explored in the study. The cluster-wise analysis showed a number of regions of significant association. Only those for CRP and ICAM remained significant after multiple comparison correction. The regions corresponding to those that survived multiple testing are labelled in Figure 3-2. CRP and ICAM showed a significant inverse association with CT (cool colours). Although a number of regions showed up on initial analysis with the other inflammatory and clotting markers, none of them survived multiple testing corrections. Some of the associations between the markers and CT were positive (warm colours). Table 3-3 shows the details of the significant clusters that were associated with each risk factor across various thresholds. Figure 3.3 shows the scatter plots of individual predictors plotted against CT pertaining to the most significant voxel that survived multiple testing corrections.

Figure 3-2: The relationship between emerging risk factors and cortical thickness.



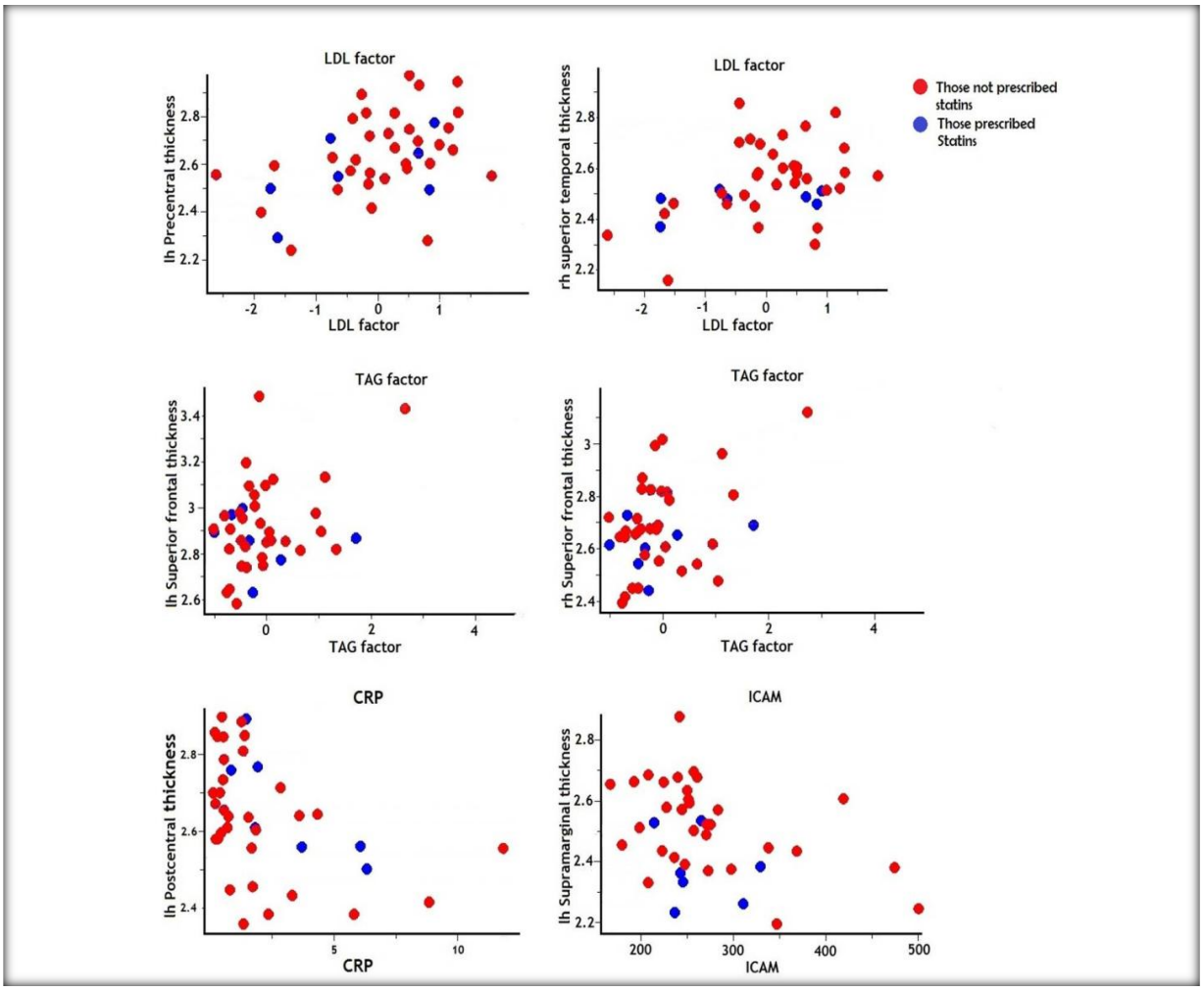
a) hsCRP – C-reactive protein; b) ICAM – Intercellular adhesion molecule; c) Fibrinogen; d) IL6 – Interleukin 6; e) tPA – tissue plasminogen activator ; f) vWF – von Willebrand Factor – Only the relationship between ICAM and cortical thickness and IL-6 levels and cortical thickness in the lateral aspects of the brain survived multiple correction using the Monte Carlo Null-Z simulation technique. The regions that survived are labelled. The cortical surface is inflated, and the dark grey areas represent sulci, and light grey represents gyri.

Table 3-3: Details of the clusters that survived the Monte Carlo Z simulation at various thresholds.

Risk factor	Threshold p value	Cluster No	Region	Size of cluster (mm^2)	Tal X	Tal Y	Tal Z	Number of vertices
LDL factor	0.05	1	lh Superior frontal	16225.72	-11.5	-7.7	47.3	34785
	0.01	1	lh Precentral	2997.09	-57.2	-0.1	10.7	6863
	0.005	1	lh Precentral	2290.76	-57.2	-0.1	10.7	5209
	0.05	1	rh banks sts	9061.66	45.9	-43.8	7.5	20217
TAG factor	0.05	1	lh superior frontal	4291.85	-6.6	33.8	49.8	6781
	0.05	1	rh precentral	9071.69	27.7	-14.4	60.2	15680
	0.05	2	rh superior temporal	5446.62	47.5	5.0	-27.2	9395
	0.01	1	rh Rostral middle frontal	3269.26	27.7	57.8	-9.5	4964
	0.01	2	rh Superior temporal	2343.63	47.5	5.0	-27.2	3610
	0.005	1	rh Superior Frontal	2064.68	9.3	41.1	30.0	3149
	0.005	2	rh Superior temporal	1701.21	47.5	5.0	-27.2	2572
hsCRP	0.05	1	lh Precentral	6662.41	-57.2	-0.1	10.7	14860
	0.01	1	lh Post central	1543.87	-62.6	-15.0	19.0	3489
ICAM	0.05	1	lh precentral	4890.49	- 57.2	- 0.1	10.7	11789
	0.01	1	lh Superior temporal	2134.62	-52.1	-24.0	-4.0	5232
	0.005	1	lh Supramarginal	1353.83	-48.6	-28.2	19.3	3334

Threshold p value represents Tal – Talairach coordinates of the vertex corresponding to the strongest association; lh – left hemisphere; rh – right hemisphere; hsCRP – C-reactive protein; b) ICAM – Intercellular adhesion molecule; LDL factor -LDL and Apo B; TAG factor – VLDL and TAG

Figure 3-3: Scatter plots of relationship between cortical thickness and risk factors.



Scatterplots pertaining to the vertex showing the strongest association are shown. Those prescribed statins are shown in blue. Details of the clusters and vertex location are shown in table 2.

3.3.3 Covariate analysis

The results are shown in Table 3-4. The relationship between LDL factor, ICAM, CRP and CT remained even after including FTE as a covariate in the model. However, the size of the clusters was reduced. Similarly, the relationship between LDL factor, CRP and CT remained even after including smoking status as a covariate in the model. With LDL, the size of the clusters reduced, while with CRP, the size of the cluster increased. However, the relationship between and ICAM and CT disappeared completely when smoking status was added as a covariate in the model.

Table 3-4: Details of the clusters that survived after including smoking status or education status as covariates

Risk factor	Region	Maxima (negative log of p value)*	Size of cluster (mm2)	Tal X	Tal Y	Tal Z	Number of vertices
Covariate : Smoking status							
LDL factor	Lh precentral	4.00	9736	-57.2	-0.1	10.7	22012
	Rh rostral middle frontal	3.39	4587.95	43.7	24.0	28.2	10309
CRP	Lh precentral	-4.00	7715.55	-57.2	-0.1	10.7	17163
ICAM	-						
Covariate: Education status							
LDL factor	Lh precentral	4.00	13069.22	-57.2	-0.1	10.7	28224
	Rh supramarginal	3.15	4438.65	55.1	-38.4	27.5	10719
CRP	Lh precentral	-2.85	4531.2 4	-52.5	-6.6	38.5	10086
ICAM	Lh insula	-2.01	3502.00	-32.4	-31.9	17.1	8525

Tal – Talairach coordinates of the vertex corresponding to the strongest association; Lh – left hemisphere; Rh – right hemisphere; *survived p<0.05 Monte Carlo z simulation; hsCRP – C-reactive protein; b) ICAM – Intercellular adhesion molecule; LDL factor – LDL and Apo B

3.4 Discussion

The aim of the analysis was to examine the relationship between cardio-metabolic risk factors and CT. I have shown that in a sample of healthy middle-aged male subjects, lipid fractions – particularly those pertaining to TAG, VLDL and LDL explained significant variance in CT across a number of regions in the brain. I have also shown that inflammatory markers, particularly CRP and ICAM-1 explained significant variance in CT across a number of regions in the brain.

3.4.1 Classic risk factors

While obesity has a strong effect on lipoprotein metabolism, recent studies have shown that the relationship between BMI and lipid fractions may not be consistent and therefore, these may indeed be independent risk factors for cerebrovascular and cardiovascular risk (Nicholls et al., 2006, Shamai et al., 2011). Hence the effect of BMI and cholesterol were examined separately. The results of the data reduction using PCA enabled us to examine the effect of broad domains within the markers that are statistically independent. The factors extracted from PCA were found to be meaningful – HDL cholesterol and Apo A1 formed a single factor (HDL) and LDL and Apo B formed a second factor (LDL). Apolipoprotein A1 and Apolipoprotein B are the major protein component of HDL and LDL respectively in plasma. VLDL and triglycerides, which contains most of the triglycerides, formed the third factor (TAG). The density of a lipoprotein that carries the cholesterol depends on the amount of triglyceride in the fraction. Therefore I found that the lipoprotein fractions with greater triglyceride fraction (TAG, LDL and VLDL) were associated with greater CT, while HDL, which contains the least triglycerides, showed the smallest association. While BMI, and HDL showed associations with CT in some regions, none of these clusters survived multiple testing corrections. These relationships were contrary to my expectations. I expected the presence of greater risk factors (LDL and TAG factors) to be associated with cortical thinning in a number of regions. Our findings are in contrast to a number of previous studies that have found a negative association between greater risk factors and grey matter morphology. Both cross sectional and longitudinal studies have shown significant negative association between BMI and regional grey matter volume (Gunstad et al., 2008,

Raji et al., 2010, Walther et al., 2010, Yokum et al., 2012). Although Ward et al, in their study found no relationship between non HDL cholesterol and grey matter volume, they found that those with decreased levels of HDL cholesterol showed GM volume reductions pertaining to a number of cortical and subcortical structures (Ward et al., 2010). All of the above studies used techniques (Voxel / tensor based morphometry) that do not decompose cortical volume into thickness and surface area. Cortical volume is a construct that is derived from two distinct properties of the cortical sheet: CT and surface area and have distinct cellular and genetic bases (Panizzon et al., 2009, Rakic, 2009). This highlights the importance of studying CT independently in morphometric studies. Further, CT appears to be highly susceptible to various environmental influences such as exposure to smoking, metabolic risk factors and cannabis, while surface area seems to be more stable (Leritz et al., 2011, Habets et al., 2011).

More recent studies that have used similar image analysis techniques have found interesting results. In the first study, Isaac et al, found a negative association between visceral abdominal fat measured using MRI and CT pertaining to frontal, parietal and occipital cortices(Isaac et al., 2011). They however, did not measure lipid fractions in the blood. A second larger study, which examined the association between CT and circulating cholesterol levels in an older population (mean age 68.3 years), found a similar relation to the present study. They found that an increase in cholesterol factor with high loadings from total cholesterol and LDL was significantly associated with an increase in CT. However, a second factor which included HDL and BMI did not show such an association (Leritz et al., 2011). They suggest that the results in their study could be explained by other studies that have shown that neurodegeneration (and hence cortical thinning) is associated with lower serum cholesterol due to disruption in brain cholesterol production (Solomon et al., 2009). The fact that I found a similar relationship in a smaller sample suggests that the relationship is more than trivial (Friston, 2012). Our sample was also younger suggesting that the relationship between risk factors and cortical morphology may be evident earlier on in life. In another study, the same group have found higher HDL and LDL cholesterol in normo-lipidemic individuals to be associated with compromised regional white matter integrity. These findings suggest that the relationship

between cholesterol, white matter and grey matter are complex (Williams et al., 2012).

Although it accounts for only 2% of total body weight, the human brain contains as much as 25% of total body cholesterol. Cholesterol in the brain is primarily synthesised by glia. It is an essential component of cell membrane and myelin in the white matter. In this context, cholesterol abnormalities have been implicated in a number of neurodegenerative illnesses, where the primary pathogenesis seems to involve deregulated cholesterol trafficking (Liu et al., 2010). Leritz et al therefore suggest that a bidirectional mechanism between cholesterol and the brain may exist, such that higher levels in the brain predispose an individual to have higher levels in circulating blood (Leritz et al., 2011). Therefore the positive relationship between cholesterol and CT is a reverse epiphenomenon. It is also not clear if the association between CT and circulating cholesterol level is related to pathological processes. Consistent with my findings, more recently, Hoogendam examined the relationship between cardiovascular risk factors and cerebral volume measured using FreeSurfer in a community dwelling non-demented elderly sample of 3962 people. They found that higher total cholesterol levels were related to a larger cerebral volume. They found no association between HDL cholesterol and cerebral volume (Hoogendam et al., 2012).

CIMT measured using carotid ultrasound is an efficient and validated method for assessing the degree of atherosclerosis in an individual. While previous studies have found an association between CIMT and a risk of stroke, I did not find a relationship between CIMT and CT (Lorenz et al., 2007). Recently, Cardenas et al (2012) examined this relationship in an older sample. Using similar technique to ours, they found no relationship between CIMT and most measures of brain volume or CT (Cardenas et al., 2012). They however found CIMT to be associated with regional thinning pertaining to the parietal cortex. In their analysis, they divided the whole cortex into lobes, considering each lobe separately as a region of interest. This may have increased the power to detect subtle changes in individual lobes. None of the clusters survived multiple corrections in the sample. This may however be due to the small sample size.

3.4.2 Emerging risk factors

There is a growing body of evidence linking circulating inflammatory markers to brain structure and function. Molecular imaging studies have shown that circulating inflammation immune/metabolic markers are directly associated with greater inflammation markers in the brain. Drake et al used PET tracer PK 11195 to examine the relationship between cerebrovascular risk factors (but no stroke) and brain inflammation. They found that people with greater risk factors showed greater microglial activation in the brain, compared to normal controls (Drake et al., 2011). Similarly, a few studies have shown that modulating circulating inflammation is associated with both functional and structural changes in the brain. Harrison et al showed that inducing a circulating inflammatory response using typhoid vaccine in normal adults is associated with change in BOLD signals in regions pertaining to modulation of mood in the brain(Harrison et al., 2009a). More recently, Hannestad et al found that systemic inflammation induced by endotoxin in humans was associated with higher normalised glucose metabolism in the insula. This change was associated with change in peak cytokine levels and also changes in social interest, suggesting that these may be linked to each other (Hannestad et al., 2012b). Previous research has shown that modulating circulating inflammation in patients with Rheumatoid arthritis, using adalimumab (a TNF-α inhibitor) is associated with a reduction in serotonin transporter availability in the brain (Cavanagh et al., 2010). A few structural MRI studies have shown an association between inflammatory markers and cortical and subcortical grey matter volume. Jefferson et al in the Framingham cohort showed that inflammatory markers including IL6 were inversely associated with total brain volume (Jefferson et al., 2007). Marsland et al, showed an association between circulating IL-6 levels and smaller grey matter volume in the hippocampus in middle aged adults (Marsland et al., 2008). Although I found an association between IL6 levels and CT pertaining to the parahippocampal gyrus, these clusters did not survive multiple testing corrections.

My findings reflect those of a more recent study that found an association between hsCRP and cortical grey matter volume pertaining to perisylvian regions in a large cohort of healthy aging subjects (Taki et al., 2012). I have replicated the finding in a smaller and younger sample. However, the clusters were more

extensive than those described by the study. While most of the above studies have shown an inverse relationship between circulating markers and cortical morphology, a few recent studies have suggested that the relationship between circulating risk factors and cortical morphology may be more complex than earlier thought. For example, greater circulating blood levels of TGF Beta, an anti-apoptotic and anti-proliferative factor have been associated with greater CT (Piras et al., 2012). I found similar relationships in the case of fibrinogen, IL6 and a few other clusters in other markers. None of them however survived multiple testing corrections, suggesting that the relationship between them and CT may not be as robust for these measures. However, this could be due to type 2 error due to the small sample size.

To my knowledge, no previous study has shown an association between ICAM-1 and CT. ICAM-1 is an adhesion molecule expressed by endothelial cells in response to inflammation – particularly pro-inflammatory cytokines like tumour necrosis factor (TNF-α) (which also is a potent stimulator of CRP) and physiological stress (Dietrich, 2002, Frank and Lisanti, 2008). Inflammatory activation of ICAM-1 has been shown to be associated with an increase in leukocyte migration and region specific (frontal and parietal) microglial activation in the brain (Huber et al., 2006, Dietrich, 2002). While this has mostly been demonstrated in primary inflammatory conditions of the brain, there are few studies examining the role of ICAM-1 in situations where inflammatory pathology is less manifest like major depression .A recent study has shown that selective serotonin reuptake inhibitors (SSRIs) , medications primarily used in the treatment of depression, may have cardio-protective properties by directly inhibiting TNF-α induced ICAM -1 expression (Lekakis et al., 2010). The clusters in the present study that correlated with CRP and ICAM-1 were in the inferior frontal and parietal cortices similar to that shown by Dietrich et al (Dietrich, 2002). While I could postulate that these findings could represent a region specific relation between circulating inflammatory and cell adhesion markers and the brain, causal assumptions cannot be made due to the cross sectional nature of the data.

3.4.3 Exploring Covariates in the model

I found a significant positive relationship between education status and CT. This is similar to previous studies(Liu et al., 2012b) . Since education status was also related to LDL, ICAM and CRP, I explored the relationship between the above predictors and CT with education status as a potential covariate. All the predictors remained significantly associated with CT however, the strength of some of the relationships reduced, denoted by a reduction in the cluster size, suggesting that at least part of the variance explained by education status and the risk factors were shared. With regards smoking we found a significantly strong negative association between smoking status and CT, similar to previous studies(Kuhn et al., 2010) . Just as education status, smoking status also showed significant association with LDL, ICAM and CRP. Surprisingly, the relationship between smoking status and LDL cholesterol was contrary to previous studies. Those who smoked had lower LDL cholesterol. The relationship between CRP and CT remained significant even with smoking status as covariate in the model. However, the relationship between ICAM and CT disappeared. This suggests that almost all of the variance explained by ICAM and CT was shared with smoking status. Theoretically I could argue that greater ICAM levels may be statistically mediating the relationship between smoking and CT. Smoking has indeed been shown to induce adhesion molecules in both pulmonary and circulating blood (Schaberg et al., 1996, Noguchi, 1999). While this is mechanistically plausible, causal mediation cannot be attributed to my findings due to the cross sectional nature of the study.

Seven subjects in the present study were prescribed statins. It could be argued that those with greater cholesterol who were prescribed statins, which have anti-inflammatory properties and may have contributed to an increase in regional CT. The sample was underpowered to examine the effect of statin intake. On examining the scatter plot, in general, the distribution of those on statins was similar to those not on statins. However, at least in certain cases (TAG and ICAM), there seems to be an interaction between statin intake status and the relationship between the variables and cortical thickness. My result differs from the previous study that used similar technique to ours showed no effect of medication on CT. They found that the association was purely due to

the cholesterol factor itself (Leritz et al., 2011). The relationship between medication status and CT should be further explored in studies with larger sample size.

3.4.4 Relevance of measuring the association between metabolic and inflammatory risk markers and cortical morphology

Quantifying the variance in cortical parameters that could be attributable to risk markers in health and illness is key to the understanding of the relationship between cardiovascular health and neural health. As mentioned above, PET studies have shown that greater levels of cardiovascular risk markers in the circulation are associated with greater microglial activation in neurologically normal subjects (Drake et al., 2011). This suggests that pathophysiological processes are in play even in normal subjects who are at high risk of developing cardio/cerebrovascular diseases. Interestingly, psychiatric morbidity has often associated with greater cardiovascular risk and mortality. While this has traditionally been attributed to poor health choices and health care utilisation by this population, recent research suggests that such simplistic modelling of physical health co-morbidities or multi-morbidity may not be useful and may indeed be misleading(de Jonge and Roest, 2012). There is now a call to examine the role of cardiovascular and metabolic risk markers as common endophenotypes of both physical and psychiatric illnesses. In other words, there may be common genetic and environmental factors that may contribute to chronic metabolic illnesses like obesity and psychiatric illnesses like major depression (Bornstein et al., 2006). The link between circulating cardiovascular risk markers and cortical thickness in the present study may therefore be the independent phenotypic representation of common genes or environment. It is now known that brain is not an immune privileged organ, and proinflammatory cytokines like TNF- α, which are potent stimulators of CRP, are also produced by astrocytes and microglia within the central nervous system and are important in synapse formation and synaptic neurotransmission and in turn neurocognitive function (McAfoose and Baune, 2009). Indeed, there is increasing evidence that inflammatory markers contribute to the pathophysiology of psychiatric illnesses(Krishnadas and Cavanagh, 2012) .

More importantly, the lack of progress in developing effective treatments in psychiatry has led to the search for markers that may aid diagnosis or predict treatment response in what are highly heterogeneous conditions (Krishnadas and Cavanagh, 2012). Recently there has been an emphasis on combining imaging markers with circulating blood risk markers in order to identify what may provide a "biological signature" that may help predict treatment response (Linden, 2012, Schmidt et al., 2011).

Exploring the relationship between cardio-metabolic risk factors and cortical morphology can also give us clues as to the pathophysiological link between high risk environmental situations like poor socioeconomic status (SES) and mental illnesses. For example, SES has been consistently associated with cognitive and mental health – which is thought to be the result of coordinated activity of large-scale networks across the whole cortex. SES has also been associated with greater cardio metabolic risk. Therefore an emerging question is whether the association between SES and cortical substrates of neurocognitive functions is mediated by the presence of greater cardio-metabolic risk in this population. Current evidence suggests that cumulative (both in time and across multiple physiological systems) physiological risk across the life span associated with socioeconomic deprivation may contribute to both mental and physical health over the life span (Gruenewald et al., 2012). McEwen et al describe a process of allostasis, where physiological systems operate within and outside a given range of parameters in order to maintain homeostasis (McEwen and Seeman, 1999). In this context, "Allostatic load" is thought to be the wear and tear that the body experiences as a result of activation of the above systems. This wear and tear represents either the 'excess' or the 'inefficient' operation of the above physiological systems. It is not surprising that, in this context, metabolic and inflammatory systems play a key role in the process of allostasis. A number of studies have shown a significant association between allostatic load (measured using a composite score of inflammatory and metabolic markers) and medical health (for e.g. cardiovascular disease) and neurocognitive function(Juster et al., 2010). These potential mechanisms may provide insight into how chronic adversity can affect physical and mental wellbeing(Kin et al., 2007). It is therefore reasonable to quantify any association between cardiovascular health and neural health in terms of gross cortical morphology.

In order to explore the role of causal mediators in this complex relationship, the findings should be replicated in larger study with greater variation in circulating risk markers and a longitudinal design. Further work should also involve replication of the study in a larger population, including younger population, targeting critical periods of brain growth. Finally, future work to develop a clearer biological framework of a more comprehensive investigation of metabolic and inflammatory markers may be more informative.

Chapter 4 - A composite measure of circulating Inflammatory markers mediate the relationship between neighbourhood deprivation and cortical morphology

In this chapter, I extend my findings from the previous chapter. Here, I see if the circulating cardio-metabolic and inflammatory risk factors would explain the difference in cortical morphology between people from the most deprived and and the least deprived neighbourhoods in Glasgow. Using a cross-sectional study design, I compared the cortical morphology of 42 neurologically healthy adult men from the least deprived and most deprived neighbourhoods of Glasgow. I performed surface based morphometry on 3T structural MRI images to extract the cortical morphology - volume, thickness (cortical thickness) and surface area (surface area) of regions commonly associated with language and executive control. I compared cortical morphology between the two groups. I used mediation analysis to examine whether cardio-metabolic and inflammatory risk factors mediated the relationship between deprivation status and cortical morphology. The findings of this chapter have been published in *Krishnadas R, et al. Socioeconomic deprivation and cortical morphology: psychological, social, and biological determinants of ill health study. Psychosomatic Medicine 2013 Sep;75(7):616-23*

4.1 Introduction

4.1.1 Socioeconomic deprivation and the brain

Socio-economic status (SES) refers to a multidimensional construct that is usually measured using a number of economic (e.g. income) and noneconomic (e.g. education) indicators(Hackman et al., 2010). SES can be measured at an individual/household or at a neighbourhood level. Regardless of the level of measurement (individual/neighbourhood), SES has been associated with

significant health disparities (Diez Roux and Mair, 2010). For example, adults living in the most deprived part of Glasgow perform poorly on cognitive tests of attention and executive function and are 3 times more likely to be diagnosed with a severe mental disorder (Srireddy et al., 2012, Packard et al., 2011). Studies examining this relationship in children have also shown a positive association between SES indicators and language skills(Hackman and Farah, 2009).

Two questions are crucial to our understanding of the association between SES and cognitive/mental health disparity. Firstly, is there an association between SES and neural substrates of these cognitive functions? It is widely recognised that cognitive and mental functions are the result of a number of brain regions working together as large scale networks.(Bressler and Menon, 2010) Recent neuroimaging studies that have examined the relationship between SES and the brain have shown a relationship between SES indicators and cortical and sub-cortical grey matter volumetric differences in hippocampal, amygdalar and anterior cingulate cortex in adults and in addition, language regions in children (Gianaros et al., 2007a, Hanson et al., 2011, Butterworth et al., 2011, Noble et al., 2012, Staff et al., 2012). However, the results are conflicting (Butterworth et al., 2011). One potential reason for this inconsistency is the fact that cortical grey matter volume is a function of two distinct measures – cortical surface area and cortical thickness.(Winkler et al., 2010) Regional and global measures of surface area and cortical thickness are independent of each other, and have a distinct genetic and ontogenic basis(Rakic, 2009, Panizzon et al., 2009, Raznahan et al., 2011). Automated surface based morphometric techniques enabled us to deconstruct surface area and cortical thickness from structural MRI data. It would therefore be informative to explore the relationships between SES indicators and these quite distinct cortical parameters. Secondly, what are the pathways that link SES to poor cognitive health?

4.1.2 Socioeconomic deprivation, inflammation (cardio-metabolic risk) and the brain

Pathways linking deprivation and poor health outcomes are diverse and complex (McEwen and Gianaros, 2010). Epidemiological studies have revealed an association between neighbourhood-level socioeconomic deprivation (SED) and raised cardio-metabolic risk markers (Nazmi et al., 2010, Deans et al., 2009). Cardio-metabolic risk markers have in turn been associated with inter-individual differences in cortical morphology in adults, as shown in the chapter 3 and in previous studies (Marsland et al., 2008, Taki et al., 2012, Raji et al., 2010). Therefore, exploring the relationship between deprivation, cardio-metabolic risk and cortical morphology may provide us with clues to the potential pathways that link SES with poor health outcomes. In keeping with this theme, Gianaros et al recently in a study of 155 healthy adults found that behavioural and physiological markers of cardiovascular risk mediated the relationship between SES indicators and white matter tract integrity. They concluded that "socioeconomic inequalities may relate to health disparities via inflammatory pathways impacting the structural integrity of brain networks"(Gianaros et al., 2012). In a similar vein, I embarked on an analysis exploring the relationship between SES, cardio-metabolic risk and cortical morphology of brain regions most often associated with language and executive function in men from the most and the least deprived neighbourhoods in the Greater Glasgow conurbation area.

4.1.3 The rationale and the aims of the study

In this analysis, I aimed to answer two questions. Firstly, are there cortical morphological differences pertaining to brain regions associated with language and executive function between people from the most deprived (MD) and least deprived (LD) neighbourhoods of Glasgow? The primary assumption that formed the basis of this analysis was that examining the distinct cortical parameters of cortical thickness and surface area of regions corresponding to nodes of previously defined functional networks, would inform us about the grey matter

element of the regions in question. For our analysis, I used the gyrosulcal parcellations based on the Destrieux atlas to define our regions of interest (ROIs) (FIgure 4-1) (Destrieux et al., 2010). Language regions of interest were the Wernicke's area and its right homologue which included the supramarginal gyrus, planum temporale of the superior temporal gyrus and the posterior ramus of the lateral sulcus; the Broca's area and its right homologue which included the pars opercularis, pars triangularis and pars orbitalis; and the posterior fusiform gyrus. All the above regions have been associated with language function in recent functional and effective connectivity studies (Sonty et al., 2007, Mesulam, 1990, Tomasi and Volkow, 2012). While language is thought to be predominantly left lateralised, there is increasing evidence that homologous areas on the right hemisphere also contribute to language function. See Hartwigsen and Siebner for a detailed review of the functions attributed to the right language homologous areas (Hartwigsen and Siebner, 2012). The executive control regions of interest included fronto-parietal regions that show strong co-activation during resting state fMRI and tasks that particularly involve executive function (Seeley et al., 2007, Margulies et al., 2007, Jurado and Rosselli, 2007, Nee et al., 2013). They included the Destrieux parcellations of middle frontal gyrus, posterior parietal cortex (intra-parietal sulcus and superior parietal lobule) and middle-anterior part of the cingulate gyrus and sulcus (corresponding to dorsal anterior cingulate cortex) on each side (Destrieux et al., 2010).

Secondly, can the difference in cortical morphology be explained by differences in cardio-metabolic risk factors between groups? In other words, can the relationship between SED and cortical morphology be explained by raised cardio-metabolic risk markers in the most deprived population? Here, our assumption was that exploring the relationship between SED, risk markers and the two ontogenically distinct cortical morphological parameters of cortical thickness and surface area of the aforementioned ROIs, would point us towards possible mechanistic pathways that link socioeconomic disparities to structural integrity of the brain.

4.2 Methods

4.2.1 Participants

Ethical approval for the study was obtained from Glasgow Royal Infirmary Research Ethics committee. All participants gave informed consent and were recruited as part of a larger study (Psychological, social and biological determinants of ill health (PSoBiD)) (http://www.gcph.co.uk/work_programmes/psobid). Each participant attended 3 visits to complete the study over a period of 2.5 years between winter 2005 and spring 2008. Details of the design of PSoBiD have been described elsewhere (Deans et al., 2009, Shiels et al., 2011). Selection of participants was based on the Scottish Index of Multiple Deprivation 2004 (SIMD), which ranks small areas on the basis of multiple deprivation indicators across six domains, namely: income; employment; health; education, skills, and training; geographic access and telecommunications; and housing (TheScottishGovernment, 2004). Sampling was stratified to achieve an approximately equal distribution of the 666 participants across males and females and age groups (35–44, 45–54 and 55–64 years) within the most (bottom 5% of SIMD score) and least deprived areas (top 20% of SIMD score). Participants could opt-in for the neuro-imaging component of the study. From a total of 327 male participants, 140 volunteered, and 42 (21 from LD and 21 from MD neighbourhoods) of these were selected. The present paper presents the results from the 42 neurologically healthy right handed men.

4.2.2 Mediators and other variables of interest

4.2.2.1 Cardio-metabolic risk factors

For this analysis, I examined the role of 9 blood markers - C-reactive protein (CRP), Interleukin-6 (IL-6), Intercellular Adhesion Molecule-1 (ICAM),Triglycerides, High density lipoprotein (HDL), Very low density lipoprotein (VLDL), Fibrinogen, D-dimer and Insulin – and an anthropometric measure- BMI. These were selected as we had complete data on these variables for all 42

individuals. Details of measurement of CRP, IL-6, ICAM, HDL, VLDL and Fibrinogen are detailed in 3.2.2. In addition, insulin was measured by a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) (Mercodia AB, Uppsala, Sweden; sensitivity = 1mU/L). Between batch analytical CV was 7.26% at 6.04mU/L and 7.85% at 11.2mU/L. D-dimer was measured by ELISA (Hyphen, Neuville-sur-Oise, France; sensitivity=2ng/ml). The between batch CV for D-dimer was 5.3% at a concentration of 109ng/mL.

4.2.2.2 Principal components analysis and dimension reduction of cardio-metabolic markers

While greater levels of the most of the above markers have been associated with poor cardiovascular health, greater HDL levels have been associated with better health outcomes. In order to reduce the number of independent variables and to derive factors representing broad domains within the markers that are statistically independent, I subjected the Z scores (reversed for HDL) of the above markers to a Principal components analysis (PCA). The statistical independence of the factors meant that the relationship of each factor to deprivation and cortical morphology was likely to estimate the specific association that each factor had on these variables. Varimax rotation was used and the minimum eigenvalue for extraction was set to one. The analysis revealed a set of 3 orthogonal factors which explained a total of 68.19% of the variance. The first factor called “Inflammation factor” explained 26.23% of the variance with high loadings from Fibrinogen, ICAM, IL6, CRP and D-dimer. The second factor was called a “TAG” (triacylglycerol) factor, which explained 24.05% of the variance, with high loadings from triglycerides and VLDL – the lipid fractions with the greatest amount of triglycerides. The third factor was called “BMI-HDL-Insulin” factor and explained 17.91% of the variance with high loadings from HDL, BMI and Insulin. Factor scores were extracted using the regression method. I used these as continuous measures in our analysis. Greater scores on the above indices meant poorer health. The details of the factor loadings of the principal components analysis are shown in Table 4-1.

Table 4-1: Factor loadings on the principal components analysis of cardio-metabolic risk factors of interest

Variables of interest	Factor loading		
	Inflammation factor	TAG factor	BMI-HDL-Insulin
hsCRP	**.682**	-.192	.151
ICAM	**.603**	.222	-.327
Fibrinogen	**.496**	-.326	.262
D_Dimer	**.772**	-.145	.042
IL6	**.717**	.286	.146
Triglycerides	.095	**925**	.101
VLDL	-.032	**.913**	.095
HDL	-.183	.346	**.790**
Insulin	.098	.013	**.836**
BMI	.227	-.135	**.752**

Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization; KMO test of sampling adequacy was 0.53, and Bartlett's test of sphericity was significant (Chi square = 149.21; p<0.001).

4.2.3 MRI acquisition

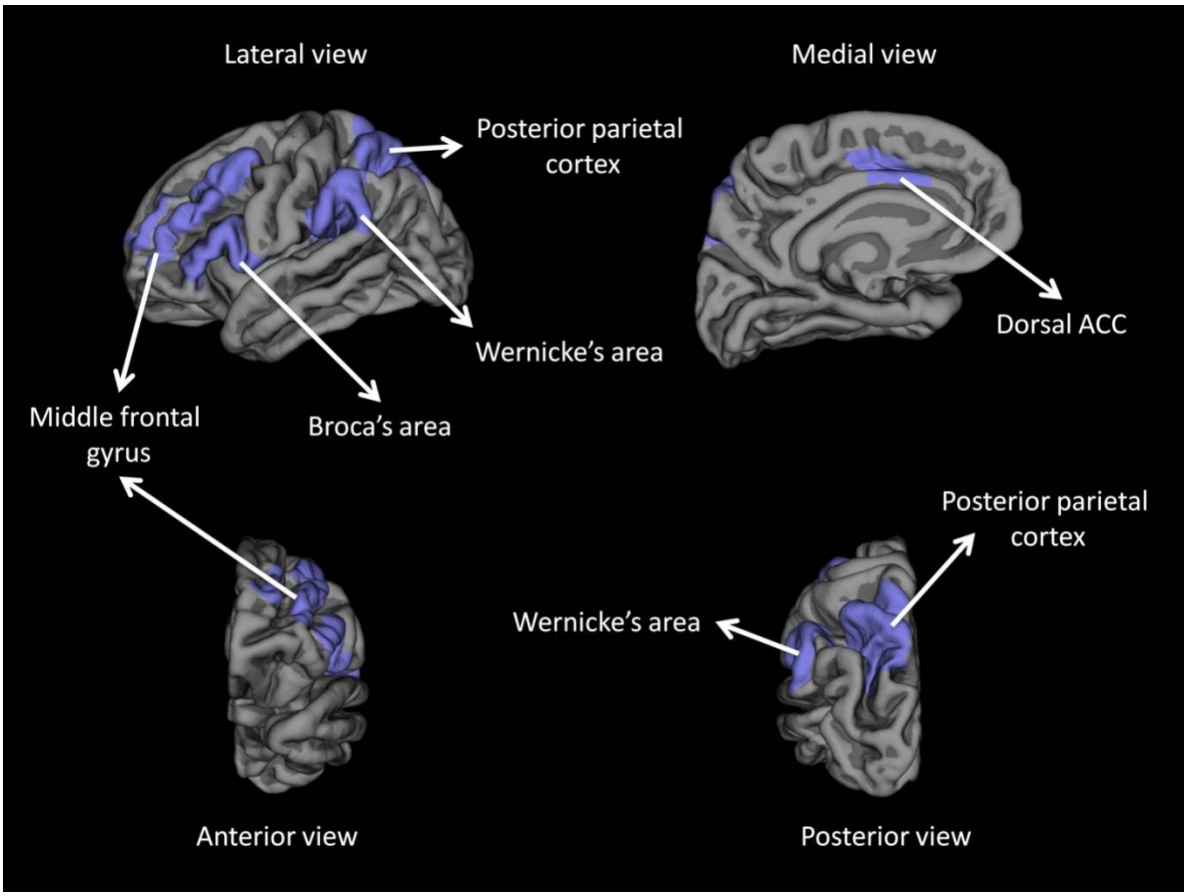
All MRI scans were performed using GE Medical systems, 3T Signa Excite HD system (Milwaukee, USA) using an eight channel phased array (receive only) head coil. The parameters of acquisition has been described in section 3.2.5.

4.2.4 FreeSurfer volume extraction and ROI (region of interest) analysis

Surface extraction, cortical parcellations and thickness computation was performed with the FreeSurfer image analysis suite. The pre-processing was carried out according to documentation, which has been validated and the description is available at (http://surfer.nmr.mgh.harvard.edu/)(Fischl and

Dale, 2000b, Fischl et al., 1999a, Dale et al., 1999). This has been described in detail in section 3.2.6. The parcellations were obtained using the Destrieux sulcogyral-based atlas, which follows the anatomical conventions of Duvernoy (Figure 4-1) (Destrieux et al., 2010). The FreeSurfer image processing pipeline was visually inspected and corrected at critical points in order to avoid errors permeating through the subsequent analyses. Procedures for the measurement of cortical thickness have been validated against histological analysis and manual measurements.

Figure 4-1: Regions of interest pertaining to language and executive function. Parcellation of interest are shown in blue on the cortical surface



4.3 Data analysis

4.3.1 Most deprived vs. least deprived

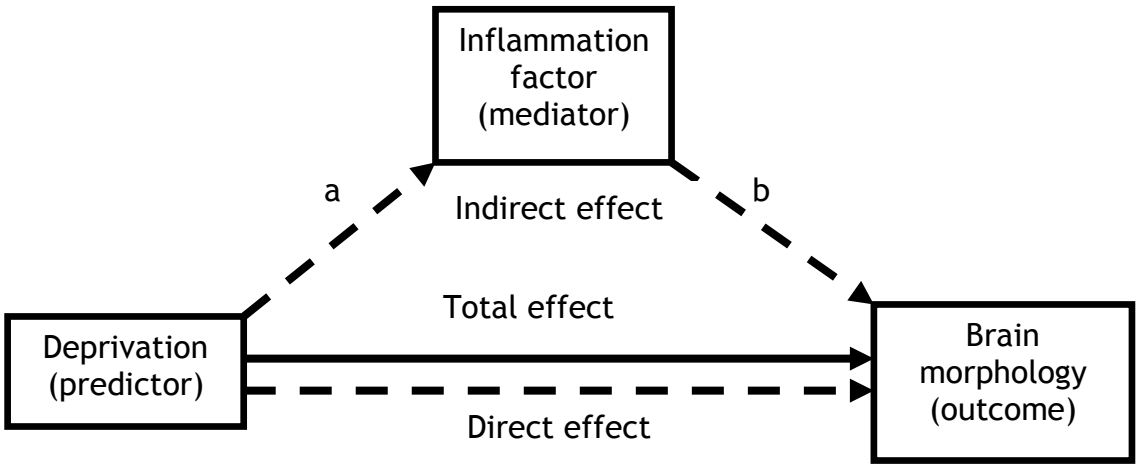
An initial Multivariate analysis of covariance (MANCOVA) was conducted with volumes of individual ROIs as dependent variables, group (deprivation status) as a fixed factor and age, intracranial volume (ICV) and self-reported measure of alcohol use in units as covariates. Since the differences in volume could reflect a change in cortical thickness or surface area, I repeated the MANCOVA with cortical thickness and surface area of ROIs as dependent variables, in order to test for between group differences. For all cortical thickness analyses, I included age, self-reported measure of alcohol use and mean whole brain cortical thickness, and for the cortical surface area analysis, I included age, alcohol use and ICV as nuisance covariates. All analyses were corrected for multiple testing using Benjamini- Hochberg False discovery rate (FDR) procedure (q=0.05) for 36 analyses (6 ROIs, 2 hemispheres and 3 morphological parameters)(Benjamini and Hochberg, 1995).

4.3.2 Mediation analysis

To explore the relationship between deprivation, cardio-metabolic risk factors, and cortical morphology, I conducted a mediation analysis with deprivation status as independent variable, “inflammation factor” as mediator and cortical morphology as dependent variable. Here, I aimed to examine if the relationship between deprivation status and cortical morphology could be explained by the inflammation factor score. In classic mediation analysis, causal models are tested using longitudinal data; such an assumption is not made here. While it could be argued that the difference in brain morphology between groups may be pathophysiologically linked to the raised risk factor score, due to the cross sectional nature of the data, such an inference is not possible with our data. This type of an approach has been taken previously to examine the contribution of MRI surface based measures to volume based measures measured using VBM (Palaniyappan and Liddle, 2012).

The analysis here, tests the hypothesis that the deprivation category accounts for variance in the mediator and in turn, this variance in the mediator accounts for a proportion of the variance in brain morphology. “The adjustable parameters of the model represent the unidirectional influence between pairs of variable in the model. The best fitting values of the parameters are estimated by using the General Linear Model to solve the linear equations that describe the relationships within the model. This analysis differs from multiple regression which estimates the proportion of variance in the dependent variable accounted for by each of several independent predictor variables while allowing for the variance accounted for by the other predictors in the model.” In other words, the mediation analysis partitions the variance explained by the predictor into a part that is independent of the mediating variable (direct), and a part that is accounted for via the mediating variable (indirect) (Figure 4-2)(Palaniyappan and Liddle, 2012).

Figure 4-2: The figure depicts the relationship between the predictor, mediator and the outcome variables.



The mediation analysis partitions the total variance (total effect) explained by the predictor into a part that is independent of the mediating variable (direct effect), and a part that is accounted for via the mediating variable (indirect effect). a represents the ‘a’ path and b represents the ‘b’ path.

I used the bootstrap method of Preacher and Hayes to estimate the indirect effect and bias-corrected 95% confidence interval (CI) for each individual mediator based on 20,000 bootstrap samples using an SPSS macro(Preacher and Hayes, 2008). I also obtained effect ratios for indirect effects (IE ratio), which express the proportion of the total effect that can be explained by the indirect (mediated) effects. In other words, an indirect effect ratio of 0.25 would mean that a quarter (25%) of the total effect of the deprivation status on the cortical morphology is explained by the mediator. This analysis requires no assumption regarding the underlying distributions since the statistical significance level is determined non-parametrically.

4.4 Results

Details of the demographic variables and cardio-metabolic risk markers are given in Table 4-2.

4.4.1 Cortical volume

The results of this analysis are shown in Table 4-3. On the multivariate analysis, deprivation status predicted cortical volumes [Wilk's lambda= 0.51;F(12,26) =2.24;p=0.04 η_p^2=0.51]. The MD group had statistically significantly smaller volumes pertaining to the left posterior parietal cortex (Cohen's d = 0.89) and right Broca's homologue (Cohen's d = 0.91) compared to the LD group. The left fusiform region volume was also smaller in the MD. However, this did not survive multiple testing corrections.

4.4.2 Cortical surface area

On multivariate analysis of cortical surface area, deprivation status predicted cortical surface area [Wilk's lambda= 0.61;F(12,26) =4.32;p=0.004;η_p^2=0.61] (Table 4-3). The MD group had statistically significantly smaller surface area pertaining to left posterior parietal cortex (Cohen's d = 0.89) and left fusiform cortex (Cohen's d = 1.05).

4.4.3 Cortical thickness

On multivariate analysis of cortical thickness, deprivation status predicted cortical thickness [Wilk's lambda= 0.52;F(12,26) =2.33;p=0.03;η_p^2=0.52] (Table 4-3). The MD group had statistically significantly thinner left Wernicke's region (Cohen's d = 0.93) and its right homologue (Cohen's d = 1.12). The left fusiform and right posterior parietal cortices were thicker in the MD. However, these did not survive multiple testing corrections.

Table 4-2: Demographic and clinical characteristics of study participants

	Least deprived n = 21 mean (s.d.)	Most Deprived n=21 mean (s.d.)	t	p
Age (years)	51.18 (8.7)	50.70 (8.75)	0.224	0.82
Alcohol units per week	15.81 (9.39)	18.61 (21.32)	-0.55	0.58
Intracranial volume (cc)	1572.94 (143.52)	1542.66 (161.72)	0.642	0.52
Mean cortical thickness(mm)	2.45 (0.08)	2.43 (0.1)	0.78	0.44
CRP (mg/L)	1.17 (1.34)	3.40 (2.94)	-3.16	0.004
IL6 (pg/ml)	2.62 (5.42)	2.53 (1.76)	0.07	0.94
Fibrinogen (g/L)	2.94 (0.61)	3.17 (0.95)	-0.89	0.37
Intercellular Adhesion Molecule-1 (ng/ml)	234.48 (25.72)	309.67 (84.19)	-3.82	0.001
D-dimer	89.81 (47.35)	150.32 (104.27)	-2.32	0.029
Inflammation factor	-0.52 (0.67)	0.52 (1.06)	-4.02	<0.001
HDL (mmol/l)	1.22 (0.20)	1.26 (0.36)	-0.46	0.64
BMI (kg/m^2)	27.02 (2.69)	28.42 (5.86)	-0.99	0.33
Insulin (uIU/ml)	7.1820 (4.82)	9.857 (68.43)	-1.23	0.22
BMI-HDL-Insulin factor	0.025(0.67)	-0.025 (1.26)	0.16	0.86
Triglycerides (mmol/l)	1.71 (0.72)	2.29 (2.23)	-1.14	0.26
VLDL (mmol/L)	1.05 (0.50)	1.08 (0.78)	-0.14	0.89
TAG factor	-0.11 (0.63)	0.11(1.27)	-0.73	0.45

t – unpaired t test ; BMI – body mass index; C-reactive protein (CRP), interleukin-6 (IL-6); ICAM VLDL - Very Low Density Lipoprotein ;HDL – High density lipoprotein; Alcohol unit defined as equivalent to 10ml or 8g of pure alcohol; TAG- triacylglycerol

Table 4-3: Difference in brain region of interest between least deprived and most deprived population.

Dependent Variable	Mean Difference Surface area (95% CI) mm^2	p[a]	Cohen's d (95% CI)	Mean Difference Cortical thickness (95% CI) mm	p [a]	Cohen's d (95% CI)	Mean Difference Cortical volume (95% CI) mm^3	p [a]	Cohen's d (95% CI)
L Middle frontal gyrus	63.0 (-188.95 – 314.95)	0.89	0.16 (-0.44 – 0.76)	0.01 (-0.05 – 0.06)	0.81	0.08 (-0.51 – 0.69)	424.02 (-466.01 – 1314.06)	0.57	0.3 (-0.3 – 0.9)
L Post Parietal cortex	471.08 (226.92 – 715.24)	0.003	1.24 (0.58 – 1.90)	-0.04 (-0.09 – 0.011)	0.29	-0.52 (-1.14 – 0.089)	1000.34 (281.38 – 1719.30)	0.04	0.89 (0.26 – 1.5)
L dACC	10.19 (-79.9 – 100.3)	0.79	0.07 (-0.05 – 0.67)	0.02 (-0.07 – 0.10)	0.86	0.12 (-0.47 – 0.73)	52.8 (-180.97 – 286.59)	0.86	0.14 (-0.46 – 0.75)
L Broca's area	95.67 (-47.25 – 238.60)	0.36	0.43 (-0.18 – 1.04)	0.007 (-0.05 – 0.06)	0.80	0.08 (-0.51 – 0.69)	383.72 (-149.35 – 916.8)	0.32	0.46 (-0.14 – 1.07)

L Wernicke's area	81.34 (-194.52 – 357.22)	0.84	0.18 (-0.41 – 0.79)	0.07 (0.02 – 0.12)	0.05	0.93 (0.29 – 1.5)	447.20 (-302 – 1197.2)	0.41	0.38 (-0.22 – 0.99)
L Fusiform gyrus	256.02 (99.5 – 412.55)	0.02	1.05 (0.41 – 1.69)	-0.12 (-0.22 - -0.011)	0.09	-0.7 (-1.3 – -0.08)	743.90 (103.78 – 1384.01)	0.09	0.74 (0.12 – 1.3)
R Middle frontal gyrus	-46.72 (-317.83 – 224.39)	0.83	-0.11 (-0.71 – 0.49)	0.019 (-0.034 – 0.072)	0.76	0.22 (-0.38 – 0.83)	105.30 (-872.15 – 1082.76)	0.79	0.069 (-0.53 – 0.67)
R Post Parietal cortex	51.31 (-236.49 – 339.13)	0.85	0.11 (-0.49 – 0.72)	-0.05 (-0.085 - - 0.007)	0.08	-0.73 (-1.35 - - 0.11)	-110.98 (-861.99 – 640.01)	0.83	-0.09 (-0.7 – 0.51)
R dACC	50.02 (-32.3 – 132.35)	0.42	0.39 (-0.21 – 1.0)	0.01 (-0.08 – 0.10)	0.77	0.06 (-0.54 – 0.66)	183.55 (-53.01 – 420.13)	0.29	0.49 (-0.16 – 1.11)
R Broca homologue	180.70 (28.13 – 333.27)	0.08	0.76 (0.13 – 1.38)	0.018 (-0.05 – 0.09)	0.86	0.16 (-0.44 – 0.76)	711.17 (209.95 – 1212.40)	0.05	0.91 (0.27 – 1.54)

R Wernicke homologue	-30.15 (-258.35 – 198.03)	0.81	-0.08 (-0.7 – 0.52)	0.09 (0.039 – 0.14)	0.01	1.12 (0.46 – 1.76)	169.82 (-585.72 – 925.37)	0.86	0.114 (-0.46 – 0.75)
R Fusiform gyrus	118.39 (-4.43 – 241.23)	0.16	0.62 (0.0007 – 0.099)	0.007 (-0.09 – 0.11)	0.78	0.05 (-0.55 – 0.6)	363.40 (-81.66 – 808.47)	0.26	0.52 (-0.09 – 1.14)

Positive values of mean difference suggest that the morphology is greater in the least deprived group. The analysis based on estimated marginal means with covariates appearing in the model evaluated at the following values: ICV (for surface area and volume) = 1557.80cc (for cortical thickness –Mean thickness = 2.44mm); Age in years = 50.94, Number of alcohol units per week = 17.21. a. All p values are adjusted for multiple comparisons using Benjamini- Hochberg FDR correction for 36 analysis (q=0.05). L – Left; R – Right ; dACC – dorsal anterior cingulate ; 95% CI - 95% confidence intervals.

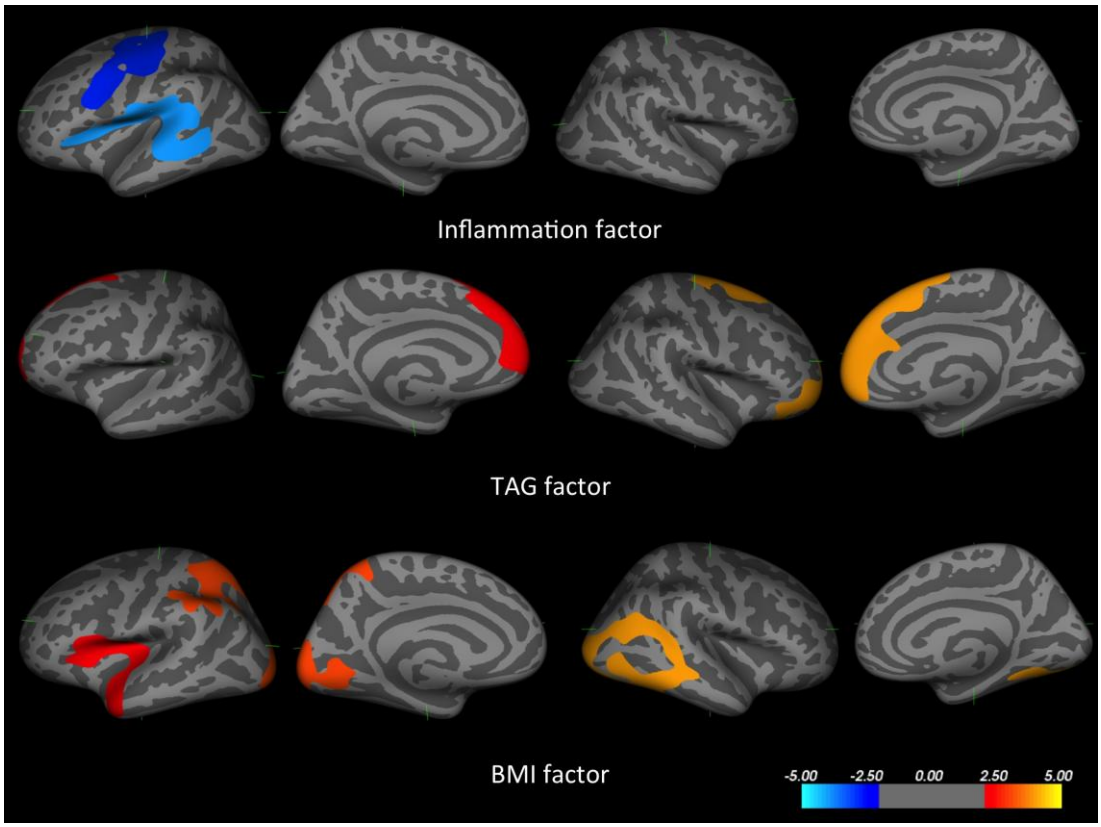
4.4.4 Correlation between inflammatory/metabolic composite factors and cortical thickness

Figure 4-3 shows the relationship between the relationship between the 3 factors derived from the principal components analysis and cortical thickness. In each of the analysis, the other factors and age were entered as covariates in the model. So, for example, when examining the relationship between inflammation factor and cortical thickness, I included TAG factor and BMI-HDL-Insulin factor as covariates. Similarly, with the other factors. These analyses characterise the b path in the mediation analysis shown below. As can be seen in the figure, the inflammation factor showed a significant negative correlation with cortical thickness along the left peri-sylvian and fronto-parietal regions. It should be noted that the 3 factors which were obtained using the PCA were orthogonal. In addition, since we added them as covariates, there is hardly any spatial overlap between the variance explained by these factors. As seen in the previous chapter, the TAG factor and the BMI factors showed significant positive correlation with cortical thickness in on both left and right side.

4.4.5 Mediation analysis

Only the inflammation factor, within the cardio-metabolic risk markers was found to be significantly different between groups (‘a’ path) (table 1). Therefore only the inflammatory factor was considered as a potential mediator. All the cortical morphological parameters that differed between the two populations (total effect) were examined. See Table 4-4 for the details of the ‘a’ path, ‘b’ path, the ‘total’ effect and the ‘indirect’ effect and the effect ratios of all mediations tested. Inflammation factor mediated the relationship between deprivation status and left Wernicke’s region cortical thickness (Beta=-0.32; SE = 0.12; 95%CI = -0.65 to -0.14). The relationship with the right homologue cortical thickness failed to reach statistical significance. None of the other regions showed a significant b path and therefore, the relationships were not tested. The detailed results of the mediation analysis are given in Table 4-4.

Figure 4-3: The relationship between inflammation, TAG and BMI factor on cortical thickness.



The regions of the brain that survived multiple correction using the Monte Carlo Null-Z simulation technique (p<0.05). The regions that survived are labelled. The cortical surface is inflated, and the dark grey areas represent sulci, and light grey represents gyri.

Table 4-4: Results of the mediation analysis

ROI	Coefficient	SE	p	Boot strap 95% CI	Effect ratio
Right Broca homologue volume	r^2 = 0.46 ; F(5,36)=6.2; p<0.0003				
a path	1.06	0.26	0.0003		
b path	0.03	0.17	0.87		
Total effect	-0.68	0.23	0.0067		
Direct effect	-0.71	0.30	0.02		
Indirect effect	0.029	0.22		-0.44 to 0.49	4.26%
Left Posterior parietal volume	r^2 = 0.53 ; F(5,36)=8.3; p<0.00001				
a path	1.06	0.26	0.0003		
b path	0.11	0.16	0.49		
Total effect	-0.63	0.22	0.007		
Direct effect	-0.74	0.28	0.01		
Indirect effect	0.11	0.16		-0.19 to 0.44	18.3%
L Posterior parietal surface area	r^2 = 0.57 ; F(5,36)=9.7; p<0.0001				
a path	1.06	0.26	0.0003		
b path	-0.009	0.15	0.95		
Total effect	-0.83	0.21	0.0004		
Direct effect	-0.82	0.27	0.004		
Indirect effect	-0.009	0.14		-0.34 to 0.24	1.08%
L Fusiform surface area	r^2 = 0.41 ; F(5,36)=4.9; p<0.0014				
a path	1.06	0.26	0.0003		
b path	0.017	0.18	0.92		
Total effect	-0.83	0.25	0.002		
Direct effect	-0.85	0.31	0.01		

Indirect effect	0.018	0.24		-0.35 to 0.36	2.16%
Left Wernicke cortical thickness	r^2 = 0.76 ; F(5,36)=23.6; p<0.00001				
a path	1.06	0.26	0.0003	-	-
b path	-0.31	0.116	0.009	-	-
Total effect	-0.49	0.17	0.007	-	-
Direct effect	-0.18	0.199	0.37	-	-
Indirect effect	-0.32	0.12		-0.65 to -0.14	65.3%
Right Wernicke homologue cortical thickness	r^2 = 0.72 ; F(5,36)=18.7; p<0.00001				
a path	1.06	0.26	0.0003	-	-
b path	-0.18	0.12	0.14	-	-
Total effect	-0.64	0.18	0.001	-	-
Direct effect	-0.45	0.21	0.04	-	-
Indirect effect	-0.19	0.17		-0.62 to 0.08	29.6%

Bootstrap samples for bias corrected bootstrap confidence intervals: 20000. Covariates in the model for the dependent variable include age, mean cortical thickness (ICV for volume and surface area) and number of alcohol units; the initial r^2 statistic shows the fit of the model with all variables including the mediator. Mediating variable – Inflammation factor; details of the path are shown in Figure 2. Coefficients are interpreted as Betas. Indirect effect is the product of coefficients of 'a' and 'b' paths.

4.5 Discussion

The aim of this analysis was to examine the relationship between neighbourhood level deprivation and cortical morphology of anatomical ROIs pertaining to language and executive functions. The rationale behind this was to examine the grey matter element most commonly associated with these cognitive functions. I have demonstrated that neurologically healthy middle-aged men from the most deprived (MD) and least deprived (LD) neighbourhoods of Glasgow show significant cortical morphological differences in the above ROIs.

The findings from the analysis are unlikely to be due to global differences in cortical morphology for the following reasons. 1) Cortical morphology was not smaller in the MD in all the regions of interest. The LD had smaller morphology pertaining to a few ROIs, although they did not survive multiple testing corrections; 2) neither ICV nor mean cortical thickness differed between groups and 3) differences in all the above regions survived even after co-varying for the above global measures. Our findings are similar to that of previous studies that have found an association between SES and regional brain anatomy, rather than global changes (Noble et al., 2012).

4.5.1 Comparison with previous studies

A few previous studies have found an association between SES and inter-individual variations in regional brain morphology in adults. Staff et al found an association between childhood SES and hippocampal volume in 235 elderly adults, even after adjusting for adult SES levels(Staff et al., 2012). They propose that their findings are consistent with a neuro-developmental trajectory where effects of childhood SES on structural brain development are detectable even after 50 years. However, not all studies have found a relationship between childhood SES and regional brain morphology. Butterworth et al found no association between childhood SES and hippocampal or amygdalar volume in 403 middle-aged individuals. They found that financial hardship over the past year was associated with smaller hippocampal and amygdalar volumes (Butterworth et al., 2011). It should however be noted that automated measurements of sub-cortical structures using FreeSurfer are less reliable than cortical surface based

measurements (Morey et al., 2009). In addition to conventional measures of SES, Gianaros et al suggest that complex psychological factors may moderate the relationship between SES and health outcomes throughout the life span (Gianaros et al., 2007a). They found that lower perceived parental social standing was associated with greater amygdalar activation in response to angry faces, even after co-varying for parental education (Gianaros et al., 2008). In a separate study, they found that self-reports of low subjective social status was associated with reduced grey matter volume in the perigenual anterior cingulate cortex (pACC), a region highly associated with affect related processes, even after co-varying for conventional SES indicators.

Although it is possible that regions like the hippocampus, amygdala and ACC that are involved in stress related processes are more sensitive to lifelong experiences associated with poverty, it can only be speculated that these processes are directly responsible for the differences in cortical morphology (Gianaros et al., 2007a, Gianaros et al., 2012, McEwen and Gianaros, 2010). The differential associations between SES and specific brain regions may explain the poor neuro-cognitive health in these individuals. I did not find an association between neighbourhood deprivation and ACC morphology. However, our parcellation was more dorsal than Gianaros et al – a region associated with cognitive control showing high connectivity with fronto-parietal regions (Bush et al., 2000, Margulies et al., 2007). While the results of the aforementioned studies are not directly related to the ROIs that I tested, Gianaros et al using fMRI along with a positive and negative feedback task, found that higher parental education predicted greater activity in pars-triangularis and the angular gyrus, both in the vicinity of language regions explored in our study (Gianaros et al., 2010).

A small number of studies have examined the associations in children. Noble et al examined the relationship between SES and volumes pertaining to language, episodic memory (hippocampus), ACC and amygdala in sixty 15 year old children. SES was found to predict differences in hippocampal and amygdalar volumes. They also observed an SES X age interaction in the left superior temporal gyrus, associated with phonological skills. Some of their negative findings may however be explained by the fact that they did not explore cortical thickness and surface area separately (Noble et al., 2012, Jednoróg et al., 2012). Previous studies have

found that regions that do not show volumetric differences, may show cortical thickness differences (Jednoróg et al., 2012). These findings emphasise the importance of selecting the appropriate phenotype to be measured when exploring inter-individual differences in cortical morphology associated with SES in both adults and children.

4.5.2 Exploring cortical thickness and surface area separately

Cortical volume is a function of surface area and cortical thickness. I found that that the MD group had smaller surface area pertaining to the left fusiform and left posterior parietal cortex. They also had smaller cortical thickness pertaining to Wernicke's area and its right homologue. Interestingly, the cortical thickness difference between groups did not show up as a volumetric difference. This is because cortical grey matter volume is almost entirely driven by surface area rather than cortical thickness (Im et al., 2006). Our findings reflect those of Jednorog et al, who found that SES in a group of 10 year old children was associated with differential involvement of cortical volume, cortical thickness and surface area pertaining to different regions and highlight the importance of examining both cortical thickness and surface area separately. (Jednoróg et al., 2012)

There are several theoretical reasons why one should examine these distinct cortical parameters separately. Firstly, cortical volume is derived from two properties of the cortical sheet with a distinct cellular basis: cortical thickness and surface area.(Panizzon et al., 2009, Rakic, 2009) Ontogenically cortical expansion (increase in surface area) is independent of cortical thickness. The radial unit hypothesis proposes that symmetrical cell division within the neural stem cell pool in the ventricular zone results in an exponential increase in the number of radial columns, contributing to an increase in surface area. This is independent of asymmetrical cell division in the neural progenitors that results in an increase in the number of neurons within a radial column, contributing to an increase in cortical thickness. Cortical structural covariance networks derived from cortical thickness and surface area shows different structural properties, suggesting that they contribute to different properties within cortical networks (Sanabria-Diaz et al., 2010).

Secondly, recent large scale studies have shown that these two parameters – cortical thickness and surface area- have an independent genetic basis. Panizzon et al found that although total cortical thickness and surface area were both highly heritable, they were essentially unrelated genetically (genetic correlation = 0.08)(Panizzon et al., 2009).

Life course trajectories of these cortical parameters seem to be different. While gyrification – a ratio of total surface area to pial surface area remains fairly stable post childhood through to early adulthood, cortical thickness changes dynamically throughout this period (Raznahan et al., 2011, Salinas et al., 2012). Cortical thickness in addition appears to be highly susceptible to various environmental influences over the life course such as smoking and alcohol dependence, while surface area appears to be influenced by various unique developmental factors (Kuhn et al., 2010, Momenan et al., 2012). Furthermore, there is some evidence that surface area and cortical thickness may be differentially affected in relation to timing of environmental events that affect cortical morphology. For example, Park et al, found that a group of congenitally blind adults had significantly reduced cortical surface area in the primary visual areas, compared in contrast to those who developed blindness after two years of age, who had cortical thinning but no difference in the surface area (Park et al., 2009).

In our study, cortical thickness differences in the Wernicke's region were mediated by the inflammation factor. It is tempting to speculate that the cortical thinning in the MD is a result of greater inflammation accumulated over the life course, while the surface area differences may have occurred in early life. While theoretically plausible, the cross sectional nature of our study precludes us from making such causal assumptions. It should also be noted that processes that affect cortical morphology may affect both parameters (cortical thickness and surface area) at different intensities, such that one may be affected more than the other. Therefore, pathological processes may affect both parameters, but may be apparent only in one. In addition, the apparent differential involvement may be due to inadequate power, rather than actual differential involvement. In fact, a number of strong relationships in our study did not attain statistical significance, suggesting that conclusions about the differential regional involvement, timing or pathological processes underlying

the associations are speculative at best. While such assumptions can only be made using longitudinal studies, examining just one parameter or a composite parameter like volume, which is primarily driven by cortical surface area, could lead to false negative findings.

4.5.3 Role of neighbourhood deprivation and mediators

Most of the studies previously mentioned have examined the association between individual level SES and brain morphology. A small number of studies have combined individual level SES indicators with neighbourhood level indicators. However, individual level explanations for poor health do not capture important social and structural determinants of ill health (Diez Roux and Mair, 2010). It is well established that social circumstances have direct biological consequences, as well as impact on health behaviours. Neighbourhood level deprivation has been associated with poor health outcomes due to inequalities in resource distribution. These neighbourhoods have physical (e.g. access to food) and social (e.g. violence) attributes that are contributors to health outcomes.

Previous epidemiological studies have found an association between SES and raised inflammatory markers (Nazmi et al., 2010). The results of the data reduction using PCA enabled us to examine the mediation effects of broad domains within the markers that were statistically independent and physiologically meaningful – inflammatory and metabolic markers. The variance explained by the inflammatory factor on cortical thickness, was significant even after including the TAG and BMI-HDL-Insulin factors as covariates. This is not surprising, as the initial PCA meant that the factors were statistically independent. Nevertheless, the results suggests that although previous results suggest that BMI, lipid markers and inflammatory markers covary significantly, they may explain significant independent variance in cortical thickness.

The “inflammation factor” mediated the relationship between deprivation status and cortical thickness in the left Wernicke’s area. A similar relationship was demonstrated by Gianaros et al who found that circulating levels of CRP mediated the relationship between SES and measures of white matter integrity (Gianaros et al., 2012). McLean et al have previously shown that a composite cardio-metabolic risk index, mediated the relationship between neighbourhood

deprivation and N-acetyl-aspartate, a measure of neuronal integrity in the hippocampus (McLean et al., 2012). Taken together, these findings suggest that SES may be related to different systems in the brain through similar pathways. Recent studies have demonstrated an association between circulating inflammatory markers and brain structure and function. Drake et al using the PET tracer PK11195 found that people with greater cerebrovascular risk factors (but no stroke) showed greater microglial activation in the brain, compared to normal controls (Drake et al., 2011). Marsland et al have previously shown an association between greater circulating IL-6 levels and smaller hippocampus, medial prefrontal cortex and cerebellar grey matter volume in middle aged adults (Marsland et al., 2008). These findings suggest that certain brain regions may be particularly associated with variations in circulating inflammatory markers. It remains unknown if a causal relationship exists between these variables or if the changes in circulating inflammatory markers and cortical morphology are a phenotypical presentation of common aetio-pathological factors. While Gianaros et al found a mediating effect of adiposity on white matter integrity, I found no such effect (Gianaros et al., 2012). While this could be due to differential relationship of these mediators on different structures, a type 2 error due to small sample size cannot be ruled out.

In conclusions, neighbourhood level deprivation is associated with differences in cortical morphology pertaining to regions of the brain commonly associated with language and executive function. I also provide evidence about potential factors that may contribute to these associations. Future longitudinal studies should enable us to explore the complex relationship between SES and variations in various cortical morphological parameters.

Chapter 5 - Inflammation mediates the difference in cortical thickness covariance network structure associated with neighbourhood deprivation

Recently, complex network analysis has been used to characterize various structural properties of the large-scale network organization of the brain. For example, the human brain has been found to have a modular architecture i.e. regions within the network form communities (modules) with more connections between regions within the community compared to regions outside it. In this chapter, examine the modular and overlapping modular architecture of the brain networks from people from the least and most deprived neighbourhoods of Glasgow, using complex network analysis. For the first time, I have shown that neighbourhood deprivation is associated with changes in network structure. More interestingly, I show that this difference may be partly driven by differences in a circulatory inflammatory index between groups. Part of this chapter, has been published as *Krishnadas, R., et al. The envirome and the connectome: exploring the structural noise in the human brain associated with socioeconomic deprivation. Front. Hum. Neurosci. 7:722. doi: 10.3389/fnhum.2013.00722*

5.1 Introduction

Overlapping large-scale networks that are organised across the cortex form the anatomical and functional foundations of complex cognitive processes (Bressler and Menon, 2010). Complex network analysis based on graph theory has been recently used on neuroimaging data (MRI, MEG and EEG) to explore different properties of these large-scale cortical network organization (Sporns, 2011). These studies have shown that human brain networks are optimally functioning systems that demonstrate small world properties, and a modular architecture (Bassett et al., 2008, He et al., 2007, Bullmore and Sporns, 2012). Modularity is an index of community structure within a large-scale network (Newman, 2006). That is, these networks have a tendency to form modules or communities with more connections between nodes within the module than between modules than expected. Structurally, modules represent discrete entities whose functions are separable from those of other modules (Hartwell et al., 1999).

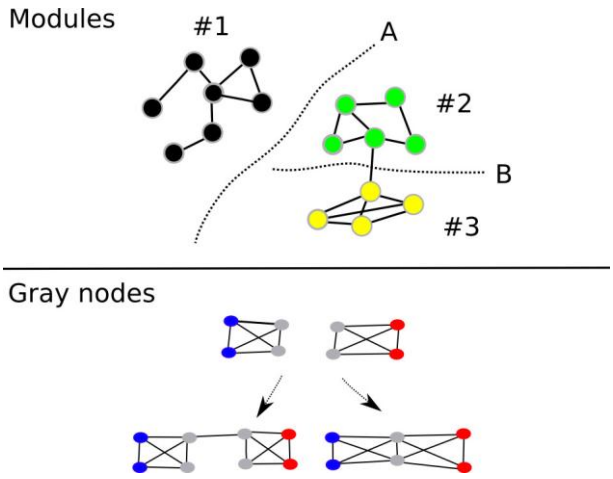
While modularity (segregation) is usually associated with robustness of the network in biological systems, complex cognitive processes (an index of performance of the network) are unlikely to occur optimally within isolated modules (Hintze and Adami, 2008). Rather, they are likely to be dependent on the coordinated activity between several modules within the large-scale network (integration). Indeed, most biological networks that survive in nature are those that achieve some balance between robustness and performance (segregation and integration). Intuitively, it would be beneficial if the human brain network demonstrated modularity – increasing its robustness - but also had an architecture that facilitates efficient information transfer between modules – thereby improving performance. Therefore, while maintaining the advantages of having a modular architecture, I propose that the human brain will also demonstrate an overlapping modular architecture, where certain nodes (I call grey nodes) are included in many modules at the same time (Figure 5-1) (Zhao et al., 2011). Within an information processing system, such an architecture, will improve information transfer between modules thereby increasing efficiency and performance of the network in terms of having lesser number of edges and shorter average path lengths. In short, while modularity represents the community architecture within a network, grey nodes represents an index of overlapping communities.

Survival in adverse environments may be associated with changes in network structure that make them less robust and reduce their performance. Neighborhood level socioeconomic status (SES) is associated with adversity and the presence of risk factors for reduced physical and neurocognitive health (Diez Roux and Mair, 2010). If indeed, cognitive functions are dependent on optimal functioning (and hence structure and topology) of large-scale brain networks, it is possible that SES is associated with changes in large-scale network structure. A small number of neuroimaging studies have shown SES to be associated with variations in individual brain anatomy and functional connectivity in adults (Gianaros et al., 2007a, Gianaros et al., 2008). While network structure and topology have been found to be disrupted in a number of mental illnesses, no study has examined the relationship between neighborhood socioeconomic deprivation and brain network structure in humans. The aim of the present study was to apply complex network analysis to examine the structural characteristics

– modularity and grey nodes – of cortical networks derived from cortical morphology correlation (Figure 1).

I also examined these structural characteristics in relation to socioeconomic deprivation. There is growing evidence that cortical morphology covariation is an indicator of connectivity between different regions of the brain (Worsley et al., 2005, Lerch et al., 2006, He et al., 2007, Bassett et al., 2008, Alexander-Bloch et al., 2013). Graph-theoretical network analyses based on morphological correlations have been used to examine brain network structure in healthy and clinical samples (He et al., 2009, He et al., 2007, Bassett et al., 2008).

Figure 5-1: Shows the modular architecture (top Figure) and grey nodes (bottom Figure), Grey nodes



Consider two fully connected networks (bottom Figure), with four nodes each and are fully connected. The two networks can be connected in two different ways. If they are connected as the first left in the bottom, then one additional edge is used. On the other hand, if they share the two nodes depicted in grey, then the combined module saves resources, i.e. there are two nodes and two edges less than the first combination. In addition, the average path lengths are shortened than the one of non-sharing combination.

Using complex network analysis of magnetic resonance imaging (MRI) surface-based morphometry I investigated the topological features of whole cortical anatomical networks in 42 neurologically healthy men from the most deprived (MD) and least deprived (LD) neighborhoods of Glasgow . The connectivity matrices in the present study were derived from region-wise cortical thickness correlations between 68 anatomical parcellations and subjected to complex network analyses. I propose that the brain networks derived thus will show an overlapping modular architecture – by the presence of modules and grey nodes. I

also sought to determine if these structural properties differed significantly between neurologically healthy people living in the most deprived (with higher risk of reduced mental health cognitive functioning) and the least deprived regions of Glasgow. Throughout the paper, “structural” refers to the network structure (e.g. modularity or proportion of grey nodes). I have used the term “anatomical” to refer to brain anatomy.

5.2 Materials and Methods

5.2.1 Participants

Participants were recruited as part of a larger study Psychological, social and biological determinants of ill health (pSoBid) and have been described in chapter 4 section 4.2.1.

5.2.2 Cardio-metabolic risk factors

For this analysis, I once again, as in the previous chapter, examined the role of 9 blood markers - C-reactive protein (CRP), Interleukin-6 (IL-6), Intercellular Adhesion Molecule-1 (ICAM),Triglycerides, High density lipoprotein (HDL), Very low density lipoprotein (VLDL), Fibrinogen, D-dimer and Insulin – and an anthropometric measure- BMI. The details of this analysis are in chapter 3 section 3.2.2 and chapter 4 section 4.2.2.1.

5.2.2.1 Principal components analysis and dimension reduction of cardio-metabolic markers

This step is same as the step in the previous chapter, and has been detailed in section 4.2.2.2.

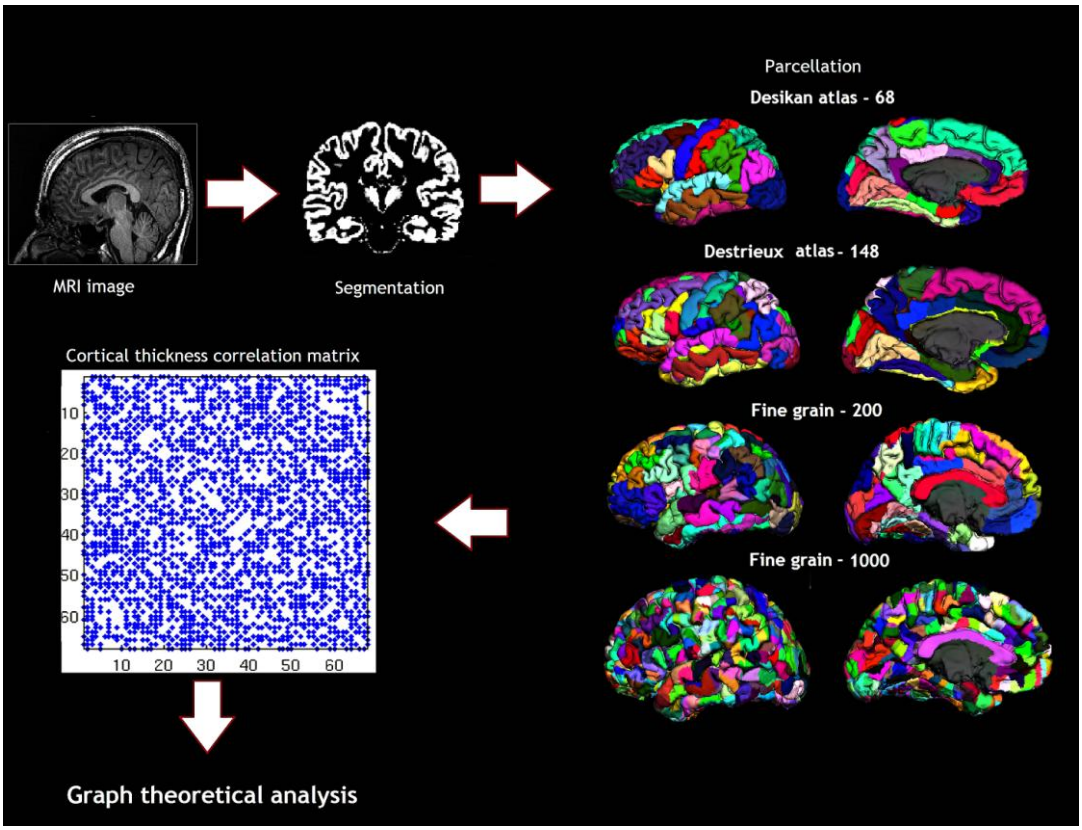
5.2.3 Image acquisition

This has been detailed in chapter 3 section 3.2.5.

5.2.4 Cortical thickness measurements and parcellations

Cortical reconstruction was performed with the FreeSurfer (FS) image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/) (Fischl and Dale, 2000b, Fischl et al., 1999a, Dale et al., 1999). This has been detailed in section 3.2.6. The parcellations were obtained using the Desikan sulcogyral-based atlas, which follows the anatomical conventions of Duvernoy. The FS image-processing pipeline was visually inspected and corrected at critical points in order to avoid errors permeating through the subsequent analyses. Procedures for the measurement of cortical thickness have been validated against histological analysis and manual measurements. The Desikan Killiany atlas produces 68 parcellations based on gyri and sulci (Desikan et al., 2006a). In addition to the Desikan Killiany atlas parcellation scheme, I also used fine-grained parcellation schemes based on anatomical sulcogyral boundaries including the Destrieux atlas, (148 parcellations) and fine-grained parcellation schemes (200, and 1000 parcellations) that did not follow anatomical conventions as described in Echtermeyer et al (Destrieux et al., 2010, Echtermeyer et al., 2011). The analysis pipeline is shown in Figure 5-2.

Figure 5-2: Analysis pipeline, including the parcellation schemes.



Desikan atlas and Destrieux atlas showing the sulcogyral parcellations and the Finegrain 200 and 1000 atlas as in (Collins et al., 2011)

5.2.5 Cortical thickness – between group comparison

Statistical comparisons of global data and surface maps were generated by computing a general linear model (GLM) of the effect of neighbourhood deprivation (independent variable) on thickness (dependent variable) at each vertex in the cortical mantle, using the Query, Design, Estimate, Contrast (QDEC) interface of FreeSurfer. Age was used as nuisance covariate in the model. QDEC is a single-binary application included in the FreeSurfer distribution that is used to perform group averaging and inference on the cortical morphometric data produced by the FreeSurfer processing stream.

(http://surfer.nmr.mgh.harvard.edu/fswiki/Qdec). Maps were created using statistical thresholds of p=.05 and were smoothed to a full width half maximum (FWHM) level of 20mm. Since this analysis involved performing a GLM analysis at 160000 vertices, these maps were corrected for multiple comparisons by means of a cluster-wise procedure using the Monte Carlo Null-Z simulation method adapted for cortical surface analysis and incorporated into the QDEC processing stream. For these analyses, a total of 10,000 iterations of simulation were performed for each comparison, using a threshold of p=.05.

5.2.6 Network construction

Network construction was based on parcellations of cortical thickness as described by He et al (He et al., 2007). I defined an anatomical connection (edge) as statistical associations in cortical thickness between cortical parcellations based on the Desikan Killiany atlas included in the FreeSurfer pipeline (nodes). The statistical similarity in cortical thickness between 2 regions was measured by computing the Pearson's correlation coefficient across subjects to create an interregional correlation matrix ($N \times N$, where N is the number of brain regions based on Desikan cortical parcellation atlas, here $N = 68$). In order to keep the analysis as close as possible to previous reports, prior to the correlation analysis, a linear regression was performed at every region to remove the effects of age, and mean overall cortical thickness; the residuals of this regression were then substituted for the raw cortical thickness values (Chen et al., 2008). In order to be consistent with the cortical thickness group difference analysis presented above, the complex network analyses were repeated without mean overall cortical thickness in the model, but the results of this analysis did not differ significantly. A separate matrix was produced for the MD (21 subjects) and the LD (21 subjects). As a first step, all negative correlations were discarded. As the correlation analysis was performed for all $68 \times 68/2 = 1431$ pairs of regions, I performed a multiple comparisons correction to test the significance of these correlations.

We applied the false discovery rate (FDR) procedure separately to each matrix in order to correct the multiple comparisons at a q value of 0.2 (this was chosen as at 0.05, both matrices were very sparse) (Genovese et al., 2002). Using this

threshold, I constructed a symmetric connection matrix (Figures 5 and 6), whose element was 1 if the cortical thickness correlation between 2 regions was statistically significant and 0 otherwise. This binarised connection matrix captures the underlying anatomical connection patterns of the human brain common to the population sample under study. I repeated all the analyses on matrices derived from the fine grained parcellation schemes described above, in order to validate my findings using multiple parcellation schemes.

5.2.7 Modularity

All the modularity metrics were calculated on the above two adjacency matrices separately and compared to corresponding random networks. Modularity is an intuitional concept and there are variations in the mathematical definitions, where each has its own advantages and disadvantages. One common property among the various ways of defining modularity, however, is accounting for the agreed intuition about modularity, i.e. a module is a subset of nodes in a graph, whose connections among the elements within the subset are much denser than the ones to nodes outside the subset. Newman suggested the following modularity measure, Q:

$$Q = \max_{s \in S} \frac{1}{4m} s^T B s ,$$

where s is a column vector and element of the set S, S is the set of all column vectors whose dimension are equal to the number of nodes in the graph, n, and each component of the vector is either -1 or +1, $(\cdot)^T$ is the transpose. B is equal to $A - kk^T/(2m)$, A is the adjacency matrix, whose dimension is $n \times n$, and the i-th column (or row) and j-th row (or column) element is 1 (or 0) if i-th and j-th nodes are connected by an edge (or if there is no edge), k is a column vector whose element is the number of edges connected for each node, i.e. the degree of node, and m is the total number of edges. Roughly speaking, B quantifies the difference between the number of edges found in a subset of the given network structure, i.e. A, and the expected average from the random graphs, whose nodes degree is the same as the one of the given graph, i.e. $kk^T/(2m)$. Hence,

positive Q values imply that there are more edges found than the expected and it is, therefore, a module.

By obtaining s that maximizes the modularity, Q, the nodes are divided into two groups, i.e. modules, depending on the corresponding values in the maximizing vector, s. The maximization problem, however, is the integer quadratic programming problem, which is NP-hard. It is even computationally very difficult to obtain the true solution, which gives the global maximum value of Q. Note that Q is always less than or equal to 1. If the condition for s is relaxed so that it can take any real numbers, then the problem becomes finding maximum eigenvalue and the corresponding eigenvector of the matrix, B. This can be solved efficiently using the power-iteration, i.e. choosing an arbitrary initial vector, s_0, and recursively updating the vector using $s_{k+1} = Bs_k$ until it converges. Then, s maximizing Q is calculated simply by taking the sign of converged s_k. To increase the chance of finding the global solution, these procedures are repeated a number of times with a different random initial vector, s_0. If the calculated maximum value, Q, is positive (or negative), then the graph is divided (or declared indivisible).

Once the graph is divided into two modules, then each module is inspected whether it can be further divided by solving the following the maximization problem:

$$\Delta Q = \max_{r \in S^g} \frac{1}{4m} r^T B^g r ,$$

where r is an element of the set s^g, s^g is the set of n_g-dimension column vectors whose element is either +1 or -1, n_g is the number of nodes in the module, which is found in the previous step, B^g is equal to $B^{ij} - \mathrm{diag}\left[k^g\right]$, B^{ij} is a matrix constructed by a part of B, where the rows and columns belong to the module, k^g is the degree of each nodes only concerning B^g, and $\mathrm{diag}[\cdot]$ is the diagonal matrix, where the diagonal terms are given by the vector in the argument and the other elements are zero. Again, if $\Delta Q > 0$ (or $\Delta Q \le 0$), then the

module is divided into two smaller modules (or declared indivisible). The above procedures are repeated on every module recursively until all modules are declared indivisible. By definition, the divisibility of a module is determined based on whether the modularity measure is positive or not. Very often, it is, hard to justify whether some subgroups of a graph are modules if the modularity contribution, i.e. Q or ΔQ, is very close to zero. As the mathematically possible maximum value is 1, the modular structure is much clearer if the modularity is closer to 1. Hence, the number of modules is calculated for various Q-threshold, which decides when modules are declared as indivisible.

5.2.8 Grey Nodes

A network, in general, is not a simple collection of modules but a combination of complicated overlapped modular structures, i.e. it demonstrates a hierarchical modular architecture. The overlapped modular structures are hard to decipher into elementary modules that pertain to the whole network. There are several methods to unravel the overlapping modular structure. In order to use a consistent measure with the modular calculation, an extended modularity (Q_e) is defined as follows:

$$Q_e = \max_{s_e \in S_e} \frac{1}{4m} s_e^T B s_e ,$$

where s_e is an element of the set, S_e, and the set S_e is the collection of vector, s_e, whose dimension is again, n, i.e. , the number of nodes, and its element is either -1, +1, or 0. Compare to the vector s in S, s_e has one more degree of freedom in possible values (Zhao et al., 2011). The nodes corresponding to zero are called grey nodes, which are included in multiple modules at the same time or are not included in any module. ΔQ_e is defined in the similar manner. Grey node is a similar concept to that of connector hub and hierarchical or overlapping modular structure. While connector hubs are defined as nodes with greater than average degree of the network and distributed between both local and long range connections, grey nodes are defined as nodes that are shared by modules. It is an index of overlapping modular architecture of the network.

Previous literature has described such overlapping architecture based on a prior definition of modularity by Girvan and Newman (Girvan and Newman, 2002) . On the other hand, “grey nodes” are a unified way to define the structure in the more recent modularity definition by Newman (Newman, 2006). This provides an advantage that I measure modular architecture, and the overlapping architecture using a consistent measure without requiring significant changes in the algorithm (Newman, 2006).

All calculations presented in this paper are based on Monte-Carlo simulations performed 1000 times. The distributions of all calculations are confirmed to be similar to Gaussian distributions (data not shown). Hence, there is no danger that the analyses based on the mean and the variance may give any false interpretations of the true distribution of the data. All graphs were compared to random graphs (with the same number of nodes and degree distribution as the corresponding brain networks).

5.3 Inflammation and network structural difference

In order to examine the role of inflammation in mediating the network structural difference between the two groups, I constructed a separate correlation matrix, to repeat the above analysis. This time, prior to the correlation analysis, I performed a linear regression at every region (based on the Desikan-Killiany atlas) to remove the effect of age, the mean overall cortical thickness and the “inflammation factor” derived from the principal component analysis in section 5.2.2.1. Among the factors from the PCA, I covaried only for the inflammation factor, as only this was different between the two groups.

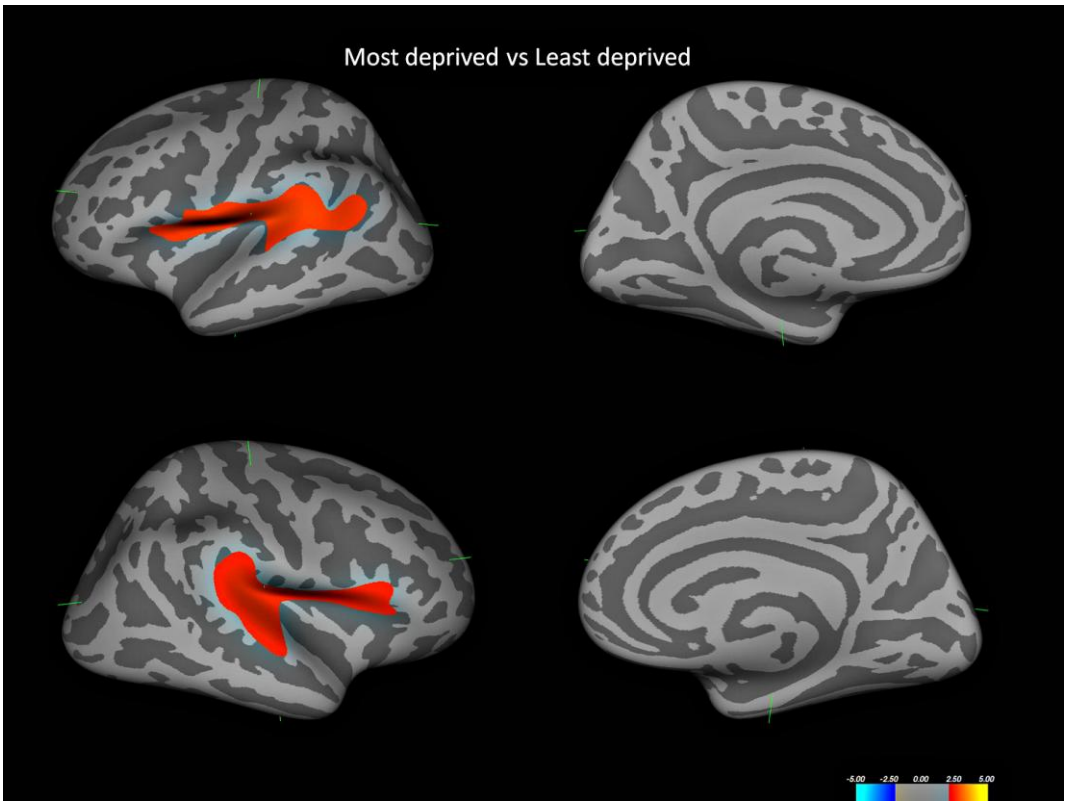
5.4 Results

Demographic details, differences in risk factors and performance on cognitive tests of the participants are shown in Table 1. In general, participants in the MD group had higher inflammatory and metabolic risk markers, poorer GHQ scores and performed poorly on a number of cognitive tests.

5.4.1 Cortical thickness differences between groups

Initial analysis of cortical thickness across groups showed that those from the most deprived population had significant cortical thinning pertaining to bilateral perisylvian cortices (Figure 5-3).

Figure 5-3: Shows the difference in cortical thickness between the most deprived and the least deprived groups.

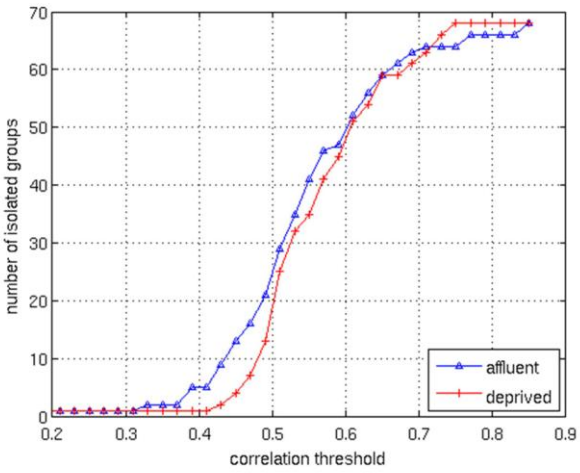


Red regions pertain to regions where the most deprived group showed cortical thinning. Covariates in the model – Age and alcohol use.

5.4.2 Network analysis

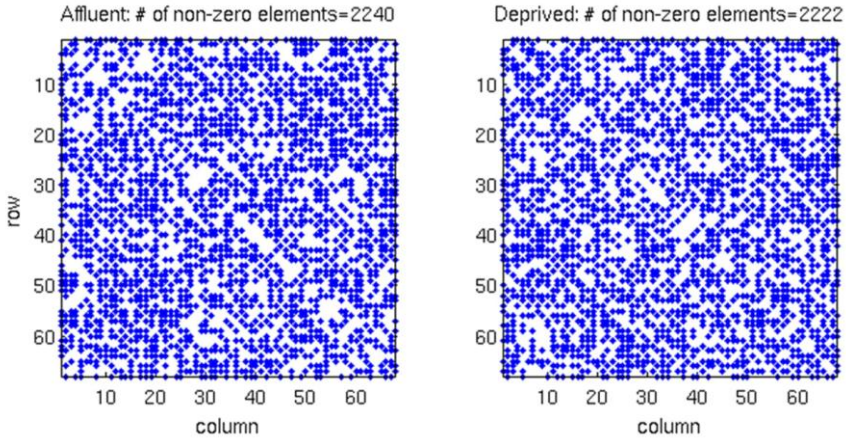
I conducted all analyses on binarised matrices derived from interregional correlations of cortical thickness. Initial examination of number of isolated modules showed that for a given correlation threshold, the least deprived group had greater number of isolated groups compared to the deprived group (Figure 5-4).

Figure 5-4: The correlation values in the matrices are distributed between 0.1 to 0.9.



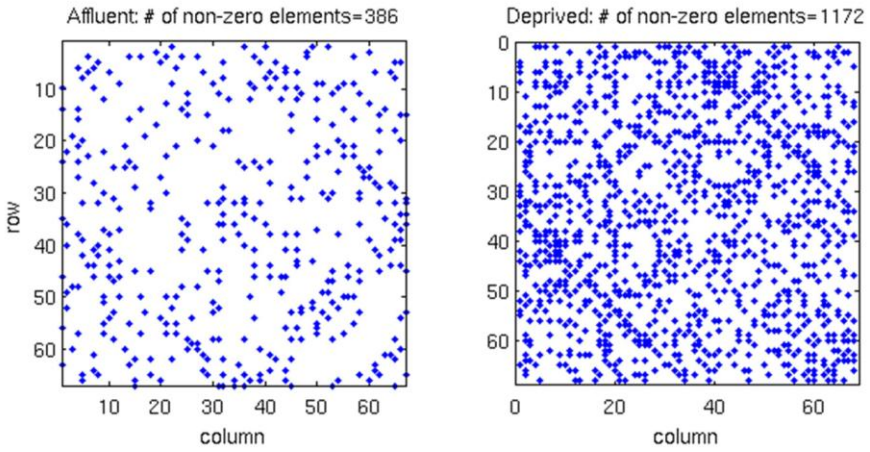
By changing the correlation threshold from 0.2 to 0.85, the number of isolated groups are counted for the both groups. The least deprived has more isolated groups than the deprived over the almost all values of the correlation threshold. The raw networks and FDR filtered networks are shown in Figure 5-5 and Figure 5-6.

Figure 5-5: The raw correlation matrix for each group shows that two groups have almost equal number of non-zero components in the matrix.



The correlation matrix for each group is a 68x68 matrix, where each value in the matrix is calculated from the cortical thickness correlation measured in 21 individuals.

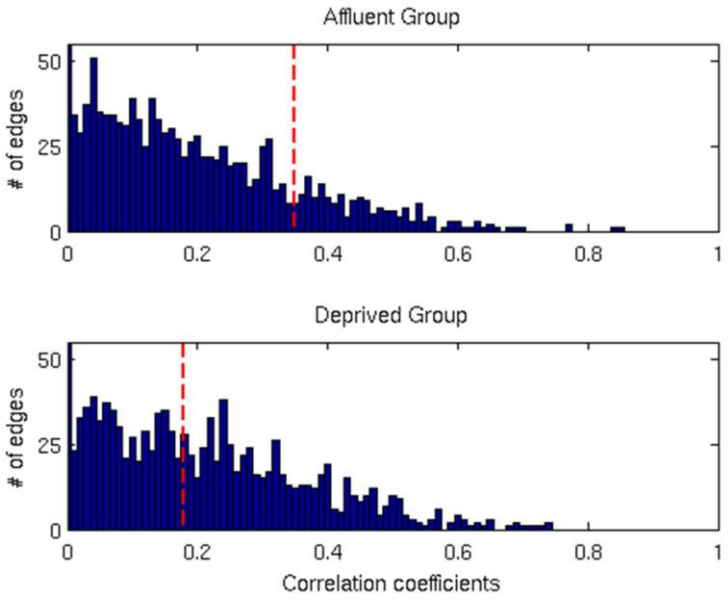
Figure 5-6: In the correlation matrix for each group, all values below the FDR threshold are set to zero.



About three-times more edges survived the FDR procedure in the most deprived than the least deprived group

The distribution of the groups' correlation coefficients is shown in Figure 5-7.

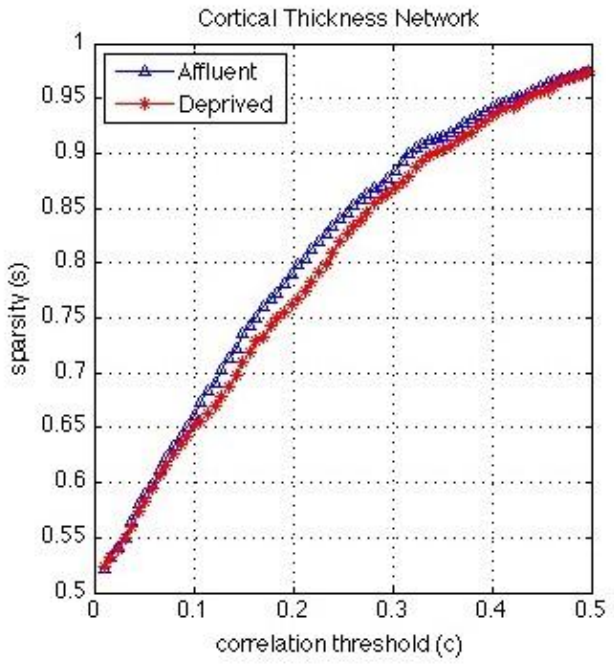
Figure 5-7: The distributions of correlation coefficients for both groups.



The vertical lines are the FDR threshold values for each group.

A direct comparison of the networks derived from the above populations, was not possible, as for a given correlation threshold, the sparsity (density) of the two networks were significantly different (Figure 5-8).

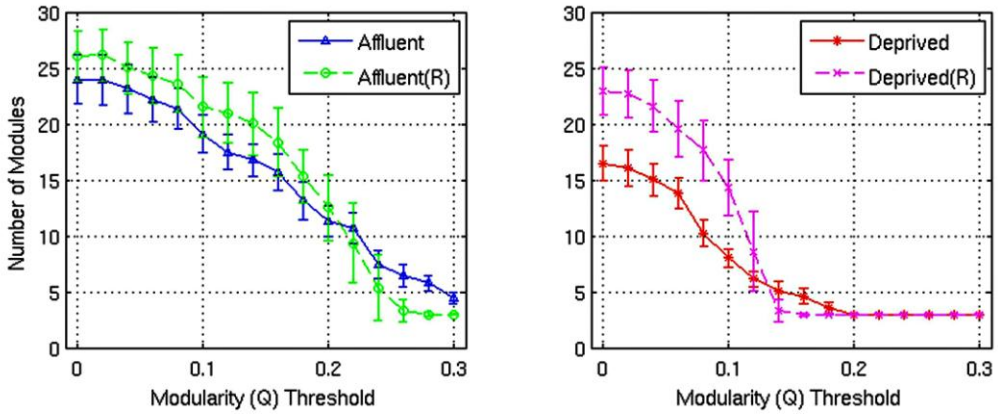
Figure 5-8: Correlation and sparsity (Number of zeros divided by Maximum possible number of edges) relations in cortical thickness network.



The most deprived group had more edges (denser network) than the least deprived for a fixed correlation threshold. On the other hand the least deprived would have more false positive edges than the deprived and/or the deprived would have more false negative edges than the least deprived for a fixed sparsity.

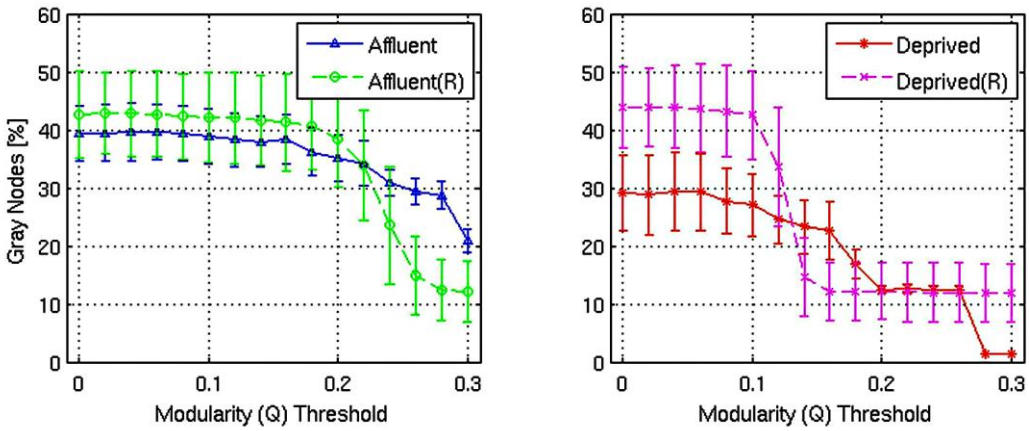
In addition, the FDR procedure thresholded the two networks significantly differently. This method of thresholding resulted in different number of edges - k - (sparsity) in the networks of the two groups because of differences in their inter-regional cortical thickness correlations. I therefore compared the network structure derived from the groups to their corresponding random networks. The results of this analysis are shown in Figure 5-9 and Figure 5-10.

Figure 5-9: Number of modules and the corresponding random graphs (indicated by "(R)") with respect to various modularity (Q) threshold



Error bars represent the 1σ-bound for each case. In the module calculation algorithm, if the module contribution, Q or ΔQ, is less than the threshold, it was declared indivisible. Higher thresholds imply strong modules.

Figure 5-10: Proportion of grey nodes with respect to the corresponding Modularity threshold



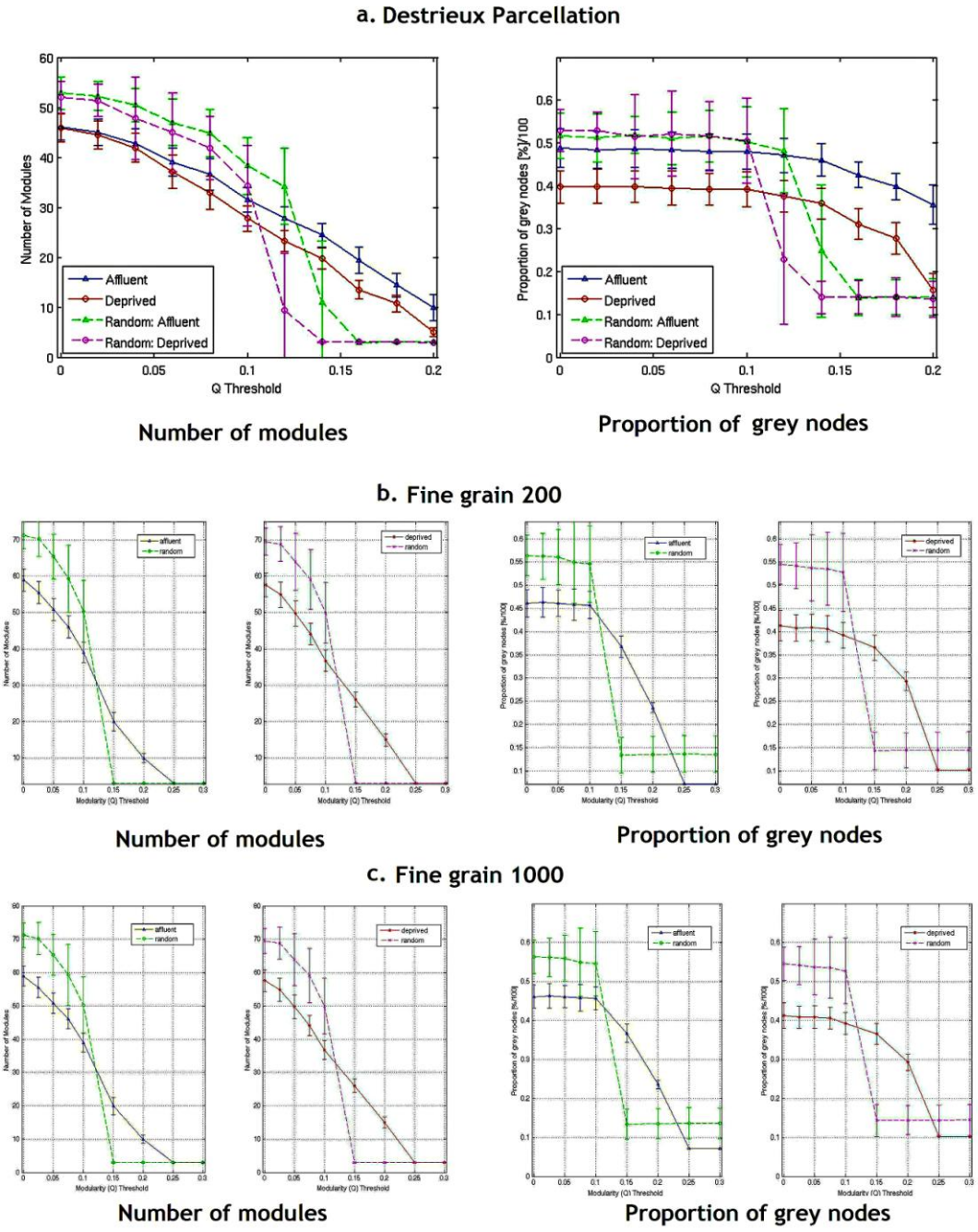
Error bars represent the 1σ-bound for each case. In the module calculation algorithm, if the module contribution, Q or ΔQ, is less than the threshold, it was declared indivisible. Higher thresholds imply strong modules. Grey nodes have two implications in the network structure: i) efficient usage of resources and ii) shorter average distance between nodes. Recycle of existing nodes and edges to combine multiple modules saves limited resources to construct the network. It is believed that reducing wiring resources is one of the major selection pressure on the brain network evolution

5.4.2.1 Modularity and grey nodes

Firstly, the networks derived from both groups showed a modular architecture, and the presence of grey nodes. Towards a modularity of 0.3 (strong modularity), the least deprived network had more modules, compared to its corresponding random network. However, the most deprived network, showed no difference from its random counterpart.

With regards the grey nodes, for a given a modularity towards 0.3, the least deprived network showed significantly greater number of grey nodes compared to the corresponding random network. However, the most deprived network showed significantly smaller proportion of grey nodes compared to its random counterpart. While the differences between groups were maintained in the Destreaux atlas (148 parcels) that followed the sulcogyral boundaries, these differences were not seen with the finer grain parcellations of 200 and 1000 parcels that did not follow the sulcogyral scheme (Figure 5-11).

Figure 5-11: The number of modules and proportion of grey nodes at a fine grain level

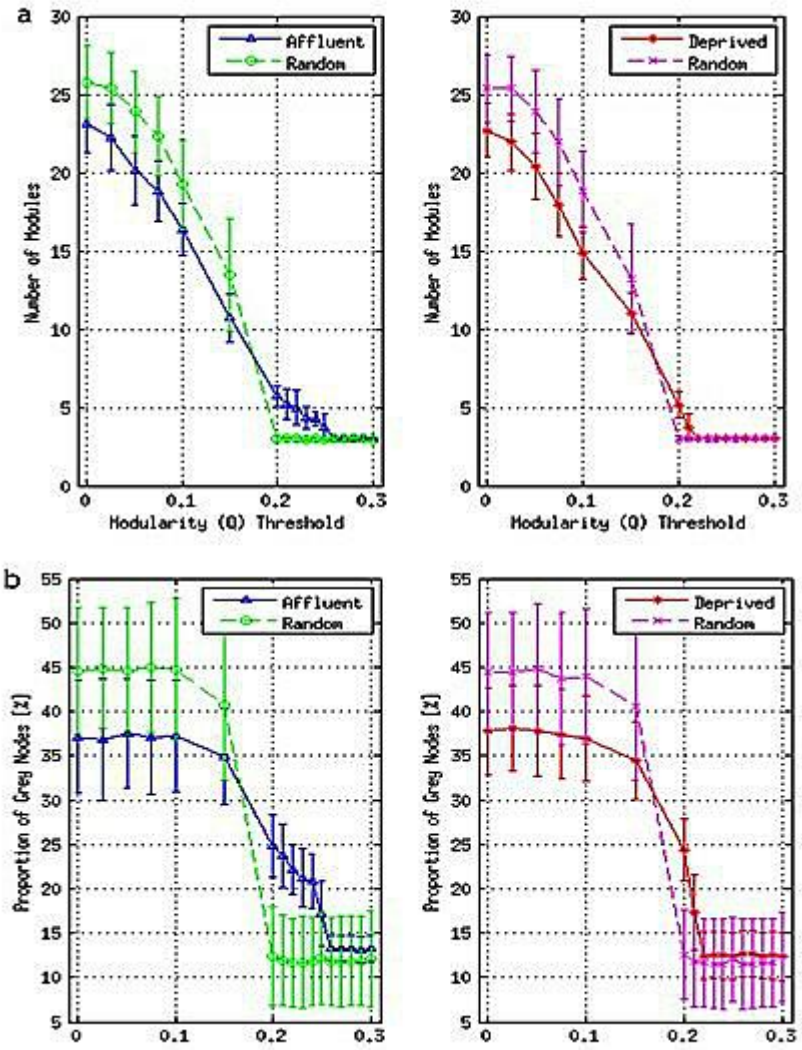


a) parcellation following sulcogyral boundaries – Destrieux atlas (148 parcels) and b) a parcellation scheme that does not follow the sulcogyral boundaries (b. 200 parcels and c. 1000 parcels).

5.4.3 Network structural difference and inflammation

I repeated the above analysis using the correlation matrix constructed after co-varying for inflammation factor. The results of this analysis are shown in Figure 5-12.

Figure 5-12: The difference in network structure derived from networks constructed from cortical thickness correction for inflammatory factor.



a) the number of modules with respect to modularity; b) the proportion of grey nodes with respect to modularity. Note that at a modularity of 0.3, the two least deprived and the most deprived networks are similar in terms of both number of modules and proportion of grey nodes.

As can be seen, towards the modularity of around 0.3, the number of modules in the affluent network is very similar to its corresponding random network and to

the deprived network. Similar is true regarding the proportion of grey nodes. These results suggest that at least part of the difference in network structure was driven by the difference in inflammatory index scores.

5.5 Discussion

I have shown here that brain networks derived from cortical morphological correlations show a modular organization, and indeed an overlapping modular architecture as demonstrated by the presence of grey nodes. I have also shown that neurologically healthy subjects from the MD regions of Glasgow differ significantly in their brain network structure from those from the LD regions in comparison to their corresponding random networks on relatively coarse parcellations schemes that followed the sulcogyral boundaries. Brain networks in the MD group showed same number of modules and smaller proportion of grey nodes compared to their corresponding random network. These differences however disappeared at fine-grained parcellation schemes that did not follow the sulcogyral schemes.

A number of recent studies have shown that human brain network structure derived from anatomical covariance demonstrates a modular architecture (Chen et al., 2011, Chen et al., 2008). There are a number of advantages in having a modular architecture. Kaiser suggests that this feature allows for low wiring costs; are time scale separable; allows for the coexistence of integration and segregation within a network; transient chimera states of desynchronisation and synchronization; and also allows for rapid and robust assembly (Kaiser, 2007). In addition, a modular architecture is robust against random attacks on the network and helps to contain the effects of these attacks to the module, rather than spreading through the network.

I compared the brain network graphs with random graphs that had similar degree to the corresponding brain network. For both the LD and MD groups, at lower modularity thresholds, the brain network graphs had fewer modules compared to their corresponding random graphs. However, this phenomenon was reversed at higher thresholds. This is possibly because within the constraints of

fixed resources (nodes/edges), brain networks enhance a few specific modules by rewiring and sacrificing unwanted modules.

In the current study, for a given number of modules, the brain networks in the LD group showed stronger modular organization than their corresponding random graphs. In other words, the networks derived from the MD group had more edges between modules, which weakened the modular architecture. Previous work by Chen et al using a similar technique showed that modules derived using correlations of cortical thickness, broadly gave out six functionally relevant modules (Chen et al., 2008). Using the same number (six modules) as Chen et al, the modules were functionally more relevant in the LD population (data not shown). For example, all anatomical regions pertaining to language function were integrated together within a given module. However, this was not the case with the MD. Anatomical regions pertaining to similar function were distributed across several modules, consistent poor functional modular organization at a given threshold. While these modularity differences may be due to anatomical differences between groups that we have shown, these may have functional implications, as anatomical networks have been found to overlap with functional networks (Alexander-Bloch et al., 2013). If we consider these networks as information processing systems, then such a difference in network structure could contribute to greater noise and less efficient information processing within the system. However, a direct interpolation of the results of the present study is not possible due to the static nature of my data.

I describe a new metric – grey node – as a measure of overlapping modular organization. While modularity improves the robustness within a system, it is unlikely that our brain network achieves optimal performance by operating as a number of different isolated modules. As stated previously, cognitive processes are likely to be the result of a number of modules interacting with each other in a fast and efficient way. The overlapping modular architecture – represented here by the presence of grey nodes - is beneficial in that given a fixed number of resources it provides the best modular architecture, maximizing the communication between modules thereby achieving a balance between robustness and optimal performance. Grey nodes have two implications in the network structure: i) efficient usage of resources and ii) shorter average distance between nodes. Recycling existing nodes and edges to combine multiple

modules saves limited resources to construct an efficient network. It is believed that reducing wiring resources is one of the major selection pressures on the brain network evolution. The results suggest that the networks derived from the MD group show much lower efficiency compared to their corresponding random network (Bullmore and Sporns, 2009, Achard and Bullmore, 2007). While metrics describing overlapping modules have been outlined previously, grey nodes have the advantage that it was derived from Newman (2006) and integrates well with the given modularity metric (Newman, 2006).

While the structural differences may be driven by the difference in cortical thickness between the two groups, the reason for the anatomical difference between the two groups is not clear. It should be noted that the groups differed on a number of variables that could potentially explain the observed difference. For example, those from the most deprived had poorer mental health and also had higher levels of inflammation. (See Table 1 in chapter 4) I have previously shown inflammatory markers to be associated with cortical thickness . The data was however underpowered to explore the role of potential mediators that could explain the difference between groups in structural properties. Previous studies have demonstrated age related changes to modularity (Chen et al., 2011). The groups were matched for age. Similarly, mental illnesses have shown to be associated with disruption to the modular architecture. A few studies have also examined this property in medical conditions like MS and epilepsy (He et al., 2009, Vaessen et al., 2012).

A number of studies have shown an association between socioeconomic deprivation and brain anatomy and function in both children and adults, though none have examined the association with network structure (Hanson et al., 2011, Gianaros et al., 2010). A key question that remains is how these anatomical differences could contribute to poorer cognitive functioning and mental health. Interestingly, the MD group performed poorly on all cognitive tests, including NART (National adult reading test) – a test that is relatively stable through age, and often considered a test of measure of the peak achieved intellectual functioning. I did not examine if less modularity was directly associated with poorer cognitive functioning as utilizing correlation coefficients to construct the matrix meant that indices of modularity could not be calculated at an individual level. However, change in network structure is a potential

mechanism by which regional anatomical brain deficits may contribute to global network topology, thereby resulting in poorer cognitive function. Previous studies have examined the relationship between intelligence quotient (IQ) and network properties. For example Li et al found a significant positive correlation between number of edges and IQ. They also found that those with greater IQ had shorter path lengths, greater clustering coefficient (similar to my findings) and in general greater global efficiency of structural networks in the brain (Li et al., 2009). Similarly using resting state fMRI to examine the overall organization of the brain network using graph analysis, van den Heuvel et al showed a strong negative association between characteristic path length of the resting-state brain network and IQ (van den Heuvel et al., 2009). They suggest that human intellectual performance is likely to be related to how efficiently the brain integrates information between various brain regions.

5.5.1 Inflammation and network structure

The finding that the difference between the two groups disappeared, when I used a correlation matrix of cortical thickness that was corrected for the inflammation index, suggests that at least in part, the difference in network structure between two groups was driven by greater inflammatory factor in the most deprived group. While no previous study has examined the effect of inflammation in cortical covariance network structure in humans, a few studies have examined patient groups where inflammation may have played a role. Inflammation is thought to play a role in the pathophysiology of cancer including the initiation, promotion, invasion and metastasis. Inflammation also affects and is affected by cancer therapy(Grivennikov et al., 2010, Tili et al., 2011). Hosseini et al examined modularity in patients with acute lymphoblastic leukemia (Hosseini et al., 2012a). They found for a given number of modules, the degree of network modularity was significantly smaller in the networks from ALL survivors. The smaller network modularity in the ALL network suggested a reduction in the balance between network segregation and integration in ALL patients. The same group also demonstrated that the clustering coefficient – the cliquishness of the network or the network segregation - was significantly smaller in the structural covariance networks from breast cancer patients than in the control network (Hosseini et al., 2012b). Although the authors did not

examine the role of inflammation in this group, they postulate that inflammatory processes may contribute to the difference in these structural properties. Indeed, acute leukemia and breast cancer has been associated with inflammatory processes (Montesano et al., 2005, Tsukasaki et al., 2001).

The relationship between inflammation and brain structural networks however, is far from clear. Interestingly, Hess et al used graph theoretical analysis to see if anti-inflammatory medications decrease pain, by directly suppressing the brain networks responsible for pain, rather than having an effect on joints. In a mouse model of arthritis, they constructed a matrix of cross-correlation of BOLD signals from structures that showed significant activation in response to nociceptive stimuli. This network showed increased clustering and modularity in arthritic mice compared to wild type mice. These changes largely resulted from the formation of tight clusters of a number of sub-cortical structures including the thalamus, periacqueductal grey and the amygdala. Interestingly, treatment with TNF- α blockade led to a rapid dissolution of this cluster, which resulted in a clustering and modularity similar to that of wild type mice. The authors suggest that neutralization of TNF-α directly affects nociceptive brain activity and network architecture in the context of arthritis, and that this occurs long before it achieves anti-inflammatory effects in the joints(Hess et al., 2011).

5.5.2 Neighbourhood level vs Individual level SES

Socio-economic status (SES) refers to a multidimensional construct that is usually measured using a number of economic (e.g. income) and noneconomic (e.g. education) indicators (Hackman et al., 2010). SES can be measured at an individual/household or at a neighbourhood level. Regardless of the level of measurement (individual/neighbourhood), SES has been associated with significant health disparities (Diez Roux and Mair, 2010). Most of the studies previously mentioned have examined the association between individual level SES and brain morphology. But individual level explanations for poor health do not capture significant social and structural determinants of ill health (Diez Roux and Mair, 2010). It is well established that social circumstances have direct biological consequences, as well as impact on health behaviours. However, relatively small number of studies have explored the contributions of individual

level SES indicators with neighbourhood level indicators to health inequalities. Neighbourhood level deprivation has been associated with poor health outcomes due to inequalities in resource distribution. These neighbourhoods have physical (e.g. access to food) and social (e.g. violence) attributes that are contributors to health outcomes. In the present study, individual level SES covaried significantly with neighbourhood level SES. Due to the nature of the sampling technique, people from the most deprived neighbourhoods also had poorer individual SES. This is partly because neighbourhood deprivation scores (SIMD) are derived from data pertaining to individuals in the area. Since the groups differed inherently in their individual SES, it was deemed inappropriate to co-vary for the effects of individual SES (Miller and Chapman, 2001). The study's relatively small sample size was also not sufficiently powered to examine if individual SES contributed significant variance over and above that explained by neighbourhood SES or vice versa. The extreme group sampling technique prevented us from examining any dose-response effect of either individual or neighbourhood level deprivation in the sample.

5.5.3 Effect of parcellation scheme on network structure

Zalesky et al have previously shown that network topology vary considerably as a function of the spatial scale of the atlas used (Alexander-Bloch et al., 2013, Zalesky et al., 2010). Previous reports that have examined cortical thickness covariance network structure in clinical and nonclinical populations have used the same parcellation scheme (Desikan-Killiany atlas) used in the present study (van Wijk et al., 2010, Romero-Garcia et al., 2012, Hanggi et al., 2011, Raj et al., 2010, Yang et al., 2012). Of note, Romero-Garcia et al in order to examine the effect of network resolution on topological properties, compared the Desikan-Killiany atlas based parcellation with finer parcellation schemes of up to 1494 parcellations . Interestingly they found that highly grained cortical scales showed enhanced local connectivity (clustering coefficient), and local efficiency, but increased path length and decreased global efficiency (Romero-Garcia et al., 2012). My findings resonate that of Romero-Garcia et al, in that, at different parcellation schemes, the network topologies differed . For fine-grained parcellation schemes that did not follow sulcogyral boundaries, the LD brain network and MD brain network were similar. At a modularity threshold of

around 0.3, both network structural properties looked similar to their random counterparts (suggesting a decrease in global properties at more fine grained schemes). (figure 1 a and 1b)

Anatomically, since cortical thickness is a continuous measure, regions that lie close to each other will show very similar cortical thickness and hence high correlation. Here, a fine parcellation schemes, may uncover local connection (or a forking-U fiber connection), while a coarse may not (see figure 1 in Zalesky et al)(Zalesky et al., 2010). In addition, regions close to each other are likely to be anatomically connected by the tangential neurons and dendrites. It is possible that in our case, the group differences disappeared when geometrically close connections were exposed at the finer parcellation schemes. In addition, at finer parcellation, where the number of parcels far exceed the number of subjects in the study, the study may have been significantly underpowered to show significant differences between groups (Zalesky et al., 2010).

It is also possible that network structure derived from relatively coarse parcellations are more representative of large scale cortical networks, while the networks derived from the fine-grained parcellations also include the meso/microscale connections representing regional/local connections. Whatever the case, it is clear that the granularity of chosen parcellations may affect the results of the network analysis. The present data suggest that when exploring connectivity, choosing the right granularity that is best suited to answer the question of interest is vital. However clear cut guidelines pertaining to this are absent. One suggestion is that in order to answer clinical questions, anatomically relevant atlases like AAL or the sulcogyral parcellations (FreeSurfer) as used in the present study may be more relevant. Interestingly for a finer (than Desikan atlas) parcellation that follows the sulcogyral boundaries (the Destreaux atlas - 149 parcellations), the difference between the brain and random networks in the most deprived group disappear at around a modularity threshold of around 0.2 (figure 11a).

5.5.4 Sparsity (density) and modularity

Although I found significant differences between the networks and their corresponding random graphs, I did not perform a direct comparison of the

network structure between the two groups, as the thresholds imposed by the FDR correction led to matrices that were significantly different in their sparsity (density). Thresholding a matrix is a problem when comparing networks that have different sparsity for a given correlation coefficient . While the reason for the sparsity difference between the groups is not known, revealing topological differences gives deeper insights into the difference in networks than just revealing the sparsity difference.

One recommended way to solve this problem is by fixing the sparsity (density) of a matrix, and comparing the networks at the same fixed sparsity threshold (van Wijk et al., 2010). This approach will however increase the false negative or false positive correlations at a given threshold. For instance, in our case, at more than 90% of correlation thresholds, the LD network was more sparse (less edges – k) than the MD. i.e. for a given correlation threshold, the networks from both the groups were different in their size (the number of edges). The difference in modularity between groups may therefore be k dependent. This difference in correlation threshold may have arisen from anatomical difference in the bilateral perisylvian cortical thickness I found between groups. While these morphological differences could have led to a reduction in correlation between regions that are actually connected, this could also have led to an increase in the number of spurious correlations (false positive), between regions that are not biologically connected, thereby contributing to noise within the network. Therefore, introducing false edges by fixing the sparsity was not thought to be meaningful.

5.5.5 Cortical thickness correlation as a measure of connectivity

While the biological meaning of structural covariance is not clear, structural covariance networks have been found to be genetically heritable, associated with cognitive function, recapitulate functional networks, and change over the life span. See Alexander-Bloch (2013) for a detailed recent review of this literature (Alexander-Bloch et al., 2013). Cortical volume is a construct that is derived from two distinct properties of the cortical sheet: cortical thickness and surface area and have distinct cellular and genetic basis(Rakic, 2007). Rakic's radial unit hypothesis proposes that symmetrical cell division within the neural

stem cell pool in the ventricular zone causes an exponential increase in the number of radial columns – that result in surface area (SA) expansion. This is independent of asymmetrical cell division in the founder cells that is responsible for a linear increase in the number of neurons within a radial column, contributing to cortical thickness (CT) (Rakic, 2007).

Complex network analysis using graph theory using cortical structural covariance networks derived from CT and cortical SA shows different structural properties, suggesting that they contribute to different properties within cortical networks (Sanabria-Diaz et al., 2010). Cortical grey matter volume is almost entirely driven by differences in the cortical SA rather than CT (Im et al., 2006). Secondly, recent large scale studies have shown that these two parameters – CT and SA- have independent genetic basis (Panizzon et al., 2009).

Thirdly, life course trajectories of these cortical parameters seem to be different. While gyrification – a ratio of total SA to pial SA remains fairly stable post childhood through to early adulthood, CT changes dynamically through this period (Raznahan et al., 2011, Rathbone et al., 2011, Salinas et al., 2012). However, more recent studies suggest that the relation between age and cortical parameters in adulthood, are complex (Hogstrom et al., 2012). CT in addition appears to be highly susceptible to various environmental influences over the life course such as smoking, alcohol dependence, and marijuana use while SA appears to be influenced by various unique developmental factors (Kuhn et al., 2010, Lopez-Larson et al., 2011, Momenan et al., 2012). I restricted our analysis to cortical thickness as I was examining the association between what an environmental variable (deprivation) and a cortical parameter (cortical thickness) that has previously shown to be influenced by environmental factors. Further analysis using other parameters may reveal differences in structural properties that are contributed by factors that may be influenced early in life.

In summary, people from the MD population show less modular and overlapping modular architecture of the brain networks derived from cortical morphology compared to their corresponding random graphs at a coarse sulcogyral parcellation scheme. At fine-grained parcellation scheme that did not follow sulcogyral boundaries, this difference disappeared. While the difference in network structure at the coarse level may be the result of anatomical

differences at a large scale level, the exact aetiopathogenesis and the consequence of this difference is not clear. Taken together I propose that brain networks associated with MD group may be less efficient in information and signal processing at a large-scale level. Greater inflammatory markers within the MD group may drive these differences. Future studies should look at longitudinal functional and effective connectivity studies using MRI and EEG/MEG to explore the effect of socioeconomic status on development.

Chapter 6 - A composite measure of circulating inflammatory markers mediates the relationship between neighbourhood deprivation and morphological changes within the limbic stress circuit

In this chapter, I explore if the same circulating inflammatory index - that was found to mediate cortical thickness difference pertaining to the Wernicke's region and the network structure difference associated with neighbourhood deprivation in the previous chapters – mediated the association between neighbourhood deprivation and a putative limbic stress circuit. I used the same dataset I examined earlier, to explore the relationship between neighbourhood deprivation and volumes pertaining to a group of limbic forebrain structures - hippocampus, amygdala and medial prefrontal cortex - implicated in the top-down regulation of the stress response. Then I go on to explore if an index of peripheral inflammation mediated the relationship between neighbourhood deprivation and the stress circuitry volumetry. The findings of these chapters have been submitted for publication.

6.1 Introduction

The social and physical environments of disadvantaged neighbourhoods impinge adversely upon individual health outcomes (Diez Roux, 2001, Aneshensel, 2009, Pickett and Pearl, 2001) over and beyond the effects of individual-level socioeconomic status (SES) (Schwartz et al., 1999, Susser, 1994, Haan et al., 1987, Pickett and Pearl, 2001). Chronic psychosocial stress associated with socioeconomic disadvantage has been proposed as the pathway mediating the effects of lower SES on increased disease risk (Schulz et al., 2012, Steptoe and Marmot, 2002, Baum et al., 1999, Brunner, 1997). Chronic stress enhances the excitability of the hypothalamic-pituitary-adreno-cortical (HPA) and sympathoadrenomedullary (SAM) axes and leads to the prolonged secretion of stress hormones (Ulrich-Lai and Herman, 2009). An influential model – Allostatic Load (McEwen and Stellar, 1993) - proposes that the cumulative effects of biological adjustments induced by stress hormones result in a cascade of multi-systemic physiological dysregulations that culminate in higher morbidity and mortality (Juster et al., 2010).

A group of limbic forebrain structures - the hippocampus, the amygdala and the medial prefrontal cortex - are implicated in the top-down regulation of the HPA axis and autonomic nervous system (ANS), in response to environmental stressors (Ulrich-Lai and Herman, 2009). These regions also process aversive and appetitive environmental cues and condition the HPA axis and ANS to motivationally salient stimuli (Smotherman et al., 1981, Sullivan et al., 2004, Ulrich-Lai and Herman, 2009). McEwen and Gianaros recently summarised a large body of evidence from both animal and human studies indicating that chronic stress is associated with structural remodelling of the limbic stress circuitry (McEwen and Gianaros, 2010). Consistent with the idea that lower socioeconomic status influences neuroplasticity in the limbic stress circuitry, a few neuroimaging studies have documented an association between lower individual-level SES and the hippocampus and amygdala volumes (Staff et al., 2012, Noble et al., 2012, Hanson et al., 2011, Butterworth et al., 2011).

The mechanistic pathways underlying the restructuring of the limbic stress circuitry in the context of neighbourhood deprivation remain unclear. So far, animal studies of chronic stress have implicated corticosteroids, neurotrophins, excitatory neurotransmitters and other endogenous mediators in the structural remodelling of the hippocampus and to a lesser extent of the amygdala, with divergent patterns of dendritic arborisation (Vyas et al., 2002). Additional evidence from human data suggests that immune mechanisms (Kohman and Rhodes, 2013) and metabolic factors (Gold et al., 2007) impair hippocampal neuroplasticity.

I have already reported that living in a designated deprived urban area as per the Scottish Index of Multiple Deprivation is associated with a significantly higher inflammatory load compared with an affluent neighbourhood (Krishnadas et al., 2013c). In addition, I found that greater peripheral levels of inflammatory markers such as C reactive protein (CRP) and intercellular adhesion molecule-1 (ICAM-1) were associated with cortical thinning pertaining to perisylvian regions in the left hemisphere (Krishnadas et al., 2013c).

Based on the aforementioned evidence of neurobiological correlates of socioeconomic deprivation, I explored the relationship between neighbourhood-level SES and limbic stress network morphology. In order to better understand the mechanistic pathways that link socioeconomic deprivation with brain

morphology, I examined whether the above relationship was mediated by an index of peripheral inflammation.

6.2 Methods and Materials

6.2.1 Participants

Participants were recruited as part of a larger study (Psychological, social and biological determinants of ill health [PSoBiD]) (http://www.gcph.co.uk/work_programmes/psobid). The details have been presented in chapter 4, section 4.2.1.

6.2.2 Mediators of interest

6.2.2.1 Cardio-metabolic risk markers

For this analysis, I once again, as in the previous chapters, I examined the role of 9 blood markers - C-reactive protein (CRP), Interleukin-6 (IL-6), Intercellular Adhesion Molecule-1 (ICAM),Triglycerides, High density lipoprotein (HDL), Very low density lipoprotein (VLDL), Fibrinogen, D-dimer and Insulin – and an anthropometric measure- BMI. The details are given in chapter 4 section 4.2.2.1.

6.2.2.2 Principal components analysis and dimension reduction of cardio-metabolic markers

This step is same as the step in chapter 4, section 4.2.2.2.

6.2.3 Image acquisition

This has been previously described in chapter 3 section 3.2.5.

6.2.4 FreeSurfer cortical morphology construction and parcellation

The automated procedures for cortical reconstruction, sulcogyral parcellations and volumetric measurements were performed with the FreeSurfer image analysis suite, which is documented and freely available online

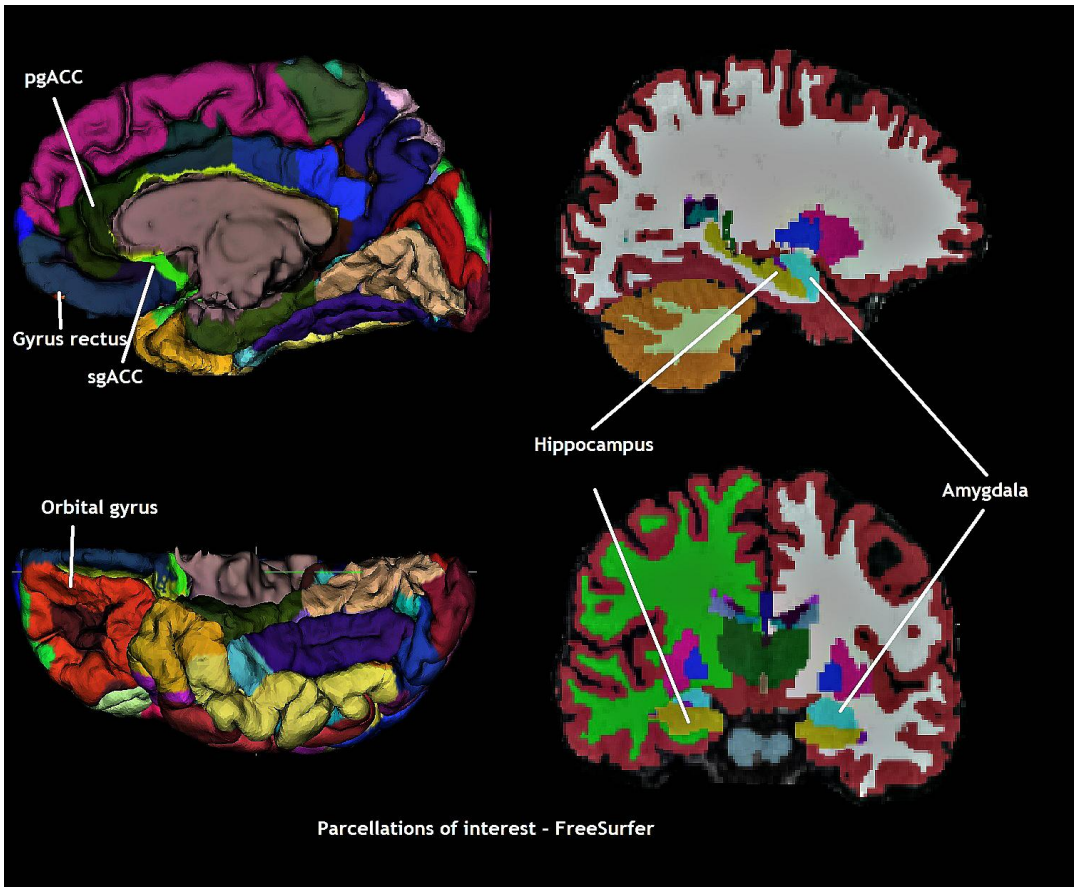
(http://surfer.nmr.mgh.harvard.edu/) (Fischl et al., 1999a, Dale et al., 1999, Fischl et al., 2002, Walhovd et al., 2005). The preprocessing steps are detailed in chapter 3, section 3.2.6. Following preprocessing, the following procedure automatically assigns a neuroanatomical label to each voxel in an MRI volume based on probabilistic information automatically estimated from a manually labelled training set. Briefly, the segmentation is carried out as follows. First, an optimal linear transform is computed that maximizes the likelihood of the input image, given an atlas constructed from manually labelled images. Next, a nonlinear transform is initialized with the linear one, and the image is allowed to further deform to better match the atlas. Finally, a Bayesian segmentation procedure is carried out, and the maximum a posteriori (MAP) estimate of the labelling is computed. The segmentation uses three pieces of information to disambiguate labels: (1) the prior probability of a given tissue class occurring at a specific atlas location; (2) the likelihood of the label given that tissue class; and (3) the probability of the local spatial configuration of labels given the tissue class. This latter term represents a large number of constraints on the space of allowable segmentations, and prohibits label configurations that never occur in the training set (e.g. hippocampus is never anterior to amygdala). The technique has been previously shown to be comparable in accuracy to manual labeling (Fischl et al., 2002). In the present paper, I report the volumes for both subcortical and cortical gray matter of the regions of interested mentioned below. The parcellations were obtained using the Destrieux sulcogyral-based atlas, which follows the anatomical conventions of Duvernoy (Destrieux et al., 2010).

6.2.5 Regions of interest

The regions of interest (ROIs) were identified from a network of limbic structures involved in regulating and coordinating the stress response (Ulrich-Lai and Herman, 2009, McEwen and Gianaros, 2010) (Figure 6-1). This limbic network comprises the hippocampus, the amygdala, the ventromedial prefrontal/orbitofrontal cortex (vmPFC/OFC) and the perigenual and subgenual ACC (pg/sg ACC). Based on the Destrieux sulcogyral-based atlas, I defined the vmPFC/OFC as a cortical region comprising the gyrus rectus (G_rectus, 31) and orbital gyri (G_orbital, 24) (Destrieux et al., 2010). The sg/pg ACC was

comprised of the subcallosal area (G_subcallosal, 32) and anterior part of the cingulate gyrus and sulcus (G_and_S_cingul-Ant, 6) (Destrieux et al., 2010).

Figure 6-1: Representative figures of the regions of interest in the current analysis



6.2.6 Statistical analysis

In order to examine the difference in ROI volumes between the least and the most deprived groups, a repeated measures analysis of covariance (rmANCOVA) was performed using the ROI volumes as dependent variables and socioeconomic group (least deprived versus most deprived) as the predictor variable of interest. Intracranial volume (ICV), age and number of alcohol units consumed per week were considered as nuisance covariates. Statistical tests were corrected for multiple testing corrections using Dunn-Sidak correction (Šidák, 1967). An independent sample t test was performed to examine if inflammatory markers and other covariates differed between the least and the most deprived groups.

A mediation analysis was conducted in order to ascertain whether the inflammatory index mediated the effect of neighbourhood-level SES on the volumes of the ROIs, which were significantly related to neighbourhood-level SES in the rmANCOVA. The mediation analysis here tests the hypothesis that a proportion of the variance in brain volumes that is predicted by variance in neighbourhood-level SES can be accounted for by the mediator variable. That is, the neighbourhood-level SES accounts for variance in the mediator variable of interest and in turn, this variance in the mediator variable of interest accounts for a proportion of the variance of the ROIs' brain volumes. In other words, the mediation analysis partitions the variance explained by the predictor into a part that is independent of the mediating variable, and a part that is accounted for via the mediating variable (Palaniyappan and Liddle, 2012). I used the bootstrap method of Preacher and Hayes to estimate the indirect effect and bias-corrected 95% confidence interval (CI) for each individual mediator based on 20,000 bootstrap samples using an SPSS macro. (8) I also obtained effect ratios for indirect effects (IE ratio), which express the proportion of the total effect that can be explained by the indirect (mediated) effects. In other words, an indirect effect ratio of 0.25 would mean that a quarter (25%) of the total effect of the deprivation status on the cortical morphology is explained by the mediator. This analysis requires no assumption regarding the underlying distributions since the statistical significance level is determined non-parametrically. Since only the inflammatory index differed between groups (a path), I only examined this relationship.

6.3 Results

On the multivariate analysis, there was a significant effect of neighbourhood deprivation on the grey matter volumes of the ROIs [$F(1,37) = 10.33$; $p = .003$; $\eta^2_p = .22$]. At the univariate level, the most deprived group had smaller right hippocampus ($t = 3.252$; $p = .002$; $\eta^2_p = .22$) and right vmPFC/OFC ($t = 2.275$; $p = .029$; $\eta^2_p = .12$) volumes. The other ROIs volumes did not differ between groups (Table 6-1).

Table 6-1: Between comparison of grey matter volumes of regions of interests

	Least deprived Mean volume mm^3 (s.d.)	Most deprived Mean volume mm^3 (s.d.)	t	p	η^2_p
Right Hippocampus	3901.81 (474.61)	3410.72 (588.33)	3.252	**.002**	.222
Left Hippocampus	3648.75 (546.46)	3416.70 (439.03)	1.46	.153	.054
Right Amygdala	1139.82 (229.82)	1086.29 (274)	0.517	.608	.007
Left Amygdala	1162.2 (243.87)	1101.36 (202.88)	0.632	.532	.011
Right sg and pg ACC	6567.3 (913.71)	6338.76 (998.32)	0.386	.702	.004
Left sg and pg ACC	6070.42 (803.01)	5658.90 (941.69)	1.427	.162	.052
Right vmPFC/OFC	8620.61 (1138.07)	8001.38 (947.27)	2.275	**.029**	.123
Left vmPFC/OFC	9011.09 (1004.42)	8501.80 (859.87)	1.776	.084	.079

Standard deviation (s.d.); Unpaired t test (t); Significance (p); Partial eta squared (); Subgenual and perigenual anterior cingulate cortex (sg and pg ACC); ventromedial prefrontal/orbitofrontal cortex (vmPFC/OFC) Covariates appearing in the model are evaluated at the following values: Age (years) = 50.9, alcohol (units per week) = 17.21, Intracranial volume (mm^3) = 1557805.39

The most deprived group had a greater inflammatory index ($t = -4.366$; $p < .001$). Results of between group comparisons for individual inflammatory markers are shown in Table 4-2.

Since both the BMI-HDL-Insulin factor and TAG factor did not differ between groups, I examined only the mediating effects of the inflammatory index. As shown in Table 6-2 on the mediation analysis, the inflammatory index mediated the relationship between neighbourhood-level SES and grey matter volume in the right vmPFC/OFC (B = -0.37; SE = .18; 95%CI = -0.83 to –0.06) but not in the right hippocampus (B = -0.05; SE = 0.29; 95%CI = -0.68 to 0.49).

Table 6-2: Results of the mediation analysis

ROI	Coefficient	SE	p	Boot strap 95% CI	Effect ratio
Right Ventral/Orbitofrontomedial cortex	r^2 = 0.19 ; F(5,36)=1.6; p=0.06				
a path	1.06	0.26	0.0003		
b path	-0.35	0.20	0.09		
Total effect	-0.68	0.29	0.028		
Direct effect	-0.30	0.36	0.4		
Indirect effect	-0.37	0.18		-0.83 to -0.06	54.46%
Right Hippocampus	r^2 = 0.48 ; F(5,36)=6.7; p<0.0002				
a path	1.06	0.26	0.0003		
b path	-0.04	0.17	0.79		
Total effect	-0.76	0.23	0.002		
Direct effect	-0.71	0.29	0.02		
Indirect effect	-0.05	0.29		-0.68 to 0.49	6.5%

Bootstrap samples for bias corrected bootstrap confidence intervals: 20000. Covariates in the model for the dependent variable include age, ICV and number of alcohol units; the initial r^2 statistic shows the fit of the model with all variables including the mediator. Mediating variable – Inflammation factor; details of the path are shown in Figure 2. Coefficients are interpreted as Betas. Indirect effect is the product of coefficients of 'a' and 'b' paths.

6.4 Discussion

In the present study, I have shown an association between greater neighbourhood deprivation and smaller right hippocampal and vmPFC/OFC volumes. Neighbourhood deprivation accounted for 22% of the variance in the volume of the right hippocampus and 10% of the variance in the right vmPFC/OFC. Greater inflammatory load mediated the association between greater neighbourhood deprivation and smaller right vmPFC/OFC volume.

6.4.1 Comparison with previous findings

Our findings are consistent with previous reports demonstrating an association between early-life and current individual-level SES and smaller hippocampi. In the PATH study, Butterworth *et al* found current financial hardship to be associated with smaller bilateral hippocampi in middle-aged adults(Butterworth et al., 2011). Others have found early-life socioeconomic disadvantage to be associated with smaller hippocampal volumes in children and adults (Staff et al., 2012, Noble et al., 2012, Hanson et al., 2011). Two of the above mentioned studies failed to find an association between parental education (an estimate of individual-level early-life SES) and hippocampal volume (Noble et al., 2012, Hanson et al., 2011). They however found an association between household income and hippocampal volumes. The authors therefore concluded that as household income was an indirect measure of limited access to basic goods, this and not parental education was particularly relevant for hippocampal development. Although our sample was comprised of middle-age adults, this observation appears to be consistent with our data given that the deprivation indicators used in our study are representative of inequalities in area-level material resource distribution. It should be noted that hippocampal neurogenesis continues throughout adulthood (Eriksson et al., 1998, Spalding et al., 2013). In addition, in adult rodents, chronic stress has been associated with hippocampal atrophy, through suppression of neurogenesis (Gould et al., 1997), remodelling of the CA1 and CA3 hippocampal subfields with loss of dendritic spines (Pawlak et al., 2005) and diminished dendritic arborisation (McKittrick et al., 2000).

To the best of our knowledge, this is the first human study to find an association between neighbourhood deprivation and smaller volume in the right

vmPFC/OFC. Our findings are strikingly similar to those documented by Gianaros *et al* who used prospectively measured chronic life stress and not SES as predictor variable (Gianaros et al., 2007b). The right-lateralized effect of chronic stress on hippocampal and OFC morphology is in accord with both animal and human data supporting a dominant role of right-sided limbic structures in the activation and coordination of stress regulatory systems (Sullivan and Gratton, 2002, Wittling, 1997). It has been proposed that disruptions in this right sided asymmetry of stress regulation may predispose to psychopathology and physical ill health (Wittling and Schweiger, 1993a, Wittling and Schweiger, 1993b). Finally, it is worth noting that whereas the hippocampus exerts inhibitory feedback on HPA axis function, the vmPFC upregulates HPA axis and sympathetic activity (Sullivan and Gratton, 1999) and mediates hippocampal influences on autonomic function (Ruit and Neafsey, 1990).

There are no data on the cellular correlates of socioeconomic deprivation in the vmPFC/OFC. Of potential relevance are previous conflicting accounts from animal studies regarding the effects of chronic stress on neural plasticity in the vmPFC/OFC. Murmu *et al* reported that prenatal stress was associated with reduced spine densities on basal and apical dendrites in both female and male rat pups and atrophy of apical dendrites only in male rats (Murmu et al., 2006). However, in another rat experiment chronic restraint stress resulted in a 43% increase of apical dendritic arborization in the lateral orbitofrontal cortex but a 20% retraction of apical dendritic arbors in the mPFC (Liston et al., 2006).

While the hippocampus is involved in associative learning and retrieval of recent memories (Squire and Zola-Morgan, 1991, Squire et al., 2004), the vmPFC/OFC supports adaptive decision making and long-term memory storage (Noonan et al., 2012, Rangel et al., 2008, Walton et al., 2011, Padoa-Schioppa and Cai, 2011, Euston et al., 2012). The coordinated activity in these two structures are necessary for early and late memory consolidation (Euston et al., 2012). It is therefore, tempting to speculate that the observed structural abnormalities in the above regions may provide a neurobiological account of the cognitive and behavioural deficits which have been linked to lower SES (Farah et al., 2006).

I found no association between neighbourhood deprivation and amygdalar volumes. Previous research in animals has shown chronic stress to be associated with increased dendritic branching within the basolateral amygdala (Vyas et al.,

2002). In keeping with this, lower parental education (a proxy measure of individual-level parental nurturance and warmth) has been associated with greater amygdalar volume . Based on this account and bearing in mind our data represent an adult sample, it could be argued that our findings are reflective of the fact that neighbourhood deprivation fails to capture the effects of individual-level deprivation and parenting style.

I found no association between neighbourhood deprivation and sg/pACC volume. Previously Gianaros *et al* demonstrated that lower perceived social standing, but not conventional indicators of SES, was associated with smaller pgACC grey matter volumes (Gianaros et al., 2007a). They proposed a dissociation between the neural substrates of subjective (perceived) and conventional indicators of SES (as used in our study). I however did not measure perceived social standing in our sample.

6.4.2 The role of circulating inflammatory markers.

Our result of an association between neighbourhood deprivation and inflammatory index is consistent with earlier accounts (Schulz et al., 2012, Gruenewald et al., 2009, Alley et al., 2006). Indeed, exposure to stressful neighbourhood conditions is associated with elevated systemic inflammatory burden both in children (Broyles et al., 2012) and adults (Pollitt et al., 2007, Petersen et al., 2008, Pollitt et al., 2008). Psychological stress and subsequent activation of ANS and HPA axis have been shown to result in the production of pro-inflammatory cytokines and activation of mononuclear cells (Bierhaus et al., 2003). This finding has been replicated in a number of studies and provides a viable mechanistic molecular pathway by which lower neighbourhood-related chronic stress may give rise to increased peripheral inflammatory load.

Of particular interest is our finding that peripheral inflammation mediates the effect of chronic stress on the morphology of the right vmPFC/OFC. Only two previous studies have documented that activation of inflammatory pathways mediates structural brain changes (i.e. reduced white matter integrity and smaller cortical thickness) in relation to living in disadvantaged neighbourhoods (Gianaros et al., 2012). Moreover, complementary evidence from functional neuroimaging data has revealed that experimentally induced elevation of peripheral pro-inflammatory cytokines correlates with abnormal neural

reactivity within brain regions subserving psychomotor activity, mood regulation and interoception (Brydon et al., 2008, Harrison et al., 2009b). It is worth noting that, given the correlative nature of our data, I cannot exclude that pre-existing structural abnormalities in the vmPFC/OFC gave rise to poor decision making strategies, ultimately leading to maladaptive and unhealthy behaviours which are associated with increased peripheral inflammation and social drift.

In spite of overwhelming evidence that inflammation has a detrimental effect on hippocampal neurogenesis (Kohman and Rhodes, 2013), I detected no mediation effect of inflammation between lower neighbourhood SES and smaller right hippocampus. Contrary to our finding a previous study reported that higher serum levels of IL-6 were associated with smaller grey matter volume in the left but not right hippocampal volume in middle aged adults (Marsland et al., 2008). At a molecular level, other mechanisms such as stress-induced surges in circulating corticosteroids (Sousa et al., 2000), plasminogen and tissue plasminogen activator (Pawlak et al., 2005), reduced levels of brain derived neurotrophic factor (McEwen and Gianaros, 2010), corticotrophin-releasing factor (Chen et al., 2004), decreased binding to serotonin receptors and transporters in Ammon's horn (McKittrick et al., 2000), NMDA receptor activation (Gould et al., 1997) or decreased density in the hippocampus (Pawlak et al., 2005) have been implicated in mediating neurodegenerative changes in hippocampal morphology. I can only speculate that one or more of the aforementioned mechanisms and not inflammatory mediators may have played a role in determining the observed smaller hippocampal grey matter volume.

In conclusion, I have provided evidence that in a sample of middle-aged healthy male adults neighbourhood deprivation was significantly associated with smaller grey matter volumes in the right hippocampus and right vmPFC/OFC. Moreover, an index of peripheral inflammation mediated the effects of neighbourhood deprivation on the right vmPFC/OFC grey matter volume. The results expand the existing body of knowledge on the neurobiology of socioeconomic deprivation and provide novel insights into the pathways mediating the impact of lower SES on brain morphology.

Chapter 7 - Relationship between circulating inflammatory markers and midbrain serotonin transporters

In this chapter, I present the methods and results of an experimental study that explore the relationship between circulating inflammatory markers, and serotonin transporter (SERT) availability and their modulation by biologics. I tested a sample of patients with psoriasis and psoriatic arthritis, who were about to receive Etanercept, a specific TNF-α blockade agent. Using a test-retest design, I explored the relationship between circulating inflammatory markers and midbrain SERT availability before and after Etanercept.

7.1 Introduction

7.1.1 Psoriasis and Psoriatic arthritis

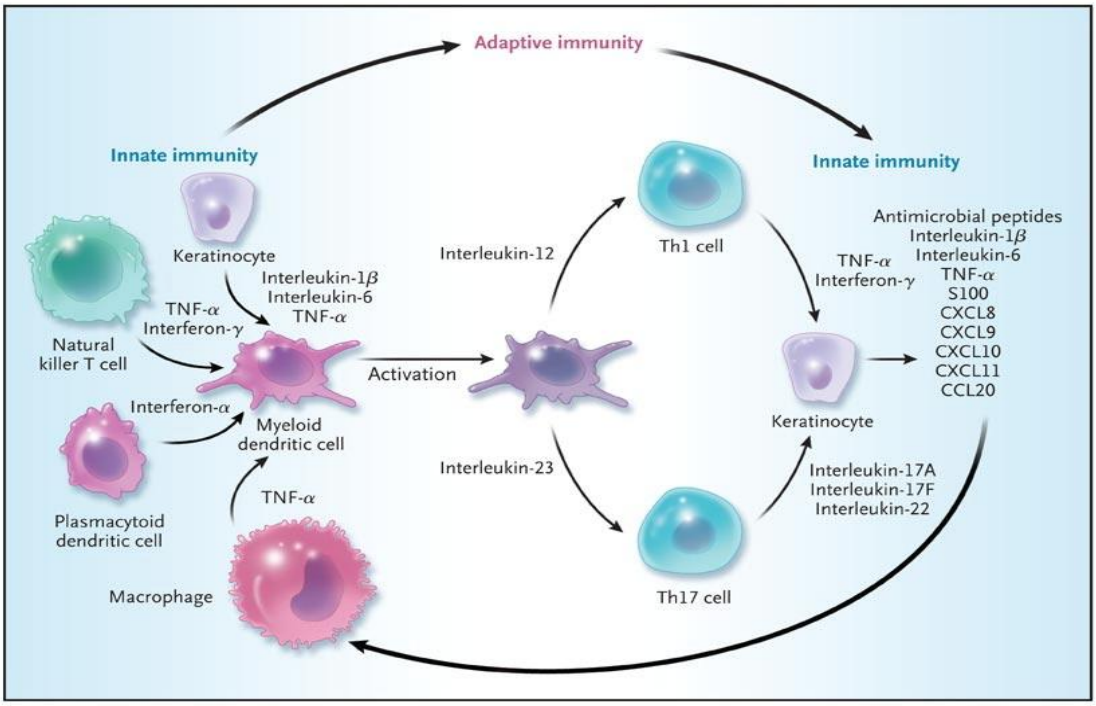
Autoimmune diseases are conditions where the body's adaptive immune response targets a cell-mediated antigen (Ag) of the host. While SLE is the prototype autoimmune illness that has been associated with neuropsychiatric symptoms, more prevalent illnesses like autoimmune arthritis are associated with significant neuropsychiatric morbidity and mortality (González et al., 2012). Psoriasis affects around 1.5% to 3 % of the population in North America and Europe. Psoriatic arthritis affects up to 30% of people with psoriasis. Although not fully clear, most of the evidence suggests that both psoriasis and psoriatic arthritis are autoimmune diseases that may share common pathogenic mechanisms (Fitzgerald and Chandran, 2012, Gladman, 1998, Gladman, 2012, Gottlieb et al., 2008, Hayes and Koo, 2010, Rieder and Tausk, 2013).

Although abnormal epidermal keratinocyte proliferation was initially considered to be the primary pathology in psoriasis, it is now thought that inflammation (particularly T cells) play an important role in initiation and maintenance of psoriasis (Coimbra et al., 2012). In addition to the T cells, number of other cells and inflammatory mediators (cytokines and chemokines) are thought to play key roles in the pathogenesis of psoriasis (Nestle et al., 2009).

In brief, the immune system is thought to consist of two components, innate and adaptive immunity. Innate immunity is primarily mediated by macrophages that have receptors that recognise families of “pathogens” and provide an early response mechanism against harm to the host (González et al., 2012). Adaptive immunity is primarily mediated by lymphocytes and recognises a host of “antigens” that are presented to them, including self-antigens. This response is usually a late / slow process. Although lymphocytes recognise self-antigens, in healthy conditions, they do not initiate an immune response towards the self, as they are either removed or inactivated – a process called tolerance(González et al., 2012).

Earlier views considered that the process, by which the immune cells responsible for adaptive immunity develop tolerance to the self-antigens, is inactive in individuals with autoimmune diseases. However, it is now known that even the innate immune system is dysregulated in psoriasis (Coimbra et al., 2012, González et al., 2012, Nestle et al., 2009). It is not clear as to what triggers the etio-pathogenic processes involved in psoriasis/psoriatic arthritis. However, it is thought that events like physical trauma or bacterial products or even stressful life events, may lead to the formation of cathelicidin-DNA complexes (this is usually triggered in response to an infection by a microbe) that activate plasmacytoid dendritic cells (macrophages) and secretion of interferon alpha (Nestle et al., 2009). The activated cells, enter the lymphatic system, where they release IL12 and IL23. These cytokines induce the differentiation of naïve T cells into effector cells like Th17 and Th1 cellls (Coimbra et al., 2012). Effector cells then enter the skin, produce IL17, IFN gamma and TNF-α. These mediators then go on to act on keratinocytes, and production of a number of downstream proteins, which in turn trigger an inflammatory response, thus maintaining the cycle (Figure 7-1).

Figure 7-1: Key Cells and Mediators in the Transition from Innate to Adaptive Immunity in Psoriasis.



Innate immune cells produce key cytokines (tumor necrosis factor α [TNF-α], interferon-α, interferon-γ, interleukin-1β, and interleukin-6) that activate myeloid dendritic cells. Activated dendritic cells present antigens and secrete mediators such as interleukin-12 and interleukin-23, leading to the differentiation of type 17 and type 1 helper T cells (Th17 and Th1). T cells, in turn, secrete mediators (e.g., interleukin-17A, interleukin-17F, and interleukin-22) that activate keratinocytes and induce the production of antimicrobial peptides (e.g., LL-37 cathelicidin and β-defensins), proinflammatory cytokines (TNF-α, interleukin-1β, and interleukin-6), chemokines (CXCL8 through CXCL11 and CCL20), and S100 proteins. These soluble mediators feed back into the proinflammatory disease cycle and shape the inflammatory infiltrate. Reused with permission from Nestle FO, Kaplan DH, Barker J. N Engl J Med 2009; 361:496-509. Copyright Massachusetts Medical Society.

7.1.2 Depression in psoriasis and psoriatic arthritis

A recent systematic review of studies examining psychological morbidity in patients with psoriasis suggest that the prevalence rates of depression in psoriasis range between 10% and 62%, largely determined by the difference in sample examined and methodology used. Table 7-1 shows the recent studies from 2005, that showing the prevalence of depression in psoriasis (Rieder and Tausk, 2013).

Similar rates of depression have been shown in patients suffering from psoriatic arthritis. A recent study by Kotsis et al found prevalence rates of 21.7% in

patients with psoriatic arthritis (Kotsis et al., 2012). Another study that used a self rated measure of HADS found a prevalence of 17.6% (Freire et al., 2011). In a cross sectional observational survey, we found that 30% of patients suffering from psoriatic arthritis fulfilled the criterion for caseness for depression, using HADS (Krishnadas et al., 2011).

Table 7-1: Studies that have explored the prevalence of depression in patients with psoriasis since 2005.

Study	*n*	Study design	Measures	Presence of depression
(Consoli et al., 2006)	93 (France)	Cross-sectional – no control	PASI and questionnaires: MINI, HADS	11.8–25.8% of patients with symptoms of depression on HAD-D
(Picardi et al., 2006)	80 (Italy)	Cross-sectional – no control	Questionnaires: GHQ-12, Skindex-29, PHQ	No psoriasis-specific statistics reported
(Sampogna et al., 2007)	414 (Italy)	Cross-sectional – no control	Questionnaires during hospitalization and 1 month after discharge: SAPASI, GHQ-12, Skindex-29	No statistics reported
(Schmitt and Ford, 2007)	265 (USA)	Cross-sectional – no control	Questionnaires: DLQI, CES-D, PLSI, SAPASI	33% of patients with history of depression 31–48.7% screened positive for depression on CES-D
(Magin et al., 2009)	27 (Australia)	Cross-sectional – 96 unmatched controls without skin disease	GHQ-12, HADS, Fenigstein Self-Consciousness Scale, Eysenck Personality Inventory	No psoriasis-specific statistics reported
(Schmitt and Ford, 2010)	3147 (Germany)	Case–control	ICD-10 codings of depression	Independent association between psoriasis and depression (OR 1.49; 95% CI 1.20–1.86)

Study	*n*	Study design	Measures	Presence of depression
(Kurd et al., 2010)	149 998 (UK)	Cohort	Clinical diagnoses of depression, anxiety, and suicidality	1.39 HR for depression (95% CI 1.37–1.41) 1.31 HR for anxiety (95% CI 1.29–1.34) 1.44 HR for suicidality (95% CI 1.32–1.57)

7.1.3 Pathogenesis of depression in psoriasis and psoriatic arthritis

The association between inflammatory conditions and depressive illness cannot be solely explained by pain or disability, because a number of people who have severe disability are not clinically depressed. More recently it has been postulated that the mediators of inflammation may be directly responsible for the pathogenesis of depression in the context of medical illness. Inflammatory processes have also been implicated in the pathophysiology of major depressive disorder in the absence of medical illnesses. At least 3 meta-analyses and 1 longitudinal study have shown an association between raised proinflammatory cytokines and depressive illness in the absence of medical illnesses (Dowlati et al., 2010, Howren et al., 2009, Liu et al., 2012a). Recent data have shown that treatments directed towards arthritis, have been associated with improvement in depression. There is also some evidence that the improvement in depression may precede the improvement in arthritis, suggesting that these two processes may be independent of each other (Gelfand et al., 2008, Langley et al., 2010, Langley et al., 2011, Tyring et al., 2006).

Of direct relevance to the link between Ps/PsA and depression is the proposal that pro-inflammatory cytokines, that mediate inflammation, also provoke changes in brain structure and function that lead to the development of co-morbid major depression (Krishnadas and Cavanagh, 2012, Capuron and Miller, 2011). Not surprisingly, most of the evidence for the effects of proinflammatory

cytokines in the proposed pathogenesis of depression come from animal studies. There is now evidence that peripheral cytokines signal the brain through various mechanisms as discussed in chapter 1. This “signalling” is thought to contribute to a number of changes that may be considered responsible for the pathogenesis of depression. (Figure 1-3)

From the above, it is clear that pro-inflammatory cytokines play a crucial role in mediating the pathogenesis of psoriasis and psoriatic arthritis. Indeed, TNF-α is thought to play a central role within the cytokine network and a number of anti-TNF-α therapies have been shown to be successful in treating Psoriasis and psoriatic arthritis. TNF-α is a pro-inflammatory cytokine, that occur in two forms - a soluble form sTNF (17kDa) and a transmembrane form tmTNF (26kDa). TNF-α has been shown to bind to at least two receptors TNFR1 and TNFR2 (Waters et al., 2013). While TNFR1 is expressed in most tissues, including neurons and glia, TNFR2 is restricted to cells of the immune system. TNFα binding to its ligands causes conformational changes in the receptors, leading to the initiation of at least 3 intracellular signalling pathways of interest(Chen and Goeddel, 2002, Wajant et al., 2003). It activates NF-kB pathways, hereby releasing a transcriptional factor – NFkB, which mediates the transcription of a vast number of proteins including cell surface receptors and intracellular receptors like glucocorticoids that are involved in mediating inflammatory response (Berthold-Losleben and Himmerich, 2008). Secondly, it activates MAPK pathways – particularly the stress related JNK groups and the p38 MAPK pathways. Thirdly, TNFR1 is also involved in death signalling – apoptosis. However, the signalling pathways show significant cross talk and other factors mediate the balance of activity within and between the pathways (Gaur and Aggarwal, 2003).

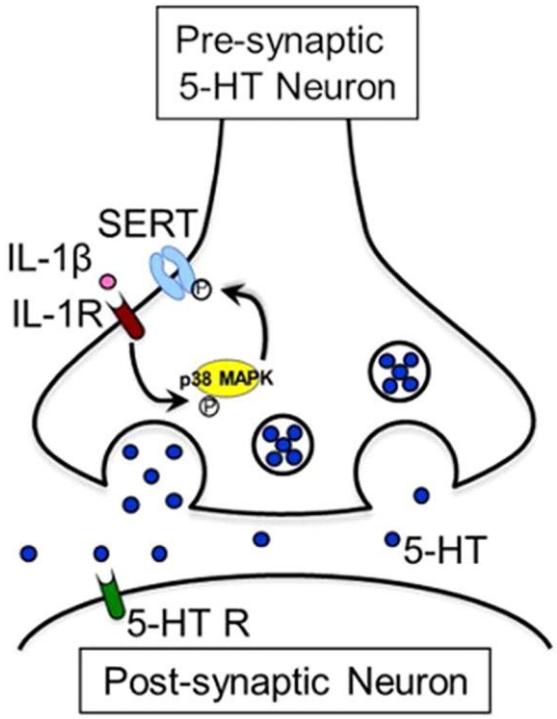
Newly developed therapeutic agents for inflammatory arthritides target proinflammatory cytokines like TNF-α (Feldmann and Maini, 2001, Feldmann and Maini, 2003, Feldmann and Maini, 2010, Feldmann et al., 2010). Blocking the activity of TNF-α, using either specific antibody or TNF-α receptor fusion constructs is highly effective in controlling inflammation and altering the course of Psoriasis/psoriatic arthritis. A common observation during anti-TNF-α treatment, which has been confirmed in trials, is a hedonic response, consistent with some antidepressant effect. For example, Etanercept (TNF-α receptor Fc

fusion protein) has been shown to relieve the symptoms / signs of psoriasis and the depression and fatigue associated with psoriasis (Tyring et al., 2006). This improvement in mood seems to occur even before any improvement in joint inflammation, suggests that this is more than an epiphenomenon associated with arthritic symptom improvement. Rather, here, I postulate that blocking circulating TNF-α (using anti-inflammatory drugs like Etanercept) would reduce the signalling of the cytokines to the CNS, thus reversing the effects of proinflammatory cytokines on the brain, which in turn, improves the mood.

7.1.4 Rationale and need for the study

The use of these anti TNF-α agents give us the ideal opportunity to explore the effect of proinflammatory cytokines on potential molecular pathways implicated in the pathophysiology of depression in the medically ill population. This study exploited the availability of these new therapeutic agents to explore specifically the relationship between circulating inflammatory markers and one such marker - SERT. A number of preclinical studies have shown that inflammatory markers (particularly IL1B and TNF-α) may have a direct effect on SERT availability through activation of MAPK pathways as shown in Figure 7-2 (Baganz and Blakely, 2013, Ramamoorthy et al., 1995, Samuvel, 2005, Couch et al., 2013, Malynn et al., 2013, Morikawa et al., 1998, Tsao et al., 2008, Katafuchi et al., 2006). Both TNF-α and IL1ß up-regulate SERT expression in in-vitro cells and animal models. The mono-amine theory of depression first proposed by Schildkraut in 1965, states that depression may the result of a functional deficit in mono-amine neurotransmission - particularly serotonin and norepinephrine(Schildkraut, 1965). SERT plays a major role in serotonin neurotransmission, and increased availability/function of SERT has been associated with depressive symptoms(Meyer, 2008, Savitz and Drevets, 2013). Indeed, the most commonly used antidepressants, show 80% SERT occupancy at therapeutic doses and inhibit SERT, thereby increasing synaptic serotonin availability(Meyer, 2012). Given the above, a key question is, will blocking TNF-α result in changes in SERT availability.

Figure 7-2: Interleukin-1 β (IL-1 β) stimulates SERT activity through an interleukin-1 receptor (IL-1R)- and p38 MAPK-dependent signalling pathway.



Reused with permission from Baganz N, Blakely RD. A Dialogue between the Immune System and Brain, Spoken in the Language of Serotonin ACS *Chem.* Neurosci., 2013, *4* (1), pp 48–63

7.2 Aim of the study

1. To study the association between circulating inflammatory markers and serotonin transporter availability in humans.

2. To ascertain whether anti-inflammatory treatment targeting TNF-α will be associated with changes in serotonin transporter availability in humans.

7.2.1 Hypothesis

Based on previous studies, I hypothesised that greater pro-inflammatory cytokines will be associated with greater SERT availability in the brain. I also hypothesise that treatment with Etanercept will be associated with a reduction in SERT availability.

7.2.2 Objectives

1. To examine the association between peripheral pro-inflammatory cytokines and SERT density in the brain measured using [^{123}I] -beta-CIT SPECT in a group of patients with Psoriasis and Psoriatic arthritis.

2. To examine the association between pro-inflammatory cytokines and change in SERT in response to treatment with Etanercept

7.3 Methods

7.3.1 Subjects

Fifteen subjects clinically diagnosed with psoriasis/psoriatic arthritis aged 30-65 years, were recruited into the study. Those who incidentally fulfilled the criteria for major depression were included in the study. Three patients dropped out - one withdrew consent, and the other two had incidental findings, and hence their data were excluded from analysis. Therefore, data from 12 individuals were analysed. All patients met disease activity criteria set by the NICE advice pertaining to the use of TNF-α blocking biologic agents for Psoriasis and psoriatic arthritis. The treating clinicians decided this. These patients were given information sheets about the study, and were contacted if they gave consent for the study. Those with a history of antidepressant intake in the previous 3 months; history of CVA, documented head trauma or neurological disorders, lifetime history of DSM Axis I psychiatric diagnoses other than depression (measured using SCID), alcohol and/or substance misuse, pregnancy, other connective tissue or systemic inflammatory disease were excluded from participation in the study. Patients with contraindications to TNF-α blockade as per NICE guidelines were also excluded. Subjects with contraindications to any type of imaging procedures in the study were not included. The study was approved by the West of Scotland Research ethics committee and the NHS Greater Glasgow and Clyde Research and Development departments. In addition, the use of ionising radiation for SPECT was approved by the regional ARSAC.

7.3.2 Sample size

Based on a pilot study by Cavanagh et al, in order to demonstrate an effect size of reduction in SERT binding of Cohen's d=1.2, with 80% power, with an alpha of

0.05, with a power of 90%, the total sample size required was 10 subjects. However that study did not look at the relationship between circulating inflammatory markers and SERT. The details of the power calculation are shown below.

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size d	=	1.23
	α err prob	=	0.05
	Power (1-ß err prob)	=	0.9
Output:	Non-centrality δ	=	3.88
	Critical t	=	2.26
	Df	=	9
	Total sample size	=	10
Actual power	**= 0.93**		

7.3.3 Study design

A single study group design was employed with 2 conditions (pre- and post-treatment with Etanercept). All subjects underwent clinical, and SPECT assessments twice during the study. Clinical, blood and SPECT measures were made before and after the commencement of treatment. The post treatment scans and clinical measurements were performed 6 - 8 weeks after commencing treatment with etanercept.

7.3.4 The study drug

The drug used to achieve TNF-α blockade in this study was Etanercept(Strober et al., 2008, Tan et al., 2007). Etanercept is a recombinant human TNF-α -receptor fusion protein with 934 aminoacids and weighs 150kDa. It interferes with the inflammatory cascade by binding to TNF-α , thereby blocking its interaction with cell-surface receptors (Feldmann and Maini, 2001, Feldmann and Maini, 2010). Etanercept is licensed for use in adults with active Psoriasis/ Psoriatic arthritis who responded inadequately to previous treatments (Fantuzzi

et al., 2008). All participants recruited into the study were then commenced on a weekly Etanercept 50 mg intramuscularly.

7.3.5 Inflammatory markers

Blood was collected and serum extracted on the same day as the SPECT scans, just before the administration of the ligand. The serum was stored in -80ºC. Cytokines were measured in plasma in duplicates using sandwich immunoassays, with all samples from the same volunteer on the same plate. The cytokines measured included TNF- α (sensitivity = 4 pg/mL), IL-1b (sensitivity = 4 pg/mL), IL-10 (sensitivity =2 pg/mL) and IL-6(sensitivity = 2 pg/mL) (Ready-set-go ELISAs, eBioscience, UK) and hsCRP (sensitivity = 1.9 ng/ml) (ELISA, Invitron, UK).

The CV% for all assays were as follows: TNF-α - 3.6% (250pg/mL) - 7.1% (3.9pg/mL) - median CV%:5.0% ; IL10 - 2.4% (150pg/mL) - 4.8% (2.34pg/mL) - median CV%:11%; IL6 - 0.4% (100pg/mL) - 3.9% (1.56pg/mL) - median CV%:7%; IL1b - 2.4% (250pg/mL) - 8.2% (3.9pg/mL) - median CV%:18%; hsCRP - 0.3% (150ng/mL) - 2% (1.9ng/mL) - median CV%:3%.

7.3.6 Clinical measures

7.3.6.1 Beck's depression inventory BDI II (Beck et al., 1996)

Beck's depression inventory II is a 21 item self-rated Likert questionnaire that measures depressive symptoms over the past 2 weeks. Each question is answered and scored on a scale of 0 to 3. Total score ranges from 0 to 63, with higher scores indicating more severe depressive symptoms. Cut off scores have been used to indicate levels of depression. However, in this study, the scores have been used as a continuous scale.

7.3.6.2 The functional assessment of chronic illness therapy (FACIT) - Fatigue scale (Webster et al., 2003)

FACIT - Fatigue scale is a short 13 item self rated scale that measures an individual's fatigue during their daily activities over the past week. The level of fatigue is measured on a four point likert scale with total greater scores indicating less fatigue. In a 2007 study, the FACIT Fatigue Scale was found to have high internal validity (Cronbach’s alpha = 0.96) and high test-retest

reliability (ICC = 0.95)(Chandran et al., 2007). The FACIT has demonstrated reliability and sensitivity to change in clients with a variety of chronic health conditions and in the general population.

7.3.6.3 The Health assessment questionnaire - HAQ pain scale (Fries et al., 1982)

The HAQ Pain Scale assesses pain severity over the past week on a double-anchored VAS (a horizontal line where each end represents opposite ends of a continuum). It obtains data on how pain has usually been over the past week, even though pain may be reported to vary over the course of a day or from day to day. The VAS line is standardized to 15 centimeters in length, which is convenient for the page and the patient. The scale is labeled from zero (no pain) at the left anchor point and 100 (severe pain) at the right anchor point. Patients are instructed to place a vertical mark on the line to indicate the severity of their pain. The distance between 0 and the mark left by the patient is then multiplied by 0.2 to get a score between 0 and 3, with greater scores representing greater pain(Bruce and Fries, 2003b, Bruce and Fries, 2003a).

7.3.7 SPECT measurement of serotonin transporter (SERT)

7.3.7.1 Serotonin transporter (SERT)

Serotonin (5-hydroxytryptamine, 5-HT), a monoamine neurotransmitter, has been implicated in many behaviours, including sleep, appetite, sexual behaviour. It has also been implicated in cognitive and emotional function. More importantly, it is implicated in the mono-amine theory of depression(Schildkraut, 1965). In short, this hypothesis suggests that depressive states are caused due to a decrease in monoamine neurotransmitters – particularly Serotonin in the synaptic cleft (Canli and Lesch, 2007, Hornung, 2010, Maximino, 2012, Savitz and Drevets, 2013, Sghendo and Mifsud, 2012).

Serotonin that is released into the synaptic cleft is either metabolised or actively transported back into the neuron by a protein in the neuronal cell membrane. This protein is called the Serotonin transporter / reuptake transporter (SERT).

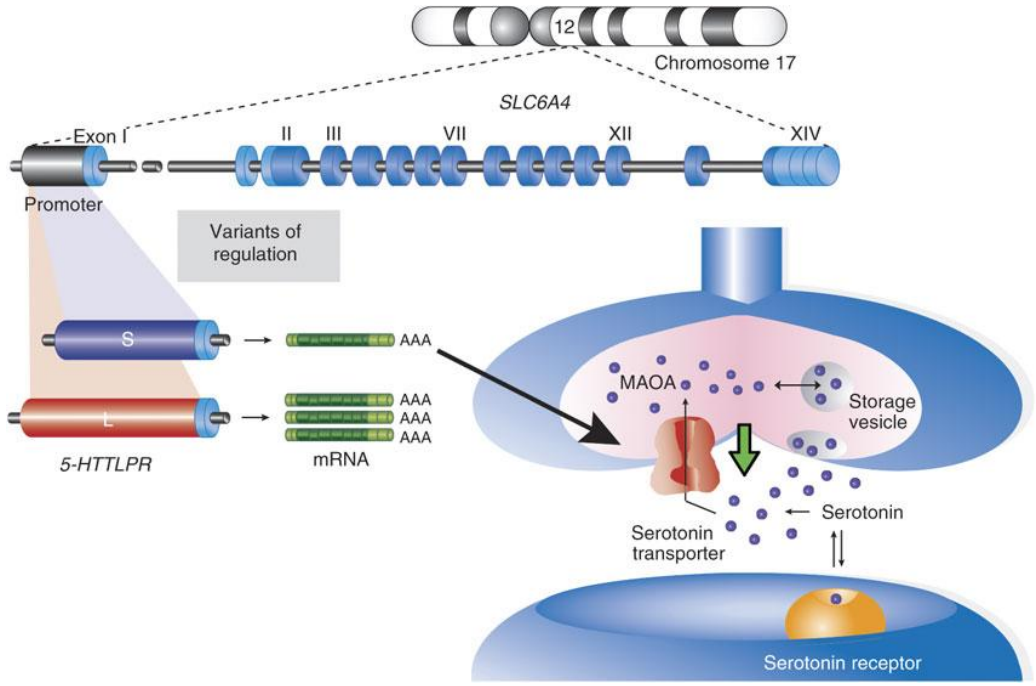
7.3.7.2 SERT location and Structure

In humans, SERT has been clearly identified in a number of brain regions, including the brain stem, hypothalamus, occipital and frontal cortices. In the neurons, SERT is located in the presynaptic terminus – i.e. the axonal nerve endings. See Figure 7-3. SERT plays a key role in scavenging serotonin transporter from synaptic cleft back to the axonal nerve ending. Surprisingly SERT is found in neuronal cells and non-neuronal cells(Hornung, 2010). In humans, SERT has been found in glia, platelets, the GIT, monocytes and the placental syncitioblast (Maximino, 2012).

Serotonin levels in the brain are controlled by a number of factors including rate of synthesis, rate of release into the synaptic cleft, rate of enzymatic breakdown, rate of diffusion through the extracellular space and active reuptake. In the brain, SERT seems to be the predominant mechanism controlling the strength and duration of serotonergic neurotransmission. Therefore it is likely that SERT availability is directly related to serotonergic neurotransmission(Paterson et al., 2013, Paterson et al., 2010, Savitz and Drevets, 2013, Sghendo and Mifsud, 2012).

Serotonin gene that codes for the SERT protein is situated on chromosome 17 (17q11.2). This gene transcription is regulated by a polymorphic promoter region – 5HTTLPR. The promoter region has two alleles – a short and a long allele. The protein itself is 630 amino acids long with 12 transmembrane domains. They are mostly located at outer cell membranes peri-synaptically and transport serotonin into the cell (Canli and Lesch, 2007). SERT also plays an important role in the trophic organisation of the somatosensory cortex in the developing brain. The evidence for this comes from the fact that the foetal brain expresses SERT in the somatosensory regions during a small window in time during development – particularly during week 12 and 14 of gestation (Daws and Gould, 2011, Sodhi and Sanders-Bush, 2004).

Figure 7-3: Shows the serotonin transporter, its genes and its promoter region alleles.



Reused with permission. Turhan Canli & Klaus-Peter Lesch. Long story short: the serotonin transporter in emotion regulation and social cognition. Nature Neuroscience 10, 1103 - 1109. Copyright Nature Publishing Group.

7.3.7.3 Measuring serotonin transporter using SPECT – [^{123}I] -beta-CIT

Brain imaging techniques, like PET and SPECT, allow us to measure SERT availability in the living human brain. While SPECT has been used extensively to assess the availability of SERT, fewer studies have used PET. It is generally considered that PET measurement give a better quantification of SERT(Paterson et al., 2013). In addition, the property of the ligand used to measure SERT also contributes to the noise in the findings. For example, a very commonly used ligand in SPECT – [^{123}I] -beta-CIT, has affinity for both SERT and DAT in the brain. Therefore it is difficult to quantify SERT completely using this ligand (Ruhe et al., 2009). Newer SPECT ligands like ADAM and MZIENT seem to be much better in quantifying this(Paterson et al., 2013, Paterson et al., 2010, Nicol et al., 2012). [^{123}I] -beta-CIT is a cocaine analogue that binds to the SERT and DAT with equal affinity(Brucke et al., 1993). However, these two transporter bindings can be differentiated due to their differential localisation. For example, DAT is more

concentrated in the striatum while SERT in the midbrain(de Win et al., 2005). In addition, there are kinetic differences that also contribute to the differential binding of [^{123}I] -beta-CIT[^{123}I] -beta-CIT to DAT and SERT. For example, DAT uptake is thought to be maximal at 20 - 24 hours of administration of Beta-CIT, while SERT binding is thought to be maximal at between 2 to 4 hours post administration (Brucke et al., 1993, de Win et al., 2005).

7.3.7.4 Radioligand [^{123}I] -beta-CIT preparation for the study

[^{123}I] -beta-CIT was prepared via electrophilic iododestannylation of the corresponding tributylstannyl precursor (Baldwin et al., 1993). Briefly, reagents were added to carrier free Na^{123}I (370-740 MBq) in approximately 10-20 µl of 0.05 M NaOH, in the following order: 30 µg tributylstannyl precursor in 300 µl glacial acetic acid followed by 10% V/V peracetic acid. The reaction proceeded for 20 minutes at room temperature. 500 µl of NaOH was then added. The mixture was purified by reverse-phase HPLC and the solvent removed by rotary evaporation. The [^{123}I] -beta-CIT was formulated as 150 MBq in 5 ml ≤6% ethanol in isotonic citrate acetate buffer and filtered through a 0.22 µm filter. Pyrogenicity tests and sterility tests were performed.

7.3.7.5 SPECT Imaging

This protocol has been previously validated and described (Cavanagh et al., 2006). [^{123}I] -beta-CIT binds with high affinity in vitro to both Dopamine transporter (DAT) and SERT. In vivo studies in both humans and nonhuman primates have shown that [^{123}I] -beta-CIT accumulates in two distinct brain regions. In the striatum, where the density of DAT is much higher than that of SERT, [^{123}I] -beta-CIT binding mainly reflects DAT density, whereas in the brainstem and diencephalon binding seems to be specific for SERT.

The kinetics of [^{123}I] -beta-CIT binding differs markedly between the DAT-rich and SERT-rich regions. The slow uptake in the striatum, which reaches a peak after 20-30 hours, is in contrast to the faster kinetics seen in the brainstem and the diencephalon, where peak activity is attained after 2-4 hours after administration. In this study, early imaging of SERT regions was performed to minimize any possible effect of DAT on the SERT measurement because DAT uptake is proportionally lower at 3 hours(de Win et al., 2005).

Brain SPECT imaging was performed with a dedicated state of the art, 12 headed Neurofocus 900 SPECT scanner (spatial resolution 7mm full width at half maximum with a line source in air; NeuroPhysics, Shirley, Massachusetts), which acquires sequential single transaxial brain sections. Up to 25 axial sections 6 mm apart were scanned and the energy window (140-178 keV) was placed symmetrically around the ^{123}I gamma energy of 159 keV. A linear attenuation correction was applied, based on an automatically detected ellipse matching the outer head surface.

Subjects were scanned starting 3 – 4 hours after IV administration of [^{123}I] -beta-CIT. To minimize thyroid uptake of radioactive iodine, 120mg of potassium iodide was administered orally to each subject at least one hour prior to [^{123}I] -beta-CIT injection. Scanning time was approximately 50 min per scan.

7.3.7.6 SPECT Image analysis

Region of interest (ROI) analysis, carried out by an investigator blinded to the subject's clinical and demographic history, was used to extract data from the [^{123}I] -beta-CIT scans. A standard ROI template was constructed with the aid of two image templates: (1) the standard magnetic resonance imaging template known as ICBM152, which is based on 152 normal MRI scans and is available from the Statistical Parametric Mapping web site of the Functional Imaging Laboratory (http://www.fil.ion.ucl.uk/spm/); and (2) and in-house cerebral perfusion template based on 32 normal SPECT scans of cerebral perfusion.

The ROI template consisted of manually drawn regions representing the striatum ROI, the brain stem/diencephalon ROI (referred to as the brain stem ROI below for brevity), and a reference ROI). The striatum ROI (7.6 cm^3 on each side) was drawn on three axial sections 5 mm apart and encompassed the head of caudate and putamen in both hemispheres. The brain stem ROI (23 cm^3) was drawn on seven axial sections 5mm apart and comprised the thalamus-hypothalamus, mid-brain, and pons. The reference ROI (35 cm^3) was drawn on three axial sections 5 mm apart in the medial and lateral occipital lobe bilaterally. The occipital region was chosen to represent nonspecific and non-displaceable [^{123}I] -beta-CIT uptake (i.e., activity not associated with binding to transporters) because it has

a negligible density of both dopamine and serotonin transporters, as does the cerebellum.

The [^{123}I] -beta-CIT uptake in each ROI was expressed as mean counts per pixel, and the specific uptake in each transporter ROI was calculated as mean counts per pixel in the ROI minus mean counts per pixel in the occipital ROI. Transporter binding ratio for SERT was then defined as the ratio of specific uptake to uptake in the occipital reference region. Under equilibrium conditions the binding ratio is proportional to transporter binding potential, and provided transporter affinity and nonspecific binding are invariant across subjects, the ratio is then a measure of transporter availability.

7.3.8 Statistical analysis.

All data were tested for normality. Where data were not normal, appropriate transformations were performed. Non-parametric tests were performed wherever possible, in order to be conservative. Differences between two independent groups were tested using unpaired t test (or Mann Whitney U Test). Differences between dependent variables were tested using paired t test (or Wilcoxon signed-rank test). Linear regression was performed to examine the relationship between variables. In addition, a mediation analysis was conducted to examine the relationship between inflammatory markers, SERT and BDI scores. All p values mentioned are 2-tailed.

7.4 Results

7.4.1 Sample demographics

We analysed data from 12 subjects. They consisted of 6 males and 6 females (mean age = 47.58; s.d.=8.8), who were initially scanned using SPECT following intravenous administration of [^{123}I] -beta-CIT (average dose = 150.08mBQ) after an average of 218.9 (s.d. = 18.5) minutes. Blood for measuring TNF-α , hsCRP, IL6, IL1B and IL10 were collected just before the [^{123}I] -beta-CIT injection.

All patients were then commenced on a weekly Etanercept 50 mg intramuscularly. All participants received the medication for an average of 6 to 8 weeks (6.58 weeks; s.d. = 0.79), and underwent a second scan using the same

protocol with [^{123}I] -beta-CIT (average dose = 148.5 mBQ) after an average of 220.4 minutes. There was no significant difference between the dose of [^{123}I] -beta-CIT between the two scans or the average delay of scan after the injection. In addition, neither the dose of [^{123}I] -beta-CIT nor the time of scan after administration, showed a correlation with SERT data.

7.4.2 Descriptive statistics

The descriptive statistics of the measures are shown in Table 7-2. Normality of continuous data was tested using normality plots and Shapiro-Wilk tests (Table 7-3). Since most of the data were not normally distributed, data were log or inverse transformed. Where data were normally distributed, they were left as it is. As shown the table below log transformation of the data rendered most of the variables normal. However, where possible, I performed non-parametric tests.

Table 7-2: Descriptive statistics of the variables measured

	Mean	Std. Deviation	Median	Percentiles		
				25	50	75
Age	47.58	8.8	49.00	41.25	49.00	55.25
sIL1B_1	5.93	5.14	3.39	2.21	3.39	7.73
sIL1B_2	8.03	5.40	7.30	2.68	7.30	12.19
IL10_1	5.83	4.52	4.81	3.04	4.81	6.81
IL10_2	6.64	5.77	4.72	3.68	4.72	6.80
TNF-α _1	61.53	108.79	12.76	2.00	12.76	85.56
TNF-α _2	198.67	147.61	248.88	20.64	248.88	293.98
hsCRP_1	8823.58	13475.50	3909.50	966.00	3909.50	10166.75
hsCRP_2	7049.58	13001.49	3264.00	781.25	3264.00	6354.00
IL6_1	16.14	42.70	2.84	1.41	2.84	6.82
IL6_2	12.23	32.63	1.79	1.06	1.79	3.23
SERT_1	1.13	.23	1.08	.99	1.08	1.18
SERT_2	1.00	.19	1.02	.81	1.02	1.14
BDI_1	11.42	7.66	10.00	5.00	10.00	16.75
BDI_2	8.08	8.05	5.00	3.25	5.00	10.00
FACIT_1	27.08	13.70	27.50	14.75	27.50	39.50
FACIT_2	33.66	13.70	38.00	23.75	38.00	44.50
Pain_1	1.31	0.64	1.42	.80	1.42	1.77
Pain_2	.74	.42	.65	.32	.65	1.10

1 refers to pre-treatment levels and 2 refers to post-treatment levels. Lower FACIT scores suggest lower fatigue. All circulating cytokine levels measured as pg/ml; hsCRP as ng/ml

Table 7-3: Normality distribution of the variables and the log transformed variables.

	Shapiro-Wilk			Shapiro-Wilk (post log transformation)		
	Statistic	df	Sig.	Statistic	df	Sig.
sIL1B_1	.82	12	.02	.95	12	.70
sIL1B_2	.93	12	.43	.92	12	.30
IL10_1	.73	12	.002	.96	12	.91
IL10_2	.57	12	.000	.81	12	.01
TNF-α _1	.63	12	.000	.91	12	.24
TNF-α _2	.90	12	.19	.76	12	.01
hsCRP_1	.65	12	.000	.98	12	.99
hsCRP_2	.52	12	.000	.96	12	.90
IL6_1	.39	12	.000	.94	12	.51
IL6_2	.39	12	.000	.97	12	.91
BDI_1	.93	12	.39	.95	12	.66
BDI_2	.77	12	.01	.98	12	.99
FACIT_1	.95	12	.65			
FACIT_2	.87	12	.07			
Pain_1	.97	12	.96			
Pain_2	.94	12	.49			

Note : p<0.05 suggests data significantly deviates from normality. 1 refers to pre-treatment levels and 2 refers to post-treatment levels

7.4.3 Relationship between diagnosis and baseline variables

Table 7-4 shows the difference in baseline continuous variables between participants with psoriasis and psoriatic arthritis. Only the fatigue scores and pain scores differed between the two groups. Fatigue (U=0.00; p=0.004) and pain (U=5.00; p=0.07) was greater in those with psoriatic arthritis.

Table 7-4: Difference in baseline variables between participants with psoriasis and psoriatic arthritis

	Psoriasis (4) Mean rank	Psoriatic Arthritis (8) Mean rank	Mann-Whitney U	Z	Exact Sig. [2 tailed]
SERT	6.75	6.38	15.00	-.17	.93
TNF-α	5.25	7.13	11.00	-.84	.46
IL6	6.63	6.44	15.50	-.08	.93
hsCRP	6.25	6.63	15.00	-.17	.93
IL10	6.50	6.50	16.00	.00	1.00
IL1B	5.00	7.25	10.00	-1.01	.36
BDI	5.00	7.25	10.00	-1.02	.36
FACIT (fatigue)	10.50	4.50	0.00	-2.71	0.004
Pain	3.75	7.78	5.00	-1.87	0.07

Lower FACIT scores suggest greater fatigue

7.4.4 Relationship between sex and baseline variables

Table 7-5 shows the difference in baseline variables between males and female participants. As can be seen, sex was not significantly associated with any of the baseline variables.

Table 7-5: Difference in baseline variables between males and females

	Males (6) Mean rank	Females (6) Mean rank	Mann-Whitney U	Z	Exact Sig. [2 tailed]
SERT	5.67	7.33	13.00	-.80	.48
TNF-α	7.00	6.00	15.00	-.48	.69
IL6	6.83	6.17	16.00	-.32	.81
hsCRP	6.50	6.50	18.00	.00	1.00
IL10	7.33	5.67	13.00	-.80	.48
IL1B	6.67	6.33	17.00	-.16	.93
BDI	5.33	7.67	11.00	-1.12	.31
FACIT (fatigue)	6.00	7.00	15.00	-.48	.69
Pain	6.17	6.83	16.00	-.32	.81

Lower FACIT scores suggest greater fatigue

7.4.5 Correlation between baseline variables

Table 7-6 shows the results of the spearman's correlation analysis used to examine if there was significant correlation between the baseline variables. As can be seen, IL1B, IL6, IL10 and CRP showed significant correlation at baseline. TNF-α did not show significant correlation with other inflammatory markers. Age correlated negatively with SERT and FACIT score and positively with BDI scores (non-significant). In addition, it can be seen that TNF-α showed a positive correlation with SERT.

Table 7-6: Correlation between baseline variables

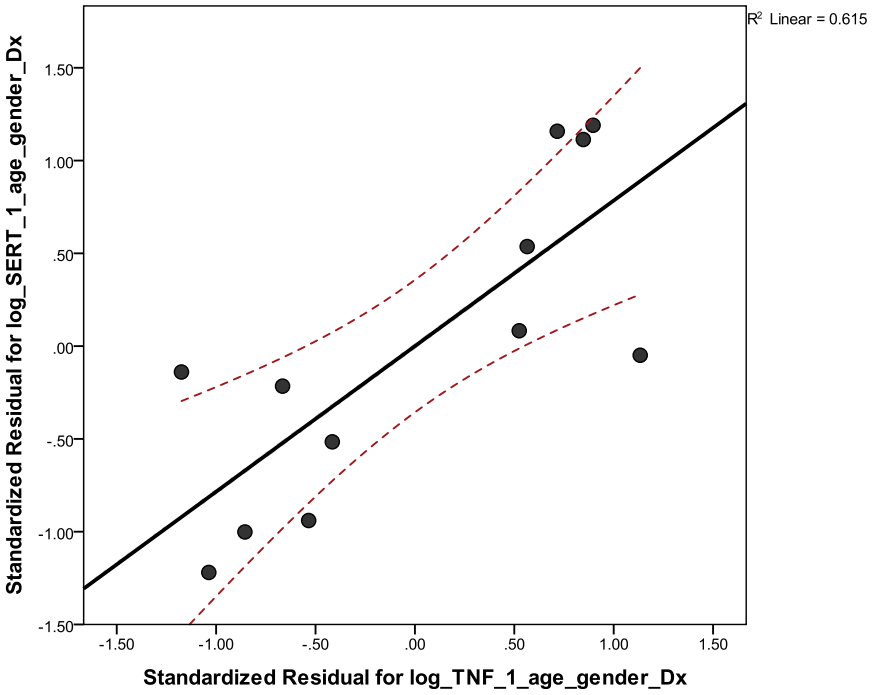
			SERT	TNF-α	IL6	hsCRP	IL10	IL1B	BDI	FACIT	Pain	Age
	SERT	rho	1									
	TNF-α	rho	.66*	1								
	IL6	rho	.004	.17	1							
	hsCRP	rho	-.21	-.22	.69*	1						
	IL10	rho	.12	.42	.64*	.28	1					
	IL1B	rho	.25	.35	.67*	.44	.25	1				
	BDI	rho	.16	.12	-.61*	-.19	-.21	-.27	1			
	FACIT	rho	-.09	-.28	.21	.04	.16	-.04	-.49	1		
	Pain	rho	.12	.37	-.07	-.26	.33	.07	.46	-.41	1	
	Age	rho	-.52	-.04	-.18	-.09	-.17	.13	.32	-.59*	.33	1

***. Correlation is significant at the 0.05 level (2-tailed); Lower FACIT scores suggest greater fatigue.**

7.4.6 Relationship between TNF-α , SERT and BDI scores at baseline

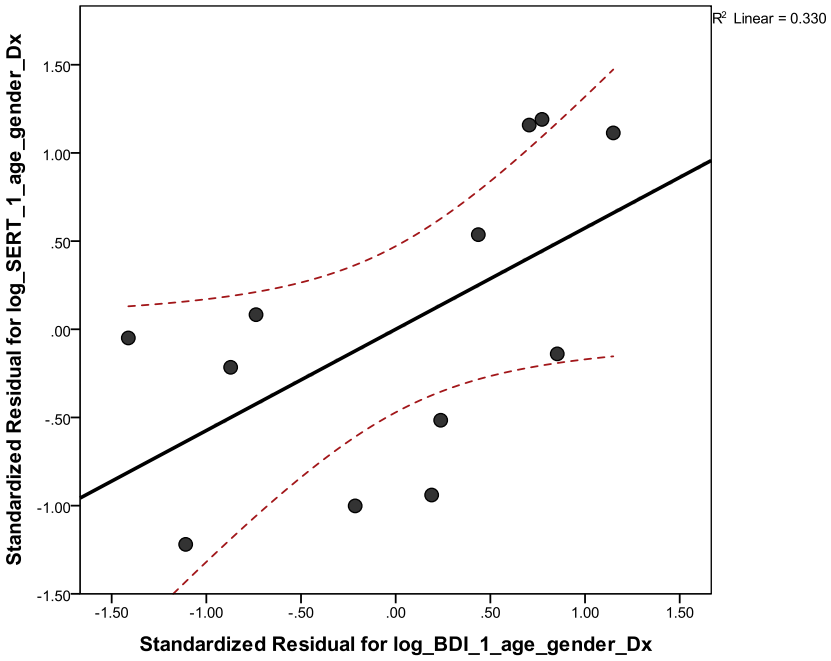
Since there was a significant association between TNF-α and SERT in the above analysis, we explored the relationship between TNF-α , SERT and BDI further. Since age, gender and diagnosis shows some association with the above variables, I corrected TNF-α , SERT and BDI for age, gender and diagnosis, using linear regression. I also corrected FACIT and Pain scores for age and diagnosis. We used the standardised residuals for further analysis.

Figure 7-4: Correlation between SERT and TNF-α levels at baseline. The dotted lines show the 95% confidence interval



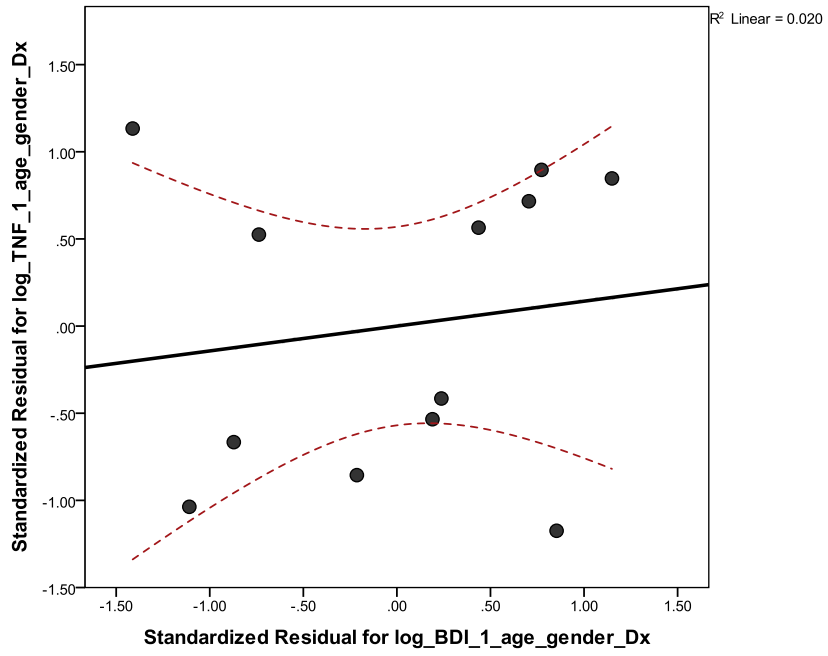
From Figure 7-4, it can be seen that baseline TNF-α explained almost 61.5% of the variance in SERT levels (rho=0.76;p=0.004). hsCRP (r2=0.03), IL1B (r2=0.018), IL10(r2=0.01) and IL6 (r2=0.10) did not explain significant variance in SERT levels.

Figure 7-5: Correlation between SERT and BDI scores at baseline. The dotted lines show the 95% confidence interval



From the Figure 7-5 it can be seen that SERT explained almost 33% of the variance in BDI (rho=0.57;p=0.05).

Figure 7-6: Correlation between TNF-α and BDI scores at baseline. The dotted lines show the 95% confidence interval

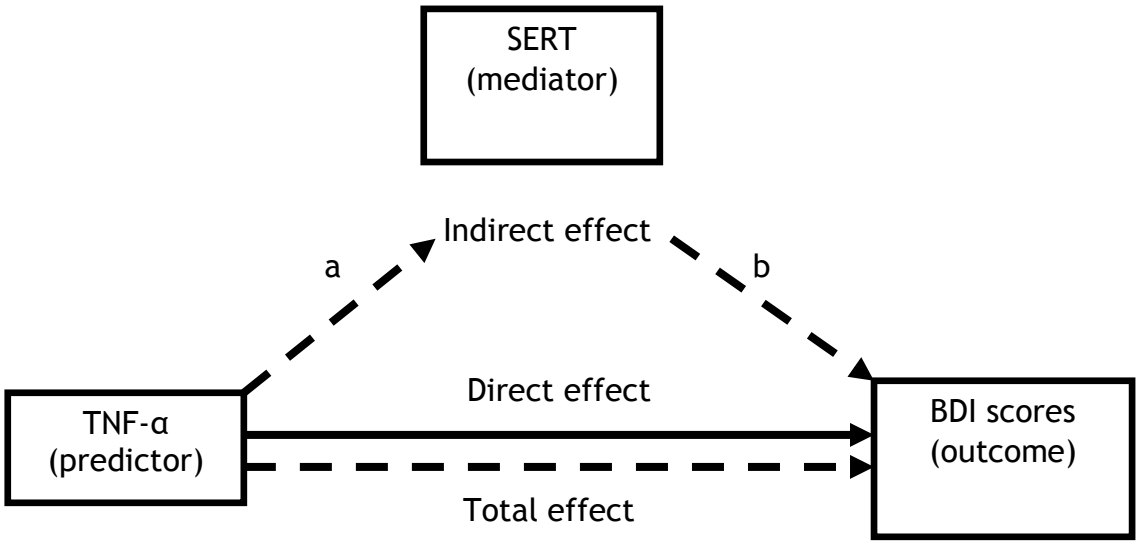


However, as can be seen from Figure 7-6, TNF-α accounted only for around 2% of the variance in BDI scores (rho=0.15;p = 0.63).

Although TNF-α explained only around 2% of the variance in BDI, since TNF-α explained significant variance in SERT and SERT in turn explained significant variance in BDI, a question was whether, the variance explained by SERT and TNF-α on BDI overlapped significantly.

We therefore conducted a mediation analysis to explore this relationship. To explore the relationship between TNF-α , SERT and BDI scores, I conducted a mediation analysis with TNF-α levels as independent (explanatory/predictor) variable, SERT as mediator and BDI scores as dependent variable. Here, I aimed to examine if a proportion of variance in SERT could explain the relationship between TNF-α and BDI scores. It should be noted that in classic mediation analysis, causal models are tested using longitudinal data; such an assumption is not made here. The analysis here, tests the hypothesis that the TNF-α levels accounts for variance in the mediator (SERT) and in turn, this variance in the mediator accounts for a proportion of the variance in BDI scores. “The adjustable parameters of the model represent the unidirectional influence between pairs of variable in the model. The best fitting values of the parameters are estimated by using the General Linear Model to solve the linear equations that describe the relationships within the model. This analysis differs from multiple regression which estimates the proportion of variance in the dependent variable accounted for by each of several independent predictor variables while allowing for the variance accounted for by the other predictors in the model(Palaniyappan and Liddle, 2012).” In other words, the mediation analysis partitions the variance explained by the predictor into a part that is independent of the mediating variable (direct), and a part that is accounted for via the mediating variable (indirect)(Figure 7-7).

Figure 7-7: The relationship between the predictor, mediator and the outcome variables.



The mediation analysis partitions the total variance (total effect) explained by the predictor into a part that is independent of the mediating variable (direct effect), and a part that is accounted for via the mediating variable (indirect effect). a represents the 'a' path and b represents the 'b' path.

I used the bootstrap method of Preacher and Hayes to estimate the indirect effect and bias-corrected 95% confidence interval (CI) for each individual mediator based on 20,000 bootstrap samples using an SPSS macro(Preacher and Hayes, 2008). It should be noted that this analysis requires no assumption regarding the underlying distributions since the statistical significance level is determined non-parametrically.

Figure 7-8: Indirect effect analysis shows that SERT mediates the association between TNF-α and BDI scores at baseline

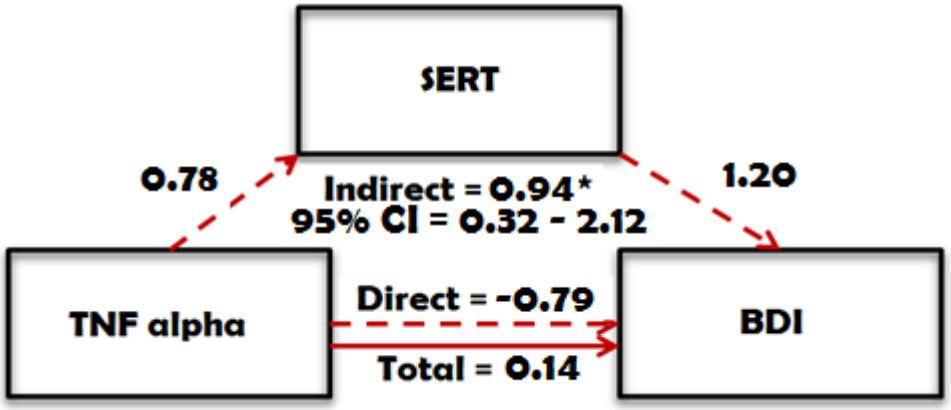
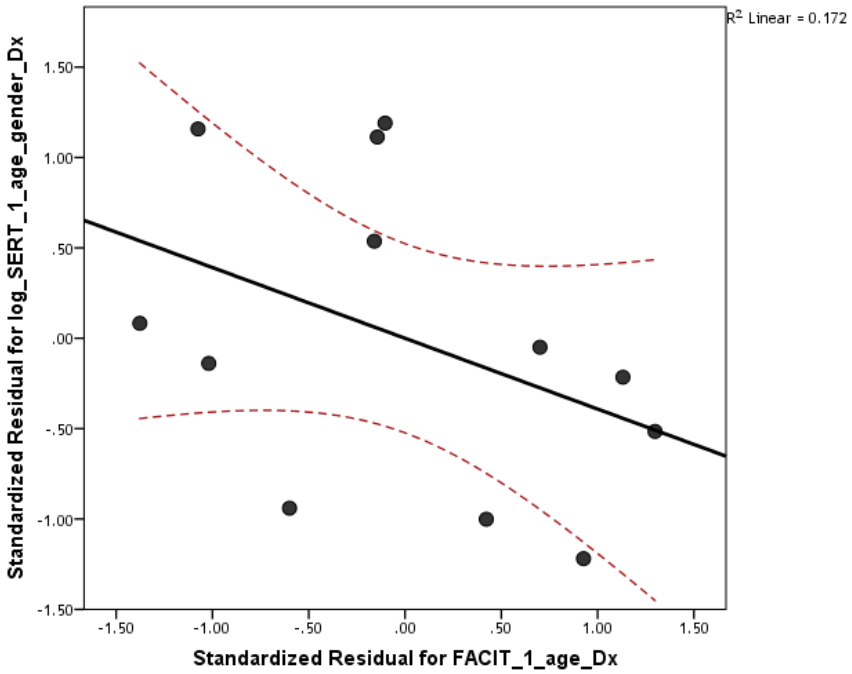


Figure 7-8 shows that SERT was a significant mediator of the relationship between TNF-α and BDI scores (Indirect effect: Beta=0.94; SE =0.50; 95% CI = .32 to 2.12). It should also be noted that neither pain scores nor fatigue scores mediated the relationship between TNF-α and BDI scores.

7.4.7 TNF-α , SERT and FACIT-fatigue

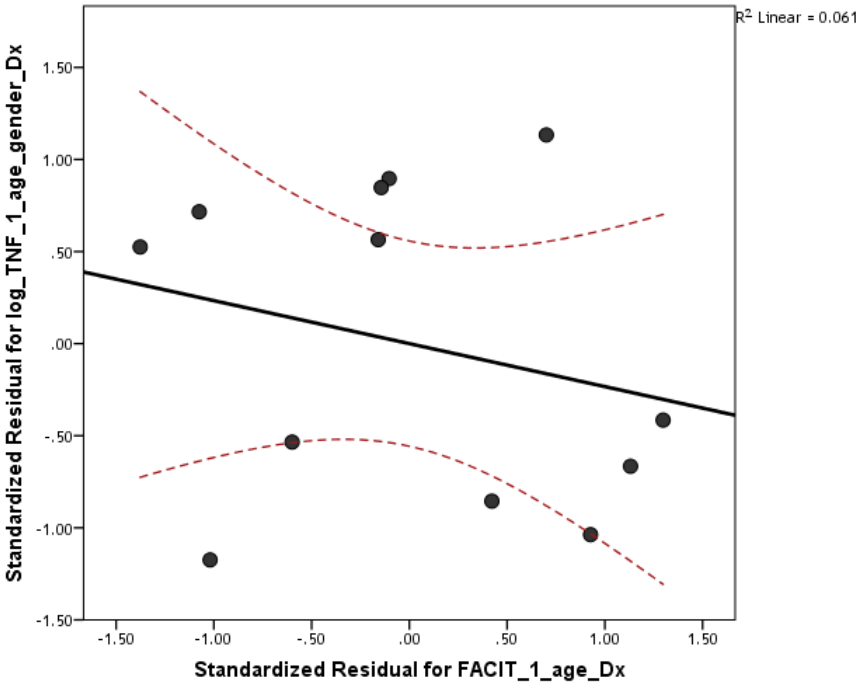
Figure 7-9 shows the relationship between SERT levels and FACIT scores. Greater SERT was associated with lower FACIT scores (greater fatigue) (r^2= 0.17; rho= -0.46 ; p=0.1).

Figure 7-9: Correlation between SERT and FACIT-F scores. The dotted lines show the 95% confidence interval



As shown in Figure 7-10 , TNF-α levels explained around 6% of variance in FACIT scores (rho=-0.25 p=0.43).

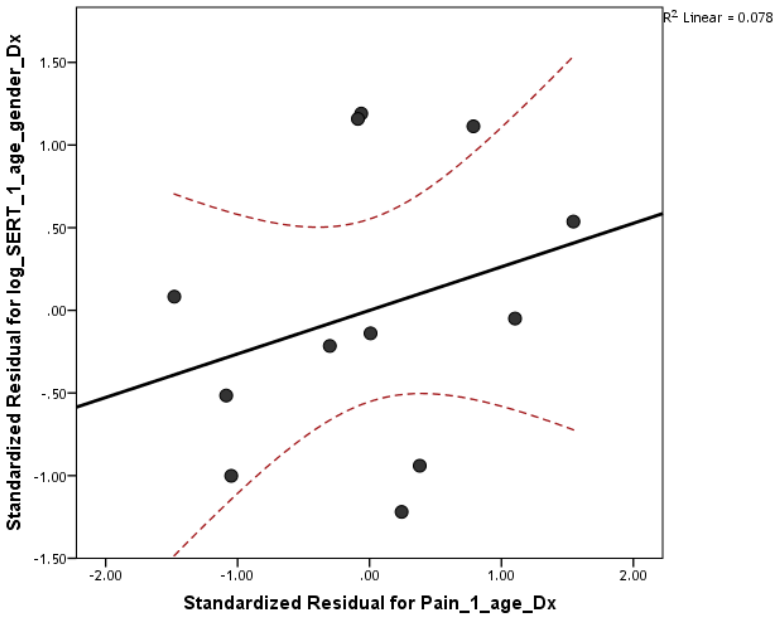
Figure 7-10: Correlation between TNF-α and FACIT scores. The dotted lines show the 95% confidence interval



However, a mediation analysis as in the previous step, showed that SERT did not mediate the relationship between TNF-α and FACIT scores (Beta=-0.47; SE=0.75; 95%CI = -1.6 to 1.2).

7.4.8 TNF-α , SERT and Pain scores

Figure 7-11: Correlation between SERT and pain scores. The dotted lines show the 95% confidence interval



As can be seen in Figure 7-11, SERT explained only around 8% of variance in pain scores (rho=0.28;p=0.37).

Figure 7-12: Correlation between TNF-α and Pain scores. The dotted lines show the 95% confidence interval

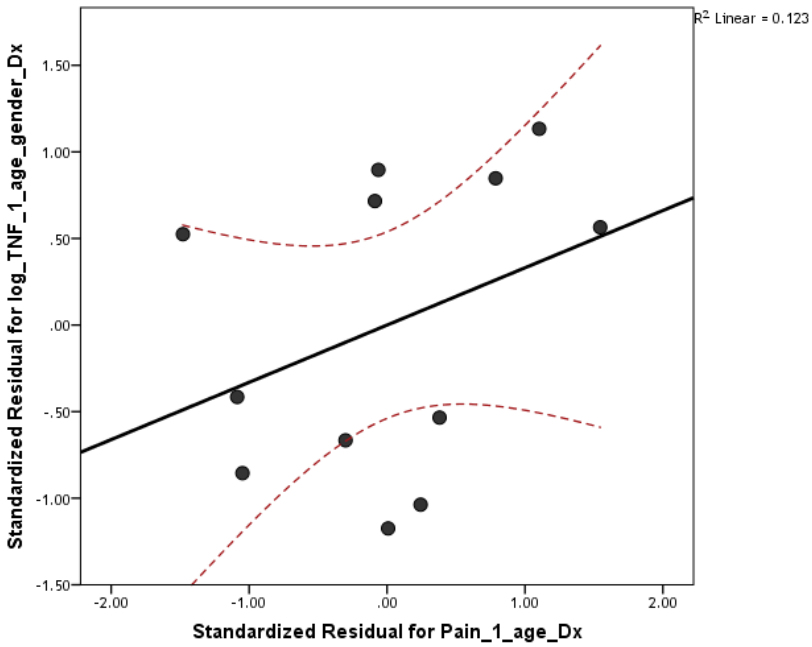


Figure 7-12 shows that TNF-α explained around 12 % of variance in pain scores (rho=0.35;p=0,26). SERT levels did not mediate the relationship between TNF-α levels and pain scores (Beta= 0.009; SE=0.65; 95%=-0.93 to 1.37).

7.4.9 Effect of TNF-α blockade using Etanercept

Table 7-7: Paired t test exploring difference between the two conditions (before and after Etanercept)

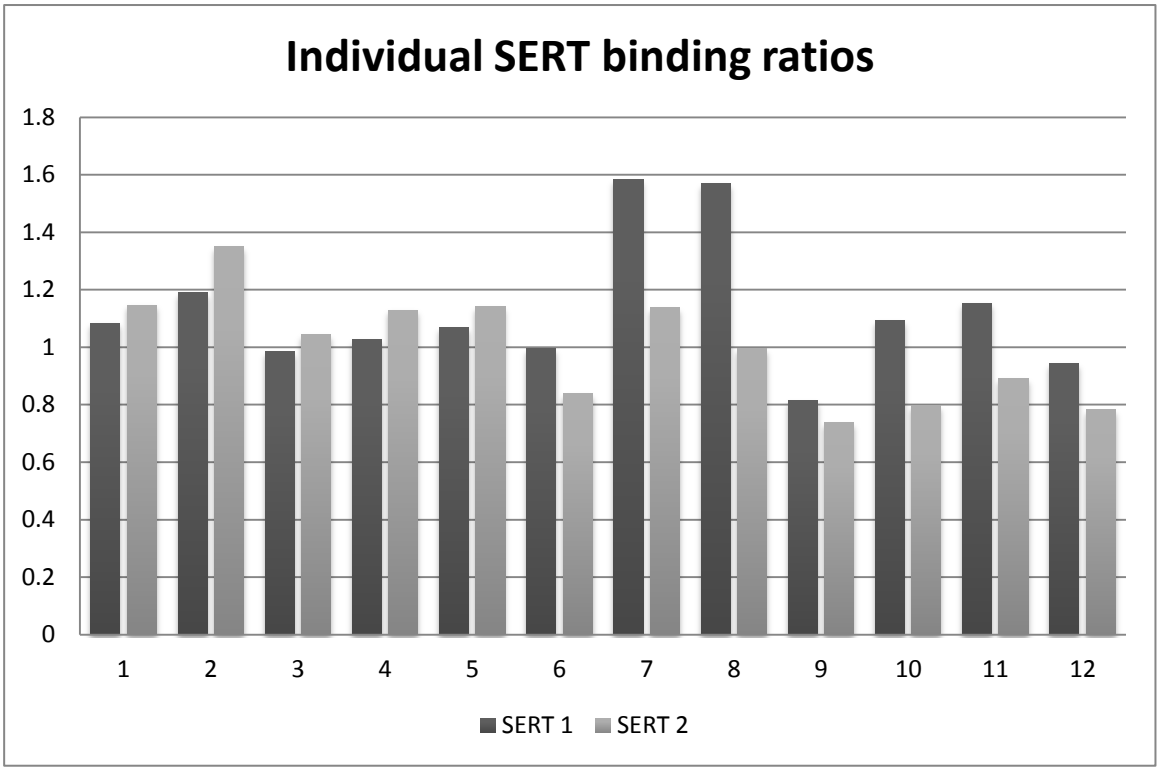
	Paired Differences (Pre - Post Etanercept)					t (d.f. =11)	p (2-tailed)
	Mean difference	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
				Lower	Upper		
SERT	0.12	0.20	0.06	-0.01	0.24	2.07	0.06
TNF-α	-1.94	1.97	0.57	-3.19	-0.69	-3.41	0.01
IL6	0.06	0.19	0.06	-0.06	0.18	1.10	0.30
CRP	0.31	0.35	0.10	0.08	0.53	3.00	0.01
IL 10	-0.14	0.48	0.14	-0.45	0.16	-1.04	0.32
IL 1B	-0.37	0.76	0.22	-0.85	0.12	-1.67	0.12
BDI	0.48	0.57	0.17	0.11	0.84	2.87	0.02
FACIT	-6.58	5.75	1.66	-10.34	-2.92	-3.95	0.002
Pain	2.86	2.22	0.64	1.44	4.28	4.45	0.001

As can be seen in the Table 7-7, SERT levels decreased following TNF-α treatment. However, this difference failed to reach statistical significance on a 2-tailed test. Interestingly, TNF-α blockade resulted in a significant increase in TNF-α levels detected using ELISA. IL 1B and IL 10 also showed minimal increase however did not reach statistical significance. Among the inflammatory markers, only hsCRP showed a significant decrease with treatment. BDI, FACIT and Pain scores also showed a reduction following treatment with Etanercept. The average reductions in the 3 variables are shown in below Table 7-8.

Table 7-8: Percentage Change in variables following treatment with Etanercept

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
BDI reduction	12	-66.67	82.35	28.5862	39.13654
SERT reduction	12	-13.29	36.66	9.5522	17.32771
CRP reduction	12	-12.42	66.75	22.4441	23.90788
FACIT change	12	-78.95	9.09	-29.39	28.07
Pain reduction	12	0	78.57	39.14	25.24

Figure 7-13: Individual SERT binding before and after treatment with Etanercept.



As can be seen in Figure 7-13, the first 5 candidates showed an increase in SERT binding. However the mean increase in SERT was 8.3%. The mean reduction in SERT binding among the 7 people who showed a reduction in SERT was 22.3%.

Figure 7-14: Individual BDI scores before and after treatment with Etanercept

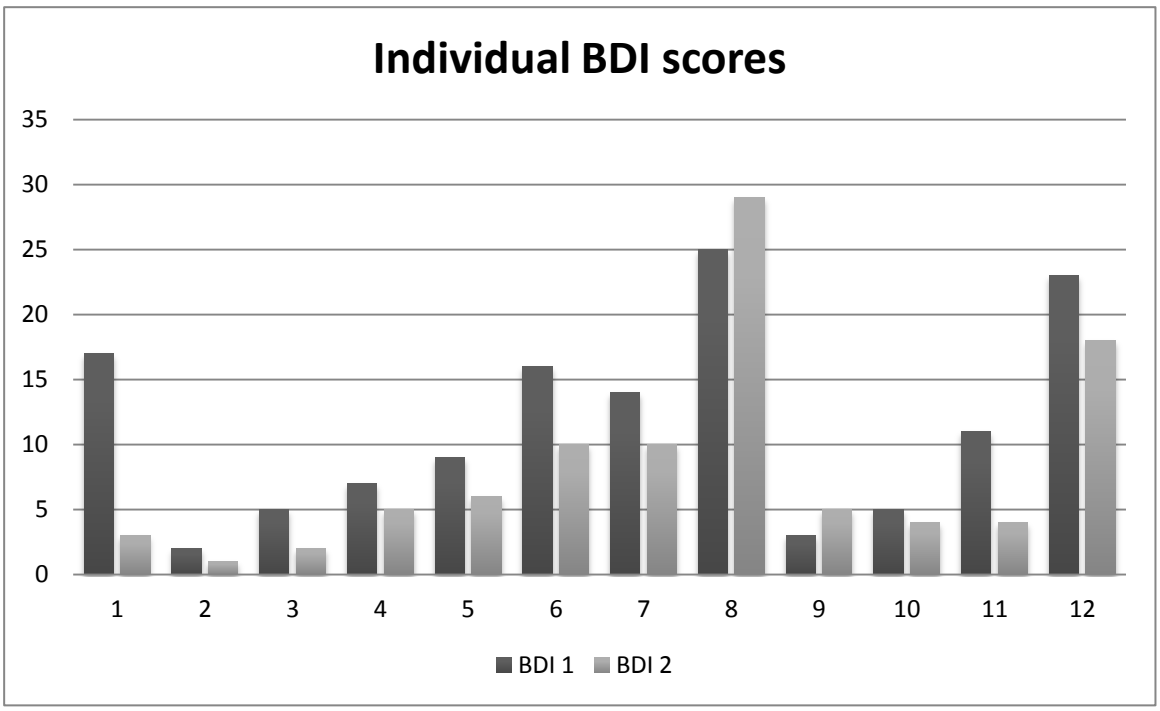
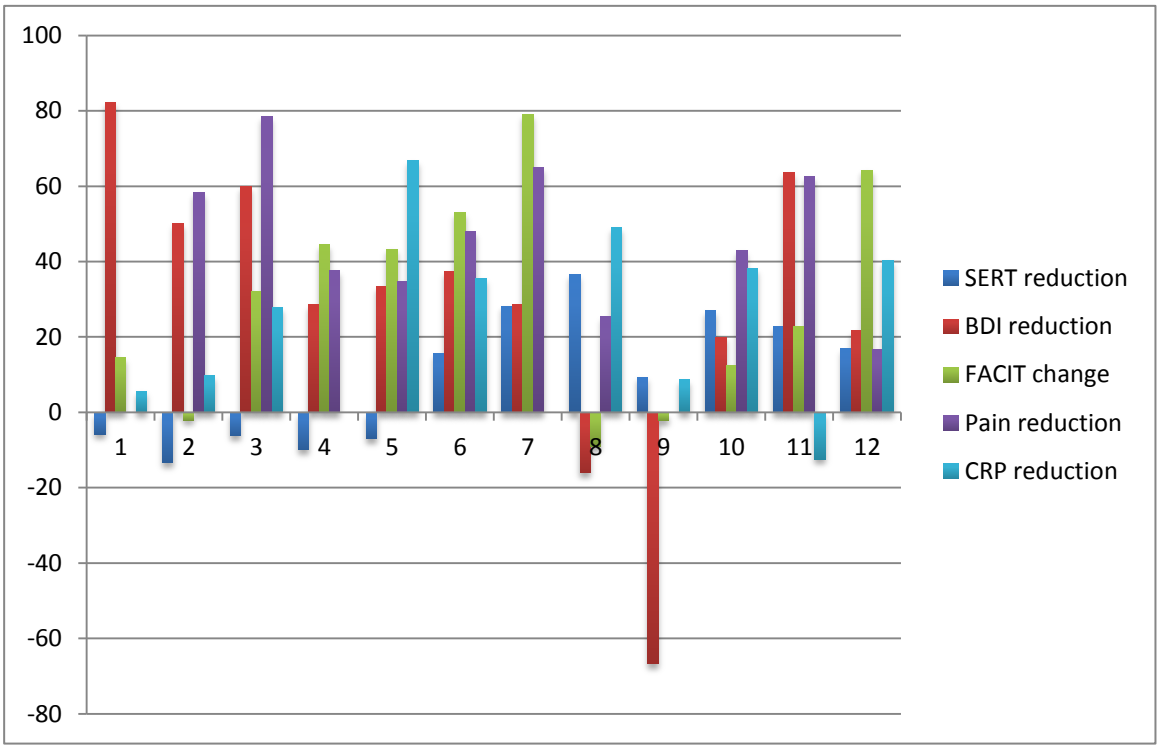


Figure 7-14 shows the individual BDI scores before and after TNF-α blockade. At baseline, 3 people met the criteria for mild mood disturbance, one person met the criteria for borderline clinical depression, and two people met the criterion for moderate depression. However, on SCID examination, none of them met the criteria for major depression requiring treatment with an antidepressant. None of them endorsed significant suicidal ideas.

As can be seen, the first 5 candidates who showed an increase in SERT binding showed a decrease in BDI scores. Among the 7 people who showed a reduction in SERT binding, two of them showed an increase in BDI scores.

Among the two people who showed an increase in BDI scores, their fatigue scores also showed some worsening. One of them did not show any change in pain scores (Figure 7-15).

Figure 7-15: Individual change in variables



As shown in the Table 7-9, there was no significant association between change in BDI, FACIT, Pain, SERT reduction and CRP reduction.

Table 7-9: Correlation between percentage change in scores following Etanercept treatment

			FACIT change	Pain reduction	SERT reduction	CRP reduction	BDI reduction
	FACIT change	Rho	1.00				
	Pain reduction	Rho	-.32	1.00			
	SERT reduction	Rho	.007	.02	1.00		
	CRP reduction	Rho	.13	-.29	.08	1.00	
	BDI reduction	rho	-.19	.40	-.41	-.38	1.00

7.4.10 Predictors of SERT reduction

Only 7 of them showed a reduction (mean reduction = 22.3%; s.d. =9.15). Those who showed a reduction in SERT had greater BDI scores at baseline. Greater baseline TNF-α predicted greater SERT levels at follow up. Not all participants showed a reduction in SERT. As can be seen in Table 7-10, the best predictor of SERT reduction was lower score on FACIT (greater fatigue). In addition, greater BDI scores predicted greater SERT reduction. Greater inflammatory markers at baseline - IL6, IL1B and hsCRP predicted lower SERT reduction.

Table 7-10: Association between baseline variables and change in SERT

		FACIT	Pain	SERT	TNF-α	IL6	hsCRP	IL10	IL1B	BDI
SERT change	rho	-.65*	.09	.37	.23	-.53	-.26	-.21	-.48	.55
	p	.02	.77	.23	.45	.07	.41	.51	.11	.06

Among those who showed a reduction in SERT binding (7 individuals), greater baseline TNF-α predicted greater SERT reduction (rho=0.61; r2=0.35; p=0.1).

7.5 Discussion

In this chapter, I have shown a significant association between circulating TNF-α and central SERT levels pre-treatment. Greater circulating TNF-α was associated with greater SERT. In addition, the greater SERT levels mediated the relationship between inflammation and depression scores (but not fatigue or pain scores). It should be noted that only a very small variance in BDI was explained by TNF-α levels. However, whatever variance was explained by TNF-α , overlapped significantly with that explained by SERT. This finding should be interpreted with caution, as all three variables were measured at the same time, and therefore, the data cannot be assumed to implicate a causal relationship. In addition, within the mediation relation tested, the direction of the relationship is speculative (but theoretically motivated). Here we are assuming that variance in TNF-α (the predictor variable) is what drives the other factors including SERT (mediator) and mood (outcome variable). However, it could be that mood is the primary driver and the other two variables may be epiphenomena (not mediated). Or SERT is the primary driver, thereby affecting mood and inflammation (not mediated). It should be noted that neither pain nor fatigue scores mediated the relationship between TNF-α and BDI scores.

The relationship between TNF-α , SERT and fatigue suggest that although SERT was significantly associated with fatigue scores, the variance in SERT did not mediate the relationship between TNF-α and fatigue. In other words, there was no significant overlap between the variance in fatigue explained by TNF-α and SERT. A similar situation obtains with pain scores.

Interestingly, TNF-α levels did not correlate significantly with the other proinflammatory cytokines. The correlation between TNF-α and IL1B was r=0.35, but did not reach statistical significance – perhaps due to the small sample size. However, it is also possible that TNF-α and IL1B have significant variance that is independent of each other. Interestingly, the anti-inflammatory cytokine, IL10 levels also correlated positively with TNF-α levels. It could be argued that greater inflammation was associated with a compensatory increase in anti-inflammatory levels. As expected, IL6 levels showed a significant association with CRP, IL1B and IL10. However, IL6 levels did not correlate with TNF-α levels.

Surprisingly, IL6 also showed a negative correlation with BDI scores. This is opposite to what was expected, in that greater inflammation was associated with lower scores on BDI. In fact, the BDI scores showed a positive association with only TNF-α . It showed a negative association with CRP, IL1B and IL10 levels. BDI scores however showed significant correlation with pain and fatigue scores.

With regards to the sample, those with psoriatic arthritis had greater fatigue and pain. However, they did not differ significantly in terms of depression scores or other inflammatory markers. A number of other measures showed an association with age and gender and diagnosis. Those variables were corrected for these, in order to account for the variance examined by these variables.

Treatment with Etanercept was associated with a reduction in SERT levels. However, not everyone showed a reduction in SERT. Those who showed a change in SERT had greater depression scores and fatigue at baseline. Those with greater baseline IL1B and IL6 showed lesser reduction in SERT. Interestingly, TNF-α levels increased following treatment with TNF-α blockade. This is in keeping with a number of previous reports(Tsimberidou et al., 2003, Suffredini et al., 1995, Zou et al., 2003, Madhusudan et al., 2004, Nowlan et al., 2006). The possible explanation for this is that the ELISA detects both bound and unbound forms of TNF-α and does not differentiate biologically active and inactive TNF-α (Bhatia and Kast, 2007). In other words, the TNF-α that was bound to Etanercept (but inactive) was also detected. This is also perhaps why, we did not find any association between TNF-α levels and SERT post-treatment.

7.5.1 The association between inflammatory markers and SERT

7.5.1.1 Comparison with preclinical studies

Most of the studies that have examined the relationship between inflammation and SERT are in animals. The relationship between circulating/peripheral inflammation and serotonergic system has been shown in a number of studies. Clement et al found that peripheral treatment with TNF-α and IL6 was associated with increased release of 5HT in the rat dorsal raphe nucleus (Clement et al., 1997). As the peripheral application of interferon gamma was

not associated with an increased release of serotonin, they suggested that the effect of peripherally elevated cytokine concentration on serotonin metabolism in the dorsal raphe nucleus was cytokine dependent. Zhang et al studied the response of peripheral administration of IL6 on in vivo microdialysis and in vivo amperometry (Zhang et al., 2001). Intraperitoneal injection of IL6 resulted in an increase in the concentration of serotonin in rat striatum associated with 5HT signals obtained from striatum, following electrical stimulation of the dorsal raphe nucleus. This in vivo amperometry signal had previously been shown to reflect specific increase in synaptic 5HT release.

We did not measure synaptic serotonin concentrations in our sample. However, it could be argued that greater SERT in at least some cases could be attributed to an up-regulation secondary to greater availability of SERT in the synaptic cleft. However, the relationship between synaptic 5HT and SERT is not clear. There is evidence to say that at least in some cases, greater synaptic 5HT results in downregulation of SERT that results in a reduction in SERT levels.

A number of studies have shown that inflammatory mediators may have a direct effect on SERT availability and activity in cell systems and in adult animals. Ramamoorthy et al showed that interleukin-1ß (IL-1ß) stimulates the activity of SERT in human JAR choriocarcinoma cells (Ramamoorthy et al., 1995). The stimulation was associated with an increase in the steady state levels of transporter mRNA and transporter density. They also showed that this increase in SERT mRNA was blocked by actinomycin D – a transcription inhibitor, thus suggesting that the increase in mRNA may be due to increased transcription of the SERT DNA. They in addition showed that this regulation may be independent of the cAMP pathway. However, they found no effect of IL-6 on SERT function. Morikawa et al examined the effects of interferon alpha and gamma on the transcriptional regulation of serotonin transporter in human choriocarcinoma cells(Morikawa et al., 1998). They showed that treatment with the interferons for around 30 minutes, led to an increase in SERT mRNA for upto 3 hours. This was found to be inhibited by treatment with the transcription inhibitor - actinomycin D. They found that treatment of the cells for a longer period (3 to 6 hours) rather than shorter period (30 minutes) was also associated with an increased SERT uptake activity. Tsao et al examined the effect of IFN alpha on MAPK phosphorylation, 5HTT mRNA expression and 5HT uptake in Jurkat T

cells(immortalized cell lines)(Tsao et al., 2008). They found that following treatment with IFN alpha, these cells showed an increase in the levels of MAPK phosphorylation, 5-HTT mRNA expression and 5-HT uptake. This was blocked in the cells that that were pretreated with MAPK inhibitors and fluoxetine. They claimed these results were evidence for an IFN-α-induced 5-HT uptake that reduces the 5-HT levels and IFN-α-regulated transcription of 5-HTT. Katafuchi et al, showed that induction of fatigue by intraperitoneal injection of polyI:C in rats, led to an increase in interferon alpha expression in the brain and an increase in 5HTT expression (Katafuchi et al., 2006). This was directly related to a reduction in 5HT in medial prefrontal cortex. All of the above are in keeping with our findings of greater association of TNF-α with SERT availability.

While the above reports suggest a SERT trafficking dependent pathway that mediate the relationship between inflammatory cytokines and SERT, there are studies, which show that the relationship between inflammation and SERT may also occur through SERT trafficking independent mechanisms. Mossner et al showed that the cytokine TNF-α modulates 5-HTT functions (Mossner et al., 1998). Using the same cell line, as Ramamoorthy et al, they found that TNF-α enhances 5-HT uptake, with a doubling of the maximal velocity of uptake. Furthermore, they showed that this effect was dose- and time- dependent, with a maximum observed after exposure to TNF-α for 48 hours. Zhu et al.showed, that both IL-1ß and TNF-α cause rapid activation of SERT in raphe neuron derived RN46A cells (Zhu et al., 2006). They also found that this increase in activity was p38 MAPK dependent. Given p38MAPK has an important role in sustaining SERT expression at the plasma membrane , immune factors that operate via this signalling mechanism could have marked effects on serotonergic neurotransmission (Samuvel, 2005). More recently, the same group showed that intraperitoneal injection of the cytokine inducer, LPS, stimulated SERT activity in vivo and increased immobility in both the tail suspension test (TST) and the forced swim test (FST) in adult mice (Zhu et al., 2010). They found that this mechanism was mediated via IL-1R and p38MAPK dependent mechanisms. They suggest that unlike previous results, which examined the direct effect of proinflammatory cytokines on SERT activity, their data provide evidence for a relationship between induction of peripheral inflammation and central expression of proinflammatory markers and SERT activity. It should be noted

that these experiments used low levels of inflammation, in order to avoid the effect of fever on behaviour.

In addition to neuronal SERT, it is recognised that SERT is also expressed in glial cells. Malynn et al showed that C6 glioma cells and primary astrocytes treated with TNF-α showed a sustained and significant increase in SERT mediated 5HT uptake(Malynn et al., 2013). They showed that this increase was due to an increase in the transporter capacity without any significant alteration in the affinity. They also found an increase in expression of SERT mRNA levels. Taken together, they argue that the increase in SERT mediated uptake was due to increased expression of the SERT gene, that is at least in part mediated through p38 MAPK dependent pathways.

We did not measure SERT activity. While it could be argued that at least some of SERT activity is dependent on its availability, there clearly exists mechanisms by which 5HT levels in the synaptic cleft is controlled by SERT activity that is independent of the availability –as described above. It is likely that at least some of the relationship between TNF-α and 5HT is mediated by changes in uptake that is independent of SERT availability.

While all the above studies have been done in animals/ human cell lines, very few studies have examined the relationship between inflammation and midbrain SERT in humans. In a pilot study, Cavanagh et al examined the effect of TNF-α blockade on midbrain SERT availability using Beta CIT SPECT(Cavanagh et al., 2010). They used adalimumab, a drug that binds to tumour necrosis factor-alpha (TNFα), preventing it from activating TNF-α receptors in patients with rheumatoid arthritis. In this study, following treatment with adalimumab, for just 4 days following last treatment, 5 out of 6 individuals showed a reduction in SERT binding of an average 20%. In their study, one participant showed an increase in SERT following treatment. They however did not measure the relationship between SERT and circulating inflammatory markers. Our findings resonate with this. However, the average binding reduction was much less. This is because 5 out of the 12 people showed an increase in SERT availability. Among those who did show a reduction, the average reduction was similar to that of Cavanagh et al(Cavanagh et al., 2010). It should however be noted that the 20% reduction in availability is far lower than that usually occurs with

antidepressants (80%). This may partly explain why a reduction in SERT availability did not correlate with reduction in BDI scores. It is likely that the reduction in depressive symptoms in our study was mediated through pathways independent of SERT availability.

Raison et al examined if anti TNF-α treatment using Infliximab reduced depressive symptoms in patients with treatment-resistant depression, and if inflammatory marker levels predicted treatment response(Raison et al., 2013). The found that after 3 infusions of infliximab vs placebo over a period of 12 weeks, there was no difference in depression scores between the two treatment groups. However, they found that those who responded to infliximab had a baseline concentration of hsCRP of more than 5mg/ l. 62% of those with a CRP of greater than 5 showed a response to infliximab, compared to only 33% who showed who showed a response to placebo. Baseline concentrations of TNF-α and its soluble receptors were also greater in infliximab responders.

7.5.2 Studies examining the relationship between inflammation and other brain markers

Two recent studies have examined the relationship between circulating inflammatory markers and other markers in the brain in the context of arthritis.

Lampa et al examined the levels of intrathecal proinflammatory cytokines in cases of rheumatoid arthritis(Lampa et al., 2012). They found that IL1B was elevated in the CSF of patients with rheumatoid arthritis, compared to healthy controls and patients with multiple sclerosis (in remission). Interestingly, they also found that CSF levels of IL1B were greater than serum levels. This suggests that at least in case of serum IL1B, serum levels may not represent levels in the brain. In addition, the presence of soluble and cell bound TNF-α in the brain, makes it difficult to reach conclusions about the CSF levels of this cytokine. They suggest that activated microglia produce cell bound TNF-α which mediate cell-cell interaction, and hardly release any soluble TNF-α detectable in the CSF. They did not find any significant difference in TNF-α levels or IL6 levels. It should also be noted that IL6 and TNF-α are very low in CSF in general. They also found that levels of anti-inflammatory cytokines – IL1Ra and IL 4 were lower in the CSF of patients with rheumatoid arthritis. They found a significant

correlation between IL1B levels and fatigue, but not with sleep/pain in these patients. In an animal model of fast-developing arthritis (serum-transferred K/BxN arthritis), they found that the spinal cord showed an increased expression of IL1B and TNF-α (but not IL6) mRNA on the day the arthritis peaked.

In another study, Hess et al aimed to establish why people with RA, who undergo TNF-α antagonist treatments perceive rapid improvement of symptoms in spite of chronic joint inflammation with structural changes(Hess et al., 2011). In patients with RA, they demonstrated that treatment with infliximab (a monoclonal antibody TNF-α blocker) was associated with reduction of nociceptive and limbic CNS activity measured using bold fMRI, as early as after 24 hours. This was independent of joint swelling and changes in acute phase reactants. They used a mouse model to show that TNF-α blockade was directly related to rapid reduction of nociceptive BOLD signals associated with thermal and mechanical hyperalgesia induced by arthritis. They used graph theoretical complex network analysis of bold signal cross-correlation to show that the pain related regions showed an increased degree of connectivity, clustering and modularity in the arthritic mice compared to the wild type mice. This tight cluster partially dissolved on TNF-α blockade administration. Their data suggest that TNF-α leads to intensive, widespread and prolonged activity on nociception, which is reversed with TNF-α blockade. They suggest that the nociceptive changes precede anti-inflammatory action of the TNF-α blockade agents.

Felger et al, examined the effect of interferon alpha treatment in rhesus monkeys(Felger et al., 2013). They treated the monkeys with interferon alpha or saline for 4 weeks. They used in vivo microdialysis to investigate dopamine release in striatum. They also used PET (C raclopride) to examine D2 receptor binding after amphetamine use, and 18F- FECNT to examine DA transporter binding. The in vivo microdialysis showed a reduction in DA release in the IFN group, compared to saline. This was associated with reduction in DA release in following amphetamines and reduced D2R binding, but no change in dopamine transporter availability. Anhedonia-like behaviour (reduced sucrose consumption) correlated with the reduction in DA release at 4 weeks.

Capuron et al measured presynaptic dopamine function in patients with hepatitis C virus who went on interferon alpha treatment (average 35 days)(Capuron et

al., 2012). They used F-dopa to measure dopamine turnover in the caudate and putamen using PET. The patients showed a significantly increased F-dopa uptake and decreased F-dopa turnover in caudate and putamen. Baseline and percentage signal change in F-dopa turnover correlated with changes in depression, fatigue and neurotoxicity. Serotonin release in the hypothalamus has been shown to be modulated by inflammatory cytokines in animal models (Wu et al., 1999). This release is thought to mediate behavioural changes associated with sickness behaviour (Guijarro et al., 2006).Raison et al demonstrated a direct relationship between cytokine levels and serotonin turnover was demonstrated in humans (Raison et al., 2009). They examined the CSF of patients being treated with interferon alpha for hepatitis C. They found that greater IL6 levels correlated negatively with 5HIAA concentrations in the CSF. This reduction in 5HIAA correlated negatively with depression severity in those who received the treatment . Higher TNF-α levels at baseline has been associated with somatic symptoms of depression in patients receiving IFN alpha treatment (Loftis et al., 2013).

Drake et al used PET tracer PK 11195 to examine the relationship between cerebrovascular risk factors (but no stroke) and brain inflammation. They found that people with greater risk factors showed greater microglial activation in the brain, compared to normal controls (Drake et al., 2011). Similarly, a few studies have shown that modulating circulating inflammation is associated with both functional and structural changes in the brain. Harrison et al showed that inducing a circulating inflammatory response using typhoid vaccine in normal adults is associated with change in BOLD signals in regions pertaining to modulation of mood in the brain (Harrison et al., 2009a) . Craig suggests that interoceptive signals from the body have a “primary viscero-cortical representation” within the Insula/SN (Craig, 2009). Essentially, interoceptive signals from vagal afferents, spinal afferents and sensory circumventricular organs are integrated in the ‘viscero-sensory hubs’ within the brainstem (Critchley and Harrison, 2013). Information from this hub is then relayed to the SN. In the case of inflammation, activated immune cells release inflammatory mediators, particularly cytokines, which activate vagal afferents and the sensory circumventricular organs (Harrison et al., 2009b). The brain stem ‘viscero-sensory hub’ responses evoke visceral reflexes that result in “sickness

behaviour", including fatigue, malaise, weight loss and anorexia. Inflammatory challenge using typhoid vaccine activates the 'brainstem hubs' and insula and disrupts affective network connectivity within the first 3 hours of administration, suggesting an early/fast neural mechanism in inflammation related viscero-cortical representation (Brydon et al., 2008, Strike et al., 2004). These changes also predict fatigue/psychomotor and sickness behaviour (Harrison et al., 2009b).

More recently, Hannestad et al found that systemic inflammation induced by endotoxin in humans was associated with higher normalised glucose metabolism in the insula. This change was associated with change in peak cytokine levels and also changes in social interest, suggesting that these may be linked to each other (Hannestad et al., 2012b). Hannestad et al , examined microglial activation using a PET TSPO ligand - PBR 28 in non-human primates. They found that following an intravenous administration of E Coli lipopolysaccharide, which induced systematic inflammation; PBR 28 binding was increased by 29% and 62% at 1 hour and 4 hours respectively(Hannestad et al., 2012a). Increased PBR binding also correlated significantly with serum IL1Beta and IL 6 levels. They conclude that systemic inflammation is associated with an increase in TSPO binding in non-human primates. The same group, however found no difference in PBR 28 binding in individuals with mild to moderate depression compared to healthy control subjects (Hannestad et al., 2013).

Functional gene polymorphisms of SERT have been examined in inflammatory cytokine-associated depression. A recent review by Felger and Lotrich, examined the relationship between the long and short allele of the SERT promoter regions and depressive symptoms in those who were receiving pegylated IFN-alfa-2a (PEGAYS) or IFN-alfa-2b (PEGINTRON) plus ribavirin for chronic hepatitis C virus (HCV) infection(Felger and Lotrich, 2013). Two studies showed no association, while two studies showed that the L/L alleles were associated with lower depressive symptoms and one study reported an association between L allele and higher depression scores in non-Hispanics, but an association between S allele and depression scores in Hispanic Caucasians (Table 7-11). In addition, this suggests that ethnicity may be a covariate of interest when examining this relationship.

Table 7-11: Relationship between functional polymorphisms of the SERT promoter region and depressive symptoms in patients receiving interferon treatment

(Bull et al., 2009)	5-HTTLPR (L/S)	*N* = 98, depression and fatigue monitored at 4, 8, 12, 24 weeks by BDI, SDS, CFQ	The L/L genotype was associated with fewer depressive symptoms, and this 'protective' effect was evident only in the presence of the 'low expression IL-6' genotype.
(Kraus et al., 2007)	5-HTTLPR (L/S)	*N* = 139, depression monitored by HADS	No significant association with cytokine-induced depressive symptoms
(Lotrich et al., 2009)	5-HTTLPR (LG, LA, and S)	*N* = 71, depression and related symptoms monitored by SCID, BDI, PSQI, NEO-Five Factor Inventory, CIRS-G at weeks 2, 4, 8, 12, and 16 weeks treatment	The LA allele was associated with a decreased rate of depression, with the LA/LA genotype being the most resilient. This genotype was also associated with better sleep quality
(Pierucci-Lagha et al., 2010)	5-HTTLPR (L/S)	*N* = 1,015, depression monitored by CIDI and BDI-II for 24 or 48 weeks	The L allele was associated with higher depression scores in non-Hispanic Caucasians and S allele associated with depression scores in Hispanic Caucasians
(Su et al., 2010)	5-HTTLPR (L/S)	*N* = 132, depression monitored by M.I.N.I., BDI and HDRS for 24 weeks	No significant association with cytokine-induced depressive symptoms, however there were only IL-6 G/G subjects in this Chinese sample and the effects of the "at risk" allele on the 5-HTTLPR could not be assessed

Reused with permission from Felger, J, Lotrich FE. Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications (Felger and Lotrich, 2013)

7.5.3 Reduction of SERT on Etanercept treatment

7.5.3.1 Does SERT play a role in the pathophysiology of depression?

The serotonin system has long been associated with depression. It is perhaps one of the most widely studied neurotransmitter systems in the context of depression. It is also the system that is most commonly affected by anti-depressants. A vast number of studies have examined the serotonin system in depression. They have demonstrated alterations in a wide range of aspects of the serotonin system. Serotonin levels in the brain are controlled by a number of factors including rate of synthesis, rate of release into the synaptic cleft, rate of enzymatic breakdown, rate of diffusion through the extracellular space and active reuptake. In the brain, SERT seems to be the predominant mechanism controlling serotonergic neurotransmission. Therefore it is likely that SERT availability is directly related to serotonergic neurotransmission (Meyer, 2008, Meyer, 2012).

Although a number of studies have examined the relationship between serotonin transporter and depression, due to the heterogeneity of the populations examined, only a few studies have the methodological rigour to address these. Meyer in a recent review of literature concluded that finding from studies that exclude subjects that have been on medications for at least 2 months, exclude those with co-morbidities, suggest either no change or an increase in regional SERT binding potential(Meyer, 2008, Meyer, 2012). Studies that have included participants, who have been recently medicated or included participants with co-morbidities, tend to report a reduction in SERT BP. More recently, Savitz and Drevets synthesised the data on SERT binding in mood disorders (Savitz and Drevets, 2013). They focused on PET studies that used 11C-DASB as the ligand, as the other ligands showed non-specific binding. Similar to Meyer, they reached the conclusion that PET based data on SERT binding in mood disorders are not definitive. However, similar to Meyer, they offer an interim summary, based on imaging studies of depression-related phenotypes, preclinical studies and the neuroimmunology literature, that SERT function is likely to be increased in depression. They then go on to provide a simplified hypothesis of SERT dysfunction in mood disorders. They suggest that increased serotonergic

neurotransmission in response to elevations in CRF and inflammation, results in an increase in SERT function, leading to chronic over-activity and decreased serotonin neurotransmission.

In our study, interestingly, we found a significant correlation between BDI scores and SERT levels. Those with greater SERT levels had greater BDI scores. SERT availability did not correlate with Fatigue scores or pain scores. It should be noted that the mean scores on BDI were below what would be considered clinical depression.

The data from the current literature reveal the complexity of this area of research. A difference in SERT binding seen in patients with depression may be due to a number of reasons (Meyer, 2008). Firstly, there may be differences in the number of serotonergic neurons - a lesion model similar to the loss of dopaminergic neurons on Parkinson's disease. Secondly, the radio-active ligands may be displaced by differences in serotonin levels. For example, an excess of serotonin can displace the radioactive ligand. Thirdly, SERT may also have undergone up/down regulation – i.e. there are differences in the number of transporters on the presynaptic neuron. Therefore, an increase in SERT binding can directly result in increased reuptake and a reduction of serotonin in the synaptic cleft. However, it can also represent a state of upregulation in response to an increase in serotonin levels. It is therefore not clear whether these are epiphenomenon or actually related to the pathophysiology of depression. In Recent research in humans have measured 5HT2A levels as a surrogate marker of extracellular serotonin in the brain. These data suggest that the relationship between SERT and 5HT2A levels seems to follow an inverted U shape. In other words, a reduction in SERT binding may represent low or high synaptic serotonin levels (Erritzoe et al., 2010). In addition to the above, serotonin levels may be regulated by SERT in two ways. Trafficking dependent activity - where the concentration of SERT molecules on the cell surface of the neurons are controlled, and a trafficking independent activity where only the activity of SERT, i.e. the substrate affinity and maximal transport is affected. There is evidence that inflammation may directly affect serotonin concentration through regulation SERT availability as well as activity, through both the above mechanisms(Ramamoorthy et al., 1995, Zhu et al., 2006).

7.5.3.2 What does a reduction in SERT availability mean?

The change in scores with treatment was more difficult to interpret. While we found no association between change in SERT and change in behavioural measures, we found that greater baseline BDI scores and greater fatigue predicted greater reduction in SERT. Interestingly, in our sample, greater inflammatory markers predicted smaller reduction in inflammatory markers. This is perhaps due to the fact that those who had greater inflammation to begin with, also showed greater inflammation (and hence SERT) following treatment, as there were no significant reductions in the inflammatory markers, except with CRP. However, CRP levels did not correlate with either pre or post etanercept SERT levels.

The reduction in SERT binding, could be due to a number of processes. It could mean that there is a direct effect of reduction of inflammation on transcription, and hence, SERT expression. It could be that greater serotonin availability (due to release of 5HT secondary to a reduction in inflammation) in the synapse, led to a down regulation of SERT. It should be noted that the relationship between SERT and 5HT follows an inverted U shape. It could also be that the greater serotonin availability just led to the displacement of beta-CIT from the transporter. It is also possible that a combination of the above factors may have led to our findings. However, using our data, the exact reason for the reduction cannot be ascertained.

7.5.3.3 How does the reduction in SERT and BDI scores compare with antidepressant treatments.

At least 3 recent studies have directly examined the effect of treatment with anti TNF-α biological agents on mood. See Table 7-12 (Rieder and Tausk, 2013). They also show the results of antidepressant trials that have directly examined the effect of antidepressants on mood in patients with psoriasis. Four studies that directly examined the effect on mood, showed significant improvement in mood scores, similar to those with antidepressant treatments.

Table 7-12: Studies examining the effect of treatment with anti-TNF-α agents or antidepressants on mood in Psoriasis

Study	*n*	Control group	Experimental group	Double-blind	Premorbid depression	Results (experimental vs. placebo)	*P* value
(Alpsoy et al., 1998)	60	Placebo	6 weeks of moclobemide, 450 mg/d	Yes	Undisclosed	Higher improvement rates in PASI, BDI, STAI-1, and HAM-A	All$P < 0.05$
(Modell et al., 2002)	11	None	6 weeks of bupropion-SR, 150–300 mg/d then 3-week washout	N/A	None	At 6 weeks, 8 of 10 patients' lesions had improved vs. baseline	<0.001 for both
(Tyring et al., 2006)	618	Placebo	Etanercept 50 mg twice weekly	Yes	77 of 308 (25%) of experimental group as measured by HAM-D 80 of 305 (26%) of placebo group as measured by HAM-D	At week 12, 42% more patients achieved 75% improvement in PASI At week 12, greater improvement in HAM-D and BDI	<0.0001 <0.0012 for both
(Gelfand et al.,	2546	Interrupted	Continued etanercept 50 mg	No	Mean BDI scores 8.1 and 8.3 in	Mean BDI scores improved by 29% at	None

Study	*n*	Control group	Experimental group	Double-blind	Premorbid depression	Results (experimental vs. placebo)	*P* value
2008)		etanercept	once weekly for second 12 weeks		continuous and interrupted treatment arms	week 12. Improvement sustained in both arms at week 24	provided
(Langley et al., 2010)	1230	Placebo	Ustekinumab 45 mg or 90 mg at weeks 0, 4, and every 12 weeks through week 52	Yes	227 of 814 (28%) of combined experimental group by HADS-D 98 of 405 (24%) of placebo group by HADS-D	At week 12, greater improvement in HADS-A (13.9%) and HADS-D (29.3%)	<0.001 for both

Recent reviews suggest that the usual minimal effective dose for SSRIs produce a SERT occupancy of around 70 to 80% measured using SPECT. Higher occupancy than this does not increase efficacy(Preskorn, 2012). de Win et al, using Beta-CIT showed that treatment with Citalopram was associated with a reduction in SERT binding of about 72% in the midbrain(de Win et al., 2005). Meyer suggest that at therapeutic doses, SSRIs and other antidepressants show SERT occupancy of around 80% (Meyer, 2012). However, the literature is not always consistent. Cavanagh et al found that SERT availability measured using Beta CIT did not differentiate treatment responders from non-responders(Cavanagh et al., 2006). Therefore the relationship between reduction SERT availability and reduction in depressive symptoms may not be linear.

In our case, the average reduction of SERT binding following TNF-α blockade was only around 9%. Among those who showed a reduction, the average reduction was around 20% and in our best case, the reduction was only 36.6%. Compared to Citalopram, the reduction in SERT binding with TNF-α treatment was considerably smaller. In our case, SERT reduction was not associated with a change in any of the clinical variables, including pain and fatigue. It was also not associated with a change in CRP levels, suggesting that this was not directly related to changes in disease/clinical state.

7.5.4 Pain and central serotonin

Figure 17: Pain modulatory pathway: The green arrows show descending inhibitory pain pathways, while the red arrows show ascending pain pathways

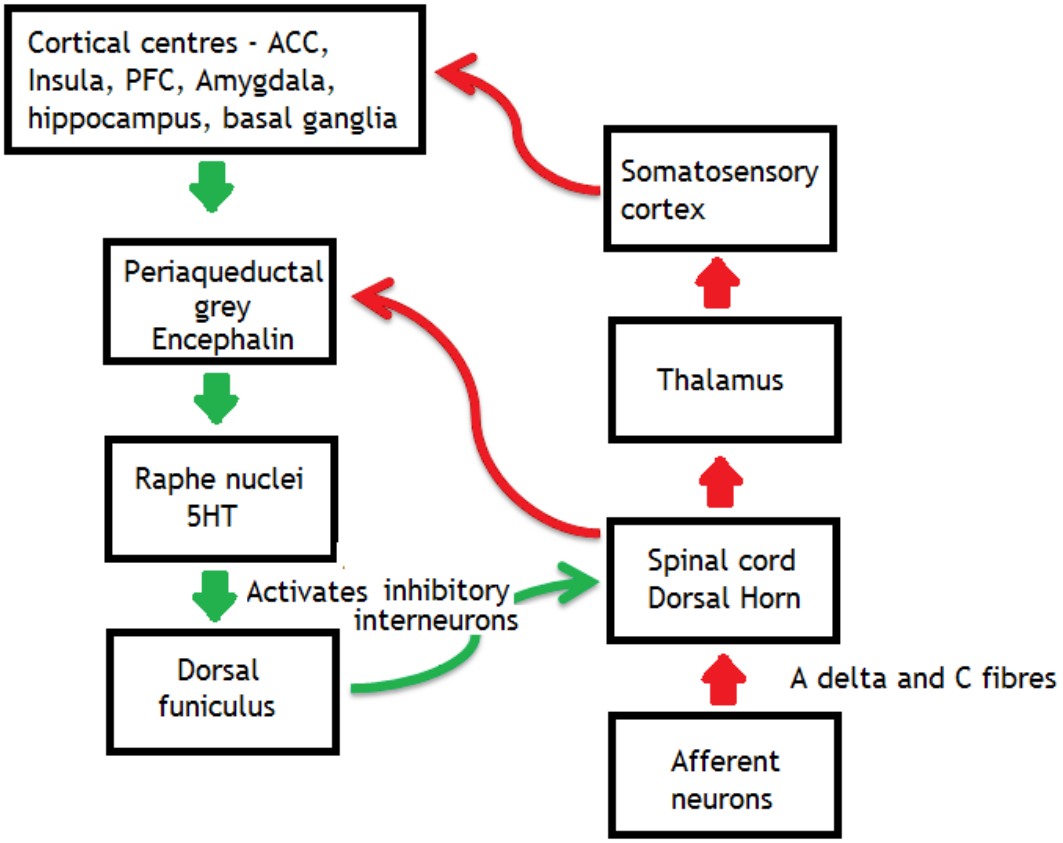


Figure 17 gives a simplistic representation of the afferent and modulatory pain pathways. Afferent neurons (A delta and C fibres) from the joints and the skin, contain nociceptors can be stimulated by inflammatory mediators like prostaglandins and cytokines(Reddi and Curran, 2013). These fibres synapse with secondary afferent neurons in the dorsal horn of the spinal cord. The primary fibres release a number of excitatory neurotransmitters including glutamate and substance P. They ascend through the spinothalamic and the spino-reticular pathways to the thalamus and third order neurons from thalamus pass on the primary somatosensory areas of the brain. These ascending pathways also project to the periaqueductal and raphe nuclei in the brain stem/midbrain. Pain also activates a number of other cortical and subcortical regions of the brain. Inhibitory pathways from the peri-aqueductal gray and the dorsal raphe nuclei, descend along the dorsal funiculus, to activate the inhibitory interneurons in the dorsal horn. These in turn interact with the afferent fibres, thereby inhibiting

further ascending neurotransmission. It can be seen that serotonin plays a major role in pain inhibition.

The reduction in pain in response to anti-inflammatory drugs could either be due to reduced stimulus of the nociceptor at the peripheral level due to reduction in inflammatory stimulus of the nociceptor, or it may be a direct central action through serotonin receptors. Anecdotal evidence say that serotonergic antidepressants have analgesic and anti-inflammatory effects. In addition, recent fMRI findings have directly examined the effect of TNF-α blockade on pain pathways in the brain(Hess et al., 2011). They suggest that the rapid reduction in pain may be modulated through pain pathways in the brain. In our data, it was difficult to interpret the relationship between changes in inflammatory markers and changes in SERT and behavioural measures.

Critchley and Harrison suggest that inflammation may communicate with centres in the brain though neural interoceptive pathways (Critchley and Harrison, 2013). These pathways include those regions that are consistently activated in pain paradigms. Therefore in conditions where pain and inflammation coexist, the neural basis of pain and inflammatory signalling are difficult to differentiate. It should be noted that all patients were also taking other NSAIDS as required, and this may have affected their pain scores and may have interfered with anti-inflammatory levels.

7.5.5 Fatigue and central serotonin

Fatigue is a common but debilitating symptom in inflammatory conditions (Capuron and Miller, 2011). While peripheral fatigue is due to neuromuscular factors, central fatigue is thought to represent a failure to complete physical and mental tasks that require self-motivation and internal cues in the absence of cognitive failure and motor weakness (Chaudhuri and Behan, 2000). Fatigue is commonly associated with sickness behaviour associated with infections, inflammatory conditions and also as a part of depressive illness (Capuron and Miller, 2011).

FACIT scores correlated with BDI scores and Pain. However, this did not reach statistical significance. Similarly, BDI scores correlated with pain scores. The

correlations suggest that they were associated, however, not fully explained by each other. Similarly inflammatory markers did not correlate significantly with BDI scores, Pain scores or Fatigue scores, suggesting that disease activity as measured using these inflammatory markers, did not significantly predict these clinical variables. It should be noted that circulating inflammatory markers have been shown to serve as a good measure of disease severity in both psoriasis with and without arthritis (Beygi et al., 2013, Dowlatshahi et al., 2013, Enerback, 2011, Fitzgerald and Chandran, 2012, Strober et al., 2008)

Although central serotonergic pathways have been linked to fatigue, the literature is not very clear about the relationship between the two. Studies in humans suggest that central fatigue correlates with an increase in serotonin in the CNS. This is somewhat counterintuitive, as 5HT is thought to boost the activity of motor neurons in the CNS.

Cotel et al suggest that the central component of the fatigue induced by serotonin is thought to be mediated by serotonergic 5HT1A receptors present in the axon initial segments (AIS) of the motor neurons(Cotel et al., 2013). They showed that focal activation of these receptors, led to inhibition of the action potential in the motor neurons. However, they also found that the AIS segments are not innervated by 5HT neurons. They found that the activation of AIS 5HT1A receptors occur only on repeated stimulation of the raphe nuclei, which leads to a spill over effect of excess serotonin into the AIS regions. In general, they suggest that during low activity, 5HT promotes the excitability of motor neurons, but during prolonged activity, this can lead to a spill over effect on the AIS, leading to greater fatigue (Cotel et al., 2013).

However, not all studies are consistent with this theory. The effect of cytokines on the relationship between serotonin and fatigue was recently examined by Katafuchi et al (Katafuchi et al., 2006). They showed that induction of fatigue by intraperitoneal injection of poly I:C in rats, led to an increase in interferon alpha expression in the brain and an increase in 5HTT expression. This was directly related to a reduction in serotonin levels in medial prefrontal cortex. This was shown to be linked to fatigue in these rats. In addition, they showed that a 5HT1A agonist reversed the fatigue in these rats. Our findings resonate with that of Katafuchi et al, in that we found an association between inflammation and

SERT and fatigue, and treatment with anti-TNF-α agent led to a reduction in fatigue. However, it is not clear whether the reduction in fatigue was part of a general improvement in mood, or an independent reduction. Although the correlations did not reach statistical significance, there seems to be significant overlap between the two.

In summary, I have shown a significant association between circulating TNF-α and central SERT levels. Greater circulating TNF-α was associated with greater SERT. In addition, the greater SERT levels mediated the relationship between inflammation and depression scores (but not fatigue or pain scores). Treatment with Etanercept was associated with a reduction in SERT levels. Future experimental studies will explore the relationship between inflammation and SERT in those who are being treated with interferons or LPS.

Chapter 8 – General Discussion

In this chapter, I will first summarise the findings of all the above chapters. Then I will discuss findings of recent studies that have explored the relationship between circulatory inflammatory markers in the context of mental illnesses.

8.1 Summary of findings presented in this thesis

In the first chapter, I reviewed the literature proposing an association between circulating inflammatory markers and the brain that may contribute to the pathophysiology of major mental illnesses, particularly major depressive disorder. I discussed findings from preclinical and clinical studies that suggest psychiatric illnesses, particularly major depressive disorder, are associated with inflammatory processes. Here I discussed that there is now evidence to suggest that inflammation may play a subtle role in the pathophysiology of major depressive disorder. Most of the evidence that links inflammation to major depressive disorder comes from three observations - a) one third of those with major depression show elevated peripheral inflammatory biomarkers, even in the absence of a medical illness; b) inflammatory illnesses are associated with greater rates of major depressive disorder; c) patients treated with cytokines are at greater risk of developing major depressive illness. I explored theoretical mechanistic pathways through which inflammatory mediators have been found to affect various substrates thought to be important in the aetiopathogenesis of major depressive disorder. These included an altered monoamine (serotonin and dopamine) and glutamate neurotransmission, glucocorticoid receptor resistance and adult hippocampal neurogenesis. At a higher level, I discussed how inflammation might affect brain signalling patterns, cognition and the production of a constellation of symptoms, termed “sickness behaviour”. I discussed findings, which suggest, how inflammation may play a role in the aetiology of depression, at least in a “cohort” of vulnerable individuals. I discussed potential pathways through which inflammation may contribute to the development of major mental illness in high-risk conditions - socioeconomic deprivation and general medical conditions. Both these conditions have been

associated with greater inflammation, and greater psychiatric morbidity, particularly major depressive disorder.

In the further chapters, I went on to present findings pertaining to observational analyses of the relationship between circulating inflammatory markers and structural MRI of the brain.

In chapter 3, I presented the results of my first observational analysis. I aimed to explore the relationship between cardio-metabolic risk factors and cortical thickness in a neurologically healthy middle aged population-based sample. T1-weighted MRI was used to create models of the cortex for calculating regional cortical thickness in adult males, selected from the PSOBID study. I examined the association between cardio-vascular risk markers – classic risk markers like BMI and lipid markers, and "emerging" markers like inflammatory markers - and cortical thickness across the whole brain, using the general linear model. I found that greater C reactive protein (CRP) and Intercellular adhesion molecule (ICAM-1) levels were associated with cortical thinning pertaining to perisylvian regions in the left hemisphere.

In chapter 4, I extended my findings from chapter 3. I examined if greater inflammatory markers mediated the relationship between neighbourhood deprivation (a high risk condition for mental illnesses) and the brain. I first compared the cortical morphology of neurologically healthy adult men from the least deprived and most deprived neighbourhoods of Glasgow. I demonstrated that the most deprived group had significantly smaller left posterior parietal cortex surface area and fusiform cortex surface area. They also had thinner left Wernicke's area and its right homologue. I then used mediation analysis to demonstrate that a composite factor comprised of circulating inflammatory markers mediated the relationship between neighbourhood deprivation and cortical thickness pertaining to the Wernicke's area. Here, I provided some preliminary evidence for a mechanistic pathway that may explain the relationship between neighbourhood-level socioeconomic deprivation and the brain.

In chapter 5, I used complex network analysis using graph theory to explore the relationship between neighbourhood deprivation, inflammation and the

structural properties, of cortical thickness covariance brain networks in the above sample. The premise of the analysis was that complex cognitive functions are widely recognized to be the result of a number of brain regions working together as large-scale networks. Here, I aimed to examine the association between neighborhood level deprivation and brain network structure (organisation) – modularity and grey nodes. I compared the structural properties of cortical thickness covariance networks derived from the same groups as described in chapter 4. I demonstrated that for a given number of modules, networks derived from the least deprived group had stronger modules and had greater proportion of grey nodes – a measure of overlapping modular structure. In other words, the structural organization of the cortical covariance brain networks from the least deprived group was consistent with a more robust and efficient information processing system compared to that of the least deprived group. More interestingly, I demonstrated that once the cortical thickness covariance networks were constructed after accounting for differences in circulating inflammatory markers, the difference in structural properties of between the groups disappeared. In other words, the structural difference was driven by differences in inflammatory markers. This is a novel finding, and one of the first studies to show an association between socioeconomic deprivation and brain network structure. In addition, I suggest that differences in circulating inflammatory markers may drive the difference in network structure in the most deprived group.

In chapter 6, I examined the potential pathways that mediate the relationship between socioeconomic deprivation and limbic stress pathways. Here, using the same sample as in the previous chapters, I investigated whether neighbourhood-level deprivation was associated with volumetric differences in a group of limbic forebrain structures -hippocampus, amygdala and medial prefrontal cortex - implicated in the top-down regulation of the stress response. I demonstrated that greater neighbourhood deprivation was associated with smaller grey matter volumes in the right hippocampus and right ventromedial and orbitofrontal cortices. I also showed that circulating inflammatory markers mediated the relationship between neighbourhood-level deprivation and the right ventromedial and orbitofrontal cortex volumes, but not hippocampal volumes. I discussed how inflammatory pathways may serve as a potential mechanism

underlying the theory of biological embedding of adverse physical and social environments.

In chapter 7, I presented the findings of an experimental study that explored the relationship between circulating inflammatory markers and serotonin transporter in the brain. The rationale for the analysis was the findings from a number of preclinical studies that have shown a direct relationship between inflammatory markers and serotonin transporter in the brain. Of particular interest in this analysis was the trafficking dependent mechanisms mediated through MAPK dependent pathways. I explored the relationship between circulating proinflammatory cytokines and serotonin transporter availability in the midbrain of 12 patients with psoriasis/psoriatic arthritis. In a group of patients suffering from psoriasis / psoriatic arthritis, I demonstrated that those who had greater circulating TNF-α had greater midbrain serotonin transporter availability. Although circulating TNF-α accounted only for a very small variance in depression scores, I found that the relationship between TNF-α and depression scores was mediated by greater serotonin transporter availability. In the next step of the analysis, I demonstrated that experimentally blocking the action of TNF-α (using Etanercept) was associated with a reduction in SERT availability. Here, I present some of the first evidence that show an association between inflammatory markers and SERT availability in humans. These findings provide a mechanistic pathway through which inflammatory pathways contribute to the pathogenesis of depression.

8.2 Limitations of the studies

With regards the findings from the observational study (chapter3,4,5 and 6), while the positive feature of the study was that I examined a well-characterized community based cohort, there are a number of limitations to be acknowledged.

The cross-sectional design means that causal inferences cannot be made. This is firstly true with regards the relationship between socioeconomic deprivation and cortical parameters. While theoretically it is possible that greater deprivation, due to various adverse factors associated with it, led to changes in cortical parameters, it could be argued that differences in cortical parameters were present even before the exposure to neighbourhood deprivation. Longitudinal

studies are required to see if these differences are present early in life, and whether they progress over the life course. Indeed, some of the recent longitudinal studies have confirmed an effect of childhood poverty on the brain and cognitive functioning in adulthood (Evans and Schamberg, 2009, Kim et al., 2013). This limitation is also crucial when considering the association between inflammation and cortical thickness. For example, throughout the literature review and discussions, I have made the assumption that inflammation drives the cortical/ subcortical parameters. However, such a causal assumption is rather speculative. Theoretically, even though this may be the direction of causality, the direction could be the other way. For example, cortical parameters can control inflammatory markers. It is known that the "inflammatory reflex", central autonomic networks and the stress (HPA axis) can have a significant impact on circulating inflammatory markers (Olofsson et al., 2012, Ulrich-Lai and Herman, 2009). Therefore, it is possible that cortical thickness/ volumes pertaining to a number of regions in the brain may influence circulating inflammatory markers. Even if we assume a direct effect of circulating inflammatory markers on cortical parameters, it is not clear how these changes are signalled. Potential pathways have been discussed in the introduction chapter, however, data from human studies are lacking (Capuron and Miller, 2011). Therefore, a number of questions remain. Do circulating inflammatory markers provide a good marker for central markers? At least in humans the relationship does not seem to be straight forward.

The observational study, reported in chapters 3, 4, 5 and 6 included only males. The rationale behind this was to decrease variability in cortical thickness that could be attributable to gender (Sowell et al., 2007). Similarly, cardio-metabolic risk factors co vary significantly with gender. In the prospective multi-ethnic study of atherosclerosis, inflammatory markers like CRP levels have been found to be significantly higher in women compared to men. This gender difference was present even after taking into account BMI and oestrogen use (Lakoski et al., 2006). Similarly, during the third and fourth decade of life, cholesterol levels show a sharper increase in men than in women. In addition, the same cardiovascular risk factors may impact differently on men and women. For example, HDL cholesterol and Triglycerides have been found to have a greater impact on cardiovascular disease risk in women compared to men (Roeters van

Lennep et al., 2002). However, this meant that the findings of the study are less generalisable to the population. The parent study – PSOBID - was designed to explore the differences in a number of biomarkers between the least and most deprived groups with the greatest power(Velupillai et al., 2008) . Therefore, the nature of the sampling technique used for recruiting subjects led to a bimodal distribution of a number of independent variables used in this study. Indeed, the mean cortical thickness (contributed by the DV) was normally distributed. Nevertheless, a sampling technique with equal distribution from all socioeconomic status with a larger sample size may have been more appropriate, in order to explore a dose dependent relationship between cardio-metabolic risk markers and cortical thickness.

In chapter 3, with regards multiple testing correction, we used a cluster wise approach when examining the relationship between risk markers and cortical thickness. This approach has been validated and used in recent studies exploring variance in cortical thickness.(Ehrlich et al., 2012, Leritz et al., 2011) We opted for this approach, as the primary aim of the study was to look for any evidence for a "signal" in the cortex that could be attributable to the risk markers. For this, a cluster wise approach was thought to be sensitive and appropriate.(Poldrack et al., 2011) In this study, I was not primarily interested in the spatial specificity or location of the significant clusters, and hence did not opt for a voxel wise approach. While this is not a limitation per se, when I repeated the analysis using a voxel wise FDR (0.05) approach for multiple testing corrections, none of the above regions survived the FDR correction. In other words, the test statistic at none of the voxels crossed the required significance threshold after multiple testing corrections using the FDR procedure. This could be either due to the small sample size or may be because the association between the risk markers and the cortical thickness is spread across a large area of the cortex, but the magnitude of the relationship is modest at best. Previous cross sectional studies suggest that cerebrovascular disease may mediate the relationship between cardiovascular risk factors like diabetes and hypertension and cortical thickness in cognitively impaired individuals. (Seo et al., 2012a, Seo et al., 2012b) It is therefore possible that the relationship between high CRP and ICAM and cortical thinning in our study was mediated by the presence of cerebrovascular pathology. None of our participants showed significant

pathology on the scans. Moreover, the small sample size and the cross sectional nature of our data precludes us from making any meaningful conclusions regarding the role of causal mediation or mediators pertaining to cerebrovascular pathology. In addition, greater LDL and TAG factor levels (generally associated with greater cardiovascular risk) were associated with greater cortical thickness. It is unlikely that this relationship was mediated by cerebrovascular pathology in our sample.

The sample size of the observational studies were relatively small. This may have led to potential type 2 errors. For instance, in chapter 4 and 6, a number of comparisons that had an effect size traditionally considered to be medium to large did not survive multiple testing corrections. It is possible that studies with greater power would have identified many of these relationships as statistically significant. It should however be noted that the groups did not differ in global measures of cortical morphology. In addition, the direction of relationship between SES and cortical morphology was not always consistent. For example, although the right posterior parietal SA was significantly smaller in the most deprived group, the posterior parietal cortical thickness was greater (not statistically significant) in the most deprived group. While this finding is in keeping with Hogstrom et al who observed a negative correlation between cortical thickness and surface area throughout the life span, these findings suggest that cortical morphology is indeed a complex construct and that the relationship between SES and the cortex could be differential in terms of morphology and direction of relationship (Hogstrom et al., 2012).

While the use of an extreme sampling technique led to a bimodal distribution of most of the variables, the mean cortical thickness (the dependent variable) did not differ between the SES groups. Therefore, the assumptions of a GLM analysis were not violated. However, most independent variables differed between the two deprivation groups. This may have led to an inflation of effect sizes in the observational studies (chapter 3,4,5 and 6). Extreme group sampling (as in the observational studies) may have potential effects on the significance, reliability and generalizability of the true effects of interest(Gianaros and Hackman, 2013).In addition, small samples can affect the statistical power to detect between-individual effects, especially when multiple comparisons are used. Yarkoni et al in a recent review, suggest that a combination of small sample size

and stringent alpha thresholds may inflate the effect sizes of relationships (Yarkoni, 2009).This is a limitation of our study. Future studies exploring these relationships should include larger sample sizes, and include participants from all socioeconomic classes.

With regards the limbic stress networks, given the lack of information on any experience of childhood trauma or other individual significant life event, which has been reported to be associated with hippocampal atrophy (McCrory et al., 2010), I cannot exclude that these individual stressful life events may be partly account for the finding. So, for example, the relationship between neighbourhood deprivation and hippocampal and orbitofrontal cortex volumes could be because those from the poorest neighbourhood are at greater likelihood of experiencing life greater life events. Indeed, there is evidence to show that individual life events may explain variance in hippocampal volumes even after accounting for socioeconomic status (Teicher et al., 2012). I did not explore the components and pathways within the construct of SES that could have explained the variance in cortical parameters. The main aim of my thesis was to explore the relationship between circulating inflammatory markers and cortical parameters. In the context of SES, my analysis emphasised what I thought was the most proximal (biologically closest to cortical parameters) biomarker (i.e. circulating inflammatory marker) that could mechanistically account for the relationship between SES and the brain. With regard to the specificity of structural brain changes to neighbourhood-level SES, it has been argued that without controlling for individual socioeconomic variables, neighbourhood-based socioeconomic measures serve to proxy for unmeasured aspects of individual socioeconomic characteristics (Geronimus and Bound, 1998). Nonetheless, another view posits that measures of neighbourhood-level SES may be a more stable marker of lifelong accumulated wealth then early and adult individual-level SES (Oakes, 2004).

Three subjects from the most deprived group and 1 person from the least deprived group were prescribed psychotropic medications during their lifetime. Although it can be argued that mental illness and its treatment are components of deprivation, removing these individuals from the analysis did not change the findings in chapters 3,4,5 and 6. None of our participants were acutely medically unwell, nor had they undergone recent surgery. However, those with chronic

physical illnesses (other than neurological conditions) were not excluded. This may have contributed to significant variance in inflammatory and cardiovascular markers. The small sample size meant that stratifying the sample further to examine these effects was not possible (Nicol et al., 2012).

There are a number of methodological challenges when exploring the relationship between SES and the brain. Hackman and Farah have enumerated these in a recent review(Hackman and Farah, 2009). Of particular concern is the separation of what constitutes confounding factors and what constitutes mediators of the effect of SES. For example, in our study, we considered inflammatory markers as mediators of the effects of SES on the brain. However, we did not explore the effects of alcohol use or cigarette smoking. It is very well know that alcohol and cigarette smoking can have an effect on cortical thickness and subcortical volumes (dependent variable). It is also known that alcohol use and cigarette smoking can covary significantly with SES (the independent variable). In addition, these factors may potentially lie in the causal pathway linking SES to brain structure. Miller and Chapman suggest that when comparing groups, including variables that covary with the independent variable (SES in our case) may be inappropriate (Miller and Chapman, 2001). It is therefore imperative to decide which of these factors are mediators and which are confounding factors. In addition to this, many potential mediators covary with each other, and it is difficult to separate out the effect of individual mediators. Another potential problem is sample selection. Do we include participants with physical and mental ill health in our sample? This is of particular concern, as we know that physical and mental ill health covaries significantly with SES (Srireddy et al., 2012). While excluding may increase the internal validity of the study, excluding these participants would decrease the external validity and generalizability of our findings(Hackman and Farah, 2009). Exploring the effect of confounding factors also requires large sample sizes.

The experimental study, presented in chapter 7, also had a small sample size. I calculated the required sample size based on the relationship between TNF-α blockade and SERT availability. However, the small sample size may have led to reduced power in calculating the correlations between circulating inflammatory markers and SERT. However, it should be noted that the presence of an association even with small numbers suggests that these results may be non-

trivial. Perhaps more importantly, a limitation of this study was the absence of a control subject group. Firstly, a healthy control group that is matched for age and sex would have enabled us to compare SERT availability in psoriasis/psoriatic arthritis patients, with that of healthy controls at baseline. Although we showed an association between TNF-α and SERT availability in the patient group, we do not know if the relationship will be present in the healthy population. Our group was a mixed group consisting of PS and PSA patients who had quite severe illness and were going to be treated with etanercept, hence, these effects may not be generalised to those with a milder illness or to the general population. This is important, as TNF-α often exerts its effect in an inverted U shape fashion. Therefore, while an adequate level of TNF-α may be necessary for normal functioning, a lower or greater than normal level may be detrimental. However, administering Etanercept to healthy controls would have been both ethically and clinically difficult. A second possible control group would have examined the role of a placebo or another anti-inflammatory medication, firstly to see if the reduction in SERT is significantly greater than that would have achieved with placebo and also to see if the effect was specific to TNF-α blockade, compared to other antiinflammatory drugs/ SSRIs. Technical difficulties prevented us from completing the originally intended study of examining the effect of other disease modifying agents. Although Beta CIT has been used and validated in examining the SERT, there are better ligands now available that are more selective for SERT.

I refer to the study presented in chapter 7 as "experimental", as there was manipulation of circulating inflammatory markers - using TNF-α blockade- to demonstrate changes in SERT availability. Here, I attempted to show a direction of causality. That is a direction from circulating inflammatory markers to central SERT. However, this does not rule out a bidirectional relationship. In other words, central SERT can in turn have an effect on circulating inflammatory markers. For example, previous research has shown that 5HT2A receptor activation suppresses TNF-α induced inflammation with extraordinary potency (Yu et al., 2008). Another concern is whether the change in SERT availability was an epiphenomenon, rather than a direct effect of the manipulation of circulating inflammatory markers. Although treatment with Etanercept was associated with a reduction in SERT and improvement in mood, it should be noted that

inflammation and pain, also significantly improved with the treatment. It could therefore be argued that rather than there being a direct relationship between reduction in inflammation and reduction in SERT, the reduction in SERT was an epiphenomenon associated with improvement in the pain and the physical condition, thereby improving mood. It should be noted that we showed a significant association between TNF- α and SERT levels at baseline. This is very much in keeping with preclinical studies.

It should also be noted that neither the reduction in pain, nor the reduction in CRP (a measure of disease activity) correlated significantly with the reduction in SERT. It should also be noted that the relationship between SERT and other markers of inflammation were not significant at baseline, suggesting that the relationship may have been unique to TNF-α, at least in my sample. However, it was not meaningful to examine the relationship between change in TNF-α and change in SERT, as the assay measured both active and inactive TNF-α in circulation, and therefore, would have not given a good picture of the relationship. Interestingly, although there was a reduction in SERT availability following TNF-α blockade, there was no association between reduction in inflammation and reduction in SERT availability. This could also be due to the fact that most circulating inflammatory markers did not show a reduction following treatment with etanercept. Only hsCRP showed a reduction. However, CRP levels did not significantly correlate with SERT even at baseline. Therefore, it is not surprising that change in CRP was not associated with changes in SERT. Interestingly, greater baseline TNF-α and IL1B also showed an association with greater SERT post treatment. This is in keeping with the fact that these two cytokines did not show significant reductions with treatment with etanercept.

8.3 Update on research

Since the start of this PhD project, a number of studies have examined the relationship between inflammation and depression. In this section, I will provide a brief and selected update. Firstly, I will review an update on the relationship between inflammatory markers and major mental illness/brain (explored in chapters 3,4, 5 and 6). Secondly, I will review research that has added evidence for the use of TNF-α blockade in the treatment of potential major

mental illness and if there is a potential for inflammatory markers to help stratify major depressive disorder, in terms of treatment response.

8.3.1 Relationship between inflammation and major mental illness

As presented in the review of literature, a number of individual studies, and at least 3 meta-analyses of cross sectional studies have shown an association between raised circulating inflammatory markers (CRP and proinflammatory cytokines) and major depression (Dowlati et al., 2010, Howren et al., 2009, Liu et al., 2012a). In addition to the traditional circulating markers of inflammation - CRP and cytokines, recent studies have also examined the role of other potential markers of inflammation, and depression. For example, Naude et al examined the determinants of plasma neutrophil gelatinase-associated lipocalin (NGAL) - a protein thought to be a neuro-inflammatory marker that is triggered by TNF-α 1 receptor signaling (Naude et al., 2013). They examined plasma levels of NGAL in older patients who are depressed. They found that depressed patients had significantly higher NGAL compared to healthy controls. They also found that those who had partially remitted had lower levels of NGAL and those with a history of recurrent depression had greater levels, compared to those who had just one episode. These levels were not affected by antidepressant medication or age of onset. Heringa et al examined the relationship between a composite score of serum inflammatory markers including CRP, SAA, IL6, IL8, TNF-α, ICAM and MPO on cognitive function in people aged 50 and 75 years (Heringa et al., 2014). They found that the composite measure of inflammation predicted poor cognitive function including information processing speed, attention and executive function, cross-sectional and prospectively.

Although a number of classic and new inflammatory markers have been linked cross-sectionally to depression, the question remains as to whether there is a causal relationship between raised inflammatory markers and major depression or even a subset of the syndrome. A few prospective studies have directly examined the relationship between depressive symptoms and inflammation. However the results are not conclusive. For example, Gimeno et al, using the Whitehall II study cohort (around 3000 participants), examined if inflammatory markers (IL6 and CRP) measured at baseline predicted cognitive symptoms of depression eleven years later (Gimeno et al., 2008, Gimeno et al., 2009). They

found that after adjusting for sociodemographic, behavioural and biological risk factors, health condition, medication use and baseline depressive symptoms, both IL6 and CRP predicted cognitive symptoms at follow up. However, in a more recent study, Stewart et al, examined the longitudinal associations between depressive symptoms and inflammatory markers (IL6 and CRP), in 263 healthy older men and women, over a period of 6 years (Stewart et al., 2009). They found that baseline BDI scores predicted changes in IL6 after 6 years. However, baseline IL 6 levels did not predict 6 year change in BDI scores. They concluded that depressive symptoms may precede and augment inflammatory processes, and that this may be a potential pathway through which depression may contribute to increased cardiovascular risk.

In order to reach a consensus, Valkonoya et al undertook a systematic review of longitudinal studies (including the study by Stewart et al) to investigate whether raised circulating inflammatory markers - CRP and IL6- was indicative of the development of subsequent depressive symptoms (Valkanova et al., 2013). They examined the relationship between CRP and IL6 on the subsequent development of depressive symptoms. They found a significant association between CRP and depressive symptoms at follow up (adjusted r=0.05). The relationship at best was thought to be modest (yet significant). As shown in Table 8-1, their results suggested that raised inflammatory markers precede the development of depressive symptoms. However, the pathways that lead from inflammation to depression remain unclear. It still remains unclear if circulating inflammatory markers represent an association, a mediating or a causal factor for depression. The relationship between circulating inflammatory proteins and those in the brain is also opaque. Their study also found moderate degree of heterogeneity among studies as well as some level of publication bias, suggesting that negative studies may have been under-reported. Once again, most of the studies examined depressive symptoms using validated scales, and not a structured clinical interview to diagnose major depression. This leaves us with a question if there are specific symptoms that are predicted by greater inflammation.

Table 8-1: Meta-analysis of longitudinal studies examining relationship between circulating inflammatory markers and depression.

	Sub groups	N studies	Correlation (weighted mean mixed effects model) 95% CI	p-value	Heterogeneity		
					Q-value	p-value	I-squared
CRP		6	0.069 (0.036 - 0.103)	<0.0005	19.23	0.002	74
CRP*		7	0.046 (0.021 - 0.07)	<0.0005	11.22	0.08	46.5
	old	3	0.079 (0.012 - 0.144)	0.02	5.7	0.06	64.89
	young	4	0.03 (0.01 - 0.05)	0.003	3.19	0.36	6.09
IL-6		2	0.045 (0.013 - 0.078)	0.007	0.54	0.46	0
IL-6*		3	0.097 (-0.005 - 0.198)	0.06	5.2	0.08	61.37
CRP—C-Reactive protein; IL—6-Interleukin-6; * fully adjusted (e.g. body-mass index, smoking, use of medication, chronic illness etc.).							

The weighted-mean effect sizes of studies and measures of heterogeneity of meta-analysis of longitudinal studies that have examined the relationship between raised circulating inflammatory markers and depression in longitudinal studies (Reused with permission from Valkanova, et al. CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. Journal of Affective Disorders, Volume 150, Issue 3, 25 September 2013, Pages 736–744)

More recent studies have tried to examine whether the link between inflammatory markers and depression are driven by somatic symptoms of depression. Considering that low-grade inflammation is perhaps a link between depression and adverse outcomes in a number of medical conditions, Duivis et al, explored if the association with inflammatory markers were primarily driven by the somatic symptoms of depression and anxiety (Duivis et al., 2013). In a cohort of 2861 participants from the Netherlands Study of Depression and Anxiety, they examined if there was a differential association of somatic and cognitive symptoms of depression with inflammation. They used linear regression to examine the association between circulating inflammatory markers – CRP, IL6

and TNF-α . They found that the association between inflammatory markers and depressive symptoms were indeed driven by the somatic symptoms.

While all the studies mentioned above examined the association between circulating inflammatory markers and depression, few studies have examined CSF cytokine variations associated with depressive illness in humans. This of course is difficult to examine. More than a decade ago, Levine et al (1999) examined CSF concentrations of cytokines in 13 un-medicated patients admitted with acute depression (Levine et al., 1999). Compared to a healthy control group, they found that those with depression had greater IL1B, but lower IL6 levels. They found no difference in TNF-α levels. Lindqvist et al more recently showed increased CSF IL-6 levels in suicide attempters compared to healthy controls (Lindqvist et al., 2009). They also found a significant association between IL6 levels and severity of depression (MADRS scores). What was most interesting in this study was that the authors did not find any association between CSF and plasma cytokine levels. This finding is in keeping with Lampa et al who found that patients with rheumatoid arthritis had greater levels of CSF IL1B compared to serum levels (Lampa et al., 2012). A fourth more recent study, Martinez et al, found no difference in CSF levels of inflammatory cytokines (IL1, IL6 and TNF-α a) between patients with depression and healthy controls. Interestingly they found that CSF IL1 levels correlated with a history of suicide attempts (r=0.53) (Martinez et al., 2012).

While the above studies have measured cytokines in the CSF, even fewer have directly measured cytokine expression in human brain. Dean et al examined if TNF-α was altered in the frontal cortices from 10 subjects with major depressive disorder and control subjects (Dean et al., 2010). They measured transmembrane and soluble TNF-α from the post-mortem tissue homogenates from the left hemisphere. They found that transmembrane TNF-α was significantly increased in BA 46 of people who had a history of depression. Similarly Shelton et al examined the gene profiles of cytokines in BA 10 from 14 subjects who had a history of major depressive disorder (Shelton et al., 2011). They found an up-regulation of a variety of pro- and anti-inflammatory cytokines in the region of interest. Steiner et al examined microglial HLA-DR expression using immunohistochemistry in the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), mediodorsal thalamus (MD) and hippocampus of

16 patients with schizophrenia, 14 with affective disorder and 10 matched healthy controls(Steiner et al., 2008). In addition, they included a subgroup of six patients with schizophrenia and seven patients with affective disorders who had committed suicide. While diagnosis was not associated with microglial density, suicide was associated with significant microgliosis in the DLPFC, ACC and thalamus. They claimed that this may represent pre-suicide stress, a causal link between the microglial activation and suicide remains speculative at best.

A few observational and experimental studies have examined the relationship between inflammation, depression and the brain using PET. Hannestad et al in their first study, showed that systemic inflammation induced by endotoxin in humans was associated with higher normalised glucose metabolism in the insula - a region thought to play a major role in inflammatory interoceptive pathways as well as key role in the central autonomic network (Hannestad et al., 2012b). This change was associated with change in peak cytokine levels and also changes in social interest, suggesting that these may be linked to each other (Hannestad et al., 2012b). Insula is also thought to play a key role as an outflow hub that sends control signals that activate frontoparietal networks in response to salient stimuli. These stimuli interestingly may be external (visual/auditory) or internal (interoceptive) stimuli. Deficient activation of insula has been shown in a recent meta-analysis of functional MRI studies of people with depression (Hamilton et al., 2012). More interestingly, the crucial role of insula as a biomarker of prediction of response was shown by McGrath et al, who found that reduced insula hypometabolism (relative to whole-brain mean) measured using FDG PET was associated with remission to cognitive behaviour therapy and poor response to escitalopram, while increased metabolism in the insula was associated with remission to escitalopram and poor response to cognitive behaviour therapy (McGrath et al., 2013).

Hannestad et al, using PET, for the first time showed that systemic inflammation induced by LPS was associated with increased binding of TSPO ligand [^{11}C]PBR28 in the brains of baboons (Hannestad et al., 2012a). They found that the increase in [^{11}C]PBR28 binding 4h after LPS administration correlated with serum IL-1ß levels at 2hours and with IL-6 and TNFα levels at 3 hours. They also found in the increase in [^{11}C]PBR28 expression occurred mostly in the microglia. Although experimental studies that induce an inflammatory state have found an

association between circulating inflammatory markers and neuronal activity in a number of regions implicated in depression, and depressive symptoms, the studies in major depression have not found consistent results (Capuron et al., 2012). For example, Hannestad et al, used positron emission tomography (PET) with [^{11}C]PBR28, to compare the level of TSPO between 10 individuals with mild to moderate depression and 10 healthy control subjects, matched for TSPO genotype (Hannestad et al., 2012a). They found no statistically significant difference in [^{11}C]PBR28 binding (VT) between the two groups. In fact, 7 of 10 individuals with depression had lower [^{11}C]PBR28 binding in all the regions of interests compared to the control subjects.

8.3.2 Anti-inflammatory medications (including TNF-α blockade agents) as a therapeutic option for major depression

Given the numerous links that have been shown to exist between inflammation and depression, it would be reasonable to surmise that treatments with anti-inflammatory agents may be beneficial in depression. There is tentative evidence that anti-inflammatory agents have antidepressant properties.

A number of recent animal studies have shown that TNF-α blockade is associated with improvement in depressive phenotype. Using a rat model of depression (repeated restrained stress), Krugel et al showed that treatment with etanercept significantly reduced the depression like behaviour induced by the repeated restrained stress. The effect was similar to that of imipramine, and significantly greater than that induced by ringer solution (Krugel et al., 2013). Karson et al examined the effect of infliximab in a chronic mild stress (CMS) model of depression in rats (Karson et al., 2013). They divided rats into three groups no stress, saline-CMS and infliximab-CMS. The latter two groups, which were exposed to CMS for eight weeks, were either administered saline or infliximab concomitantly during the eight-week period. After the eight week period, they found that the infliximab treated rats had decreased depression-like behavior compared to the saline treated group. Similarly, Bayramgurler et al examined the effect of Etanercept treatment on anxiety and depression-like behavior in rats (Bayramgurler et al., 2013). They found that etanercept significantly decreased immobility time as assessed with elevated plus maze and forced swim test. Their data suggest that etanercept treatment reduced the anxiety and

depressive-like behavior in rats even in the absence of chronic inflammation or stressful condition.

In humans, specifically, TNF-α blocking agents have been shown to improve mood, independent of improvement in the inflammatory condition. One of the first evidence is by Tyring et al who found that 55% of patients with psoriasis who were treated with etanercept showed a 50% reduction in Beck's depression inventory (BDI) scores compared to 39% on placebo, an effect-size comparable to antidepressants (Tyring et al., 2006). This improvement was found to be independent of improvement in psoriasis. Since then a few studies have explored this, which I have reviewed in chapter 7 (Table 7-12).

In a placebo controlled double blind cross over trial of 18 abstinent alcohol dependent male adults, Irwin et al found that a single dose of etanercept 25 mg produced significant decrease (normalisation) in the amount and percentage of REM sleep (Irwin et al., 2009). They also found that circulating concentration of soluble TNF-α receptor II, obtained 24 hours after the drug administration, correlated negatively with the percentage of REM sleep. They conclude that their data is consistent with the hypothesis that circulating TNF-α may have a physiological role in the regulation of REM sleep in humans, and that pharmacologically blocking the effects of TNF-α may lead to the normalisation of REM sleep in abstinent alcohol dependent patients. They suggest that the sleep disturbance in alcohol use disorder may be due to ethanol induced production and release of TNF-α into the circulation. This in turn, leads to rapid increase in the expression of brain TNF-α , which is then sustained in the brain, that leads to the sleep problems. However, what is not clear is, why do these patients have persistent increase in sleep disturbance even after abstinence from alcohol? One explanation is that the increase in brain expression of TNF-α is sustained. If that is so, how could etanercept (a large molecule that does not cross the blood brain barrier) have an effect on TNF-α in the brain.

In short, how peripherally administered Etanercept or any TNF-α blockade could cause changes in inflammation in the CNS is not clear. Bearing in mind that MDD is primarily an illness of the central nervous system, how these drugs bring about their antidepressant action is not clear (Krishnadas, 2010). At least in animal models, manipulating peripheral inflammatory cytokines have shown to reflect

changes in cytokine expression central nervous system. A possible mechanism is that the trafficking of immune cells that are already affected by HPA axis dysregulation into the CNS is being blocked by these anti-inflammatory agents. This area of research is however not explored as much. Tobinick et al have earlier shown that peri-spinal delivery of Etanercept is associated with rapid amelioration of cognitive deficits associated with Alzheimer's disease (Tobinick, 2010, Tobinick and Gross, 2008). Surprisingly, this finding has not had significant translational implication.

Raison et al examined if anti TNF-α treatment using Infliximab reduced depressive symptoms in patients with treatment-resistant depression, and if inflammatory marker levels predicted treatment response (Raison et al., 2013). They found that after 3 infusions of infliximab vs. placebo over a period of 12 weeks, there was no difference in depression scores between the two treatment groups. However, they found that those who responded to infliximab had a baseline concentration of hsCRP of more than 5mg/ l. 62% of those with a CRP of greater than 5 showed a response to infliximab, compared to only 33% who showed who showed a response to placebo. The symptoms that were more responsive to infliximab were anhedonia, psychomotor retardation, psychic anxiety, depressed mood and suicidal ideation. Baseline concentrations of TNF-α and its soluble receptors were also greater in infliximab responders. Patients showed a greater improvement of around 3.1 points on the HAM-D in the infliximab + antidepressant group, which 'corresponds to the average effect of antidepressants'. The number of needed-to-treat patients in the hs-CRP > 5mg/l group was 3.45 (as opposed to 8–10 for conventional antidepressants) (Undurraga and Baldessarini, 2012, Fond et al., 2013).

Na et al conducted a meta-analysis of studies that have examined the efficacy of adjunctive celecoxib treatment for patients with major depressive studies (Na et al., 2014). They found four relevant studies with 75 patients in the NSAID group and 75 on the placebo group. The celecoxib group showed greater mean changes in depression scores (weighted mean difference = 3.26) compared to the placebo group. Those in the celecoxib group were also more likely to show remission (OR=6.58) and response (OR=6.49) than placebo group. They suggest that NSAIDS like celecoxib could be promising treatment strategy for patients with depressive disorder.

Fond et al reviewed the effectiveness and tolerance of anti-inflammatory add on treatment for major depression (Table 8-2)(Fond et al., 2013). They reviewed the efficacy of drugs from four major antiinflammatory drug classes, particularly, COX inhibitors, TNF-α inhibitors and minocycline in depression. In particular, they reviewed the benefit/risk ratios of these groups of medications. With regards TNF-α blockade agents, they suggest that extreme care should be taken with regards the patient developing "survival compromising complication". These include immunosuppression related infectious diseases, neoplastic processes including lymphomas and hypersensitivity reactions. With regards cox-inhibitors, the major risks being serious complications like stroke, cardiovascular, raised liver enzymes, renal toxicity and gastrointestinal risks.

A continuing challenge in MDD is the lack of “stratification”, i.e. a clear way of classifying what is a highly heterogeneous disorder, in order to aid diagnosis or predicting treatment response. There has recently been an emphasis on the need to develop a “biomarker” panel for depression that aims to profile diverse peripheral factors, including cytokine levels and peripheral growth factors that may provide a “biological signature” that may help predict treatment response (Schmidt et al., 2011). Anti-inflammatory response has been associated with antidepressant effects, Persoons et al finding that those with Crohn’s and MDD with higher pre-treatment CRP levels, and had greater remission from MDD with infliximab (Persoons et al., 2005).

Some studies that have assessed the usefulness of cytokine levels in predicting treatment response in depression. Some of these studies have shown interesting patterns of findings(Janssen et al., 2010). O’Brien et al. found that raised pre-treatment plasma levels of IL-6 and TNF-α were suggestive of poor response to antidepressants (O'Brien et al., 2007). Similarly, Lanquillon et al. found greater pre treatment IL-6 levels was associated with treatment resistance (Lanquillon et al., 2000). Eller et al found that higher levels of TNF-α predicted non-response to treatment with escitalopram (Eller et al., 2008). In a more recent study that combined pharmacogenetics and imaging, Baune et al found an association between rs114643 variant of the IL1B gene and non-remission after antidepressant treatment and decreased amygdala and ACC function(Baune et al., 2009).

Table 8-2: Summary of clinical trials (open-label and double-blind randomized placebo controlled trials (RCT)) on add-on therapy of anti-inflammatory drugs in major depressive disorder (MDD)

Author	Study design	Treatment + trial duration	Outcome measures	Results
(Abbasi et al., 2012)	RCT *N* = 40 patients with HAM-D score > 17	Sertraline 200 mg/day + (celecoxib 400 mg or placebo) 6 weeks	Serum IL-6 concentrations at baseline and week 6, HAM-D scores at baseline and weeks 1, 2, 4, and 6	The celecoxib group showed significantly greater reduction in serum IL-6 concentrations (mean difference (95%CI) = 0.42(0.30–0.55) pg/ml, t(35) = 6.727, *P* < 0.001) as well as HAM-D scores (mean difference (95%CI) = 3.35(1.08–5.61), t(38) = 2.99, *P* = 0.005) than the placebo group. The patients in the celecoxib group experienced more response (95%) and remission (35%) than the placebo group (50% and 5%, *P* = 0.003 and 0.04 respectively). Baseline serum IL-6 levels were significantly correlated with baseline HAM-D scores (*r* = 0.378,*P* = 0.016). Significant correlation was observed between reduction of HAM-D scores and reduction of serum IL-6 levels at week 6 (*r* = 0.673, *P* < 0.001)
(Muller et al., 2006)	RCT *N* = 40 patients with acute MDD	Reboxetine 4 mg+ (Celecoxib 400 mg or placebo)	HAM-D	The celecoxib group showed significantly greater improvement compared to the reboxetine-alone group. (*F* = 3.220; df 2.434; *P* = 0.035)

Author	Study design	Treatment + trial duration	Outcome measures	Results
		6 weeks		
(Akhondzadeh et al., 2009)	RCT *N* = 40 out-patient with MDD (baseline HAM-D score > 17)	Fluoxetine 40 mg + (Celecoxib 400 mg or placebo) 6 weeks	HAM-D (weeks 0,1,2,4,6)	The combination of fluoxetine and celecoxib showed a significant superiority over fluoxetine alone in the treatment of symptoms of major depression. The difference between the two treatments was significant at the endpoint (week 6) (t = 3.35, df = 38, P < 0.001). There was a significant difference between two treatments in terms of the percentage of responders (at least 50% reduction in the HAM-D score) (celecoxib group: 90%, 18 of 20 and placebo group: 50%, 10 of 20; P < 0.01). Thirty-five per cent of the patients in the celecoxib group and 5% in the placebo group were remitted after 6 weeks (HAM-D < 7). The difference was significant (P = 0.04). There were no significant differences in the two groups in terms of observed side-effects
(Mendlewicz et al., 2006)	Open-label *N* = 24 non-responder patients with MDD	SSRI + aspirin 160 mg/day 4 weeks	HAM-D	The combination SSRI-ASA was associated with a response rate of 52.4%. Remission was achieved in 43% of the total sample and 82% of the responder sample. In the responder group, a significant improvement was observed within week 1 (mean HAM-D at day 0 = 29.3 ± 4.5, at day 7 = 14.0 ± 4.1;P < 0.0001) and remained sustained until day 28. These

Author	Study design	Treatment + trial duration	Outcome measures	Results
				preliminary results are in favour of an accelerating effect of ASA in combination with SSRIs in the treatment of major depression
(Raison et al., 2013)	RCT *N* = 60 out-patients with stable depression (37 with antidepressant and 23 medication free)	Three infliximab administrations (week 0,2,6) 12 weeks	HAM-D at weeks 0 (baseline), 1, 2, 3, 4, 6, 8, 10 and 12. -high-sensitivity C-reactive protein (hs-CRP), TNF-α, and its soluble receptors at weeks 0 (baseline), 1, 2, 3, 4, 6, 8, 10 and 12	No overall difference in change of HAM-D scores between treatment groups across time was found. However, there was a significant interaction between treatment, time, and log baseline hs-CRP concentration ($P = 0.01$), with change in HAM-D scores (baseline to week 12) favouring infliximab-treated patients at a baseline hs-CRP concentration >5 mg/l and favouring placebo-treated patients at a baseline hs-CRP concentration of 5 mg/l or less. Exploratory analyses focusing on patients with a baseline hs-CRP concentration >5 mg/l revealed a treatment response (≥50% reduction in HAM-D score at any point during treatment) of 62% (eight of 13 patients) in infliximab-treated patients vs. 33% (three of nine patients) in placebo-treated patients ($P = 0.019$). Baseline concentrations of TNF-α and its soluble receptors were significantly higher in infliximab-treated responders vs non-responders ($P < 0.05$), and infliximab-treated responders exhibited significantly greater decreases in hs-CRP from baseline to week 12

Author	Study design	Treatment + trial duration	Outcome measures	Results
				compared with placebo-treated responders (*P* < 0 .01). Drop-outs and adverse events were limited and did not differ between groups. This proof-of-concept study suggests that TNF-α antagonism does not have generalized efficacy in treatment-resistant depression but may improve depressive symptoms in patients with high baseline inflammatory biomarkers
(Miyaoka et al., 2012)	Open-label *N* = 25 adult in-patients with major depression with psychotic features (psychotic depression) according to DSM-IV-TR	Fluvoxamine, paroxetine, or sertraline + minocycline 150 mg/day 6 weeks	HAM-D (baseline and week 6) CGI (baseline and week 6) BPRS (baseline and week 6)	Minocycline in combination with antidepressants provided significant improvement in depression. Mean (±SD) HAM-D was reduced to 6.7 ± 1.9 at week 6 from a baseline value of 40.4 ± 2.5. Significant improvement of psychotic symptoms (mean ± SD) was indicated by the decrease in BPRS scores from baseline (63.3 ± 8.7) to week 6 (4.6 ± 2.4) (*P* < 0.001). No serious adverse events occurred
Hs-CRP, highly sensitive C-reactive protein; 95% CI, 95% confidence interval.				

All diagnoses were carried out according to the DSM-IV or DSM-IV-TR (Chronic inflammatory diseases were excluded). HAM-D Hamilton Depression Rating Scale. CGI clinical Global Impression score. BPRS Brief Psychiatric Rating Scale (Reused with permission from Fond G, Hamdani N, Kapczinski F, Boukouaci W, Drancourt N, Dargel A, Oliveira J, Le Guen E,Marlinge E, Tamouza R, Leboyer M. Effectiveness and tolerance of anti-inflammatory drugs' add-on therapy in major mental disorders: a systematic qualitative review. DOI: 10.1111/acps.12211)

These findings imply that raised inflammatory parameters in patients with MDD may be biological markers of poor treatment response. More importantly, tackling this state of “inflammation” may be important in treating MDD in those with high pre-treatment levels of inflammatory markers. It is tempting here to hypothesise that addition of an anti-inflammatory medication may be a treatment option, and as discussed below, this seems to be the back bone of a number of translational endeavours. A few studies indeed have been successful in showing this as shown in Table 8-2. However, the evidence pointing towards this direction is inconsistent.

In fact, at least one recent study has shown that adding an anti-inflammatory drug may actually attenuate the antidepressant actions of SSRIs. Warner-Schmidt et al recently in a mouse model, showed that antidepressant treatment was associated with an increased expression of cytokines - particularly interferon gamma and TNF-α (Warner-Schmidt et al., 2011). This in turn led to an increased expression of protein p11, a member of the S100 family of proteins that interact with specific serotonin receptors to regulate their trafficking. This protein has previously been associated with behavioural markers of depressive states. For example p11 knockout mice show depression-like phenotype and over-expression of p11 has been associated with antidepressant-like responses. The authors showed that an antidepressant response to SSRIs were mediated by an increase in the above cytokines and a resultant increase in p11 expression. More interestingly they showed that treatment with NSAIDS prevented the increase in cytokines, thereby preventing the increase in p11 and the behavioural response to depression. They went on to test this hypothesis in humans. They analysed data from STAR D to show that those people who were prescribed NSAIDS had a reduced response to SSRIs taken for depression. In keeping with this , Harley et al (2010) found that patients that were treated with antidepressants with baseline CRP levels above 10 mg/L showed significantly better improvement than those who were within the normal range (Harley et al., 2010).

Anti-inflammatory agents targeting novel neurotransmitter systems (including glutamate) have been found to have some efficacy in treating psychiatric conditions. Two drugs of note are riluzole and ketamine, both of which have significant anti-inflammatory effects, and have been found to be effective in treating depression. Riluzole is a glumatergic modulator, which has both neuro-

protective and anticonvulsant properties, due to its ability to inhibit glutamate release and enhance both glutamate reuptake and AMPA trafficking. The non-competitive, high-affinity NMDA antagonist ketamine is a phencyclidine derivative that prevents excess calcium influx and cellular damage, by antagonising NMDARs(Zarate et al., 2010). Ketamine has been shown to have a very fast onset of action in relieving depressive symptoms, and is currently the focus of a number of studies in mood disorders. Other novel anti-inflammatory agents that may have promise include dietary omega 3 fatty acids, particularly eicosapentanoic acid and docosahexaenoic acid, which have been found to have significant anti-inflammatory action. Clinically important anti-inflammatory effects are suggested by trials demonstrating benefits of n-3 fatty acids in rheumatoid arthritis, psoriasis, asthma, and inflammatory bowel disorders. Addition of n-3 fatty acids to existing antidepressant therapy has been found to be effective in recurrent major depressive disorders(Logan, 2004). Finally, drugs targeting the kynurenine pathway have shown preliminary encouraging results in phase 1 trials(Malpass, 2011). Studies are also currently underway, that aim to clarify the clinical and neurobiological phenotype of depressed patients with increased inflammation. It is hoped that if an “inflammatory phenotype” of depression does emerge, this will help to individualise the diagnosis and treatment of patients with this particular phenotype.

8.4 Conclusion

In this thesis, I have shown some evidence that circulating inflammatory markers explain significant variance in brain substrates - particularly cortical thickness and serotonin transporters. I also show that inflammatory markers may mediate the association between socioeconomic deprivation (a high-risk condition for depression/ other major mental illnesses) and cortical thickness and volumes pertaining to executive function, language and the limbic stress networks in the brain. In addition, I show for the first time in humans that circulating inflammatory markers mediate the relationship between socioeconomic deprivation and cortical thickness covariance network structural properties. I also present some of the first evidence to suggest that circulating TNF-α levels correlate significantly with serotonin transporters availability in people suffering from psoriasis/ psoriatic arthritis. I also confirm the findings from an initial pilot show that treatment with TNF-α blockade is associated with a reduction in SERT

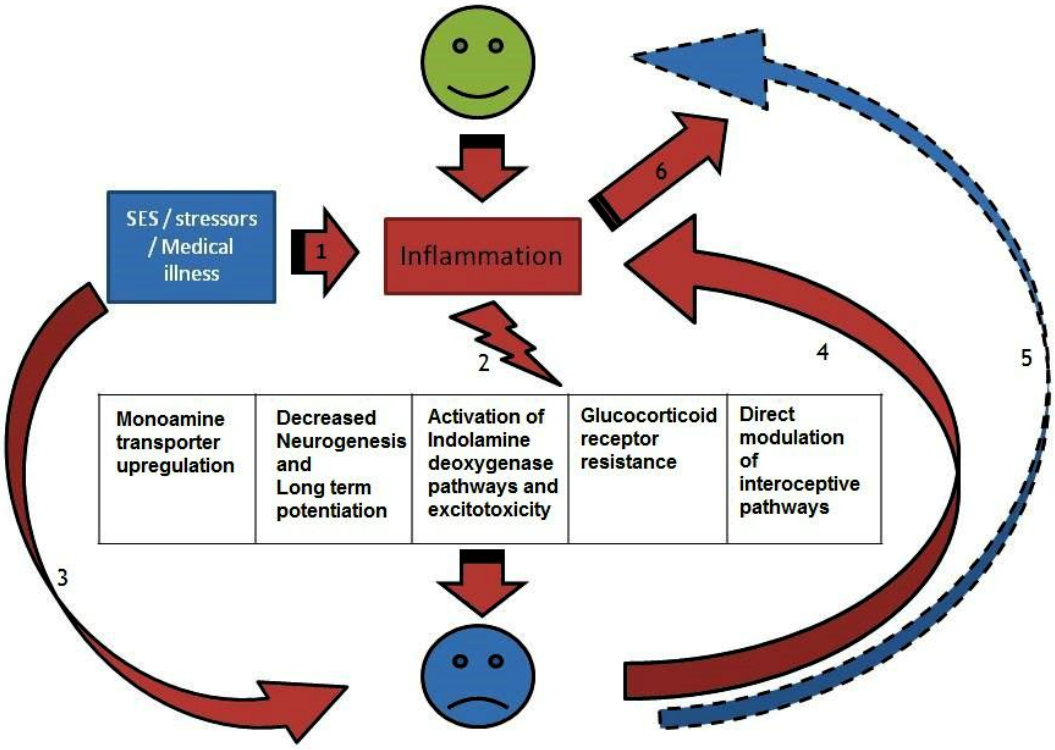
availability. It should be noted that mechanistic pathways that link inflammation with major mental illnesses seem at best speculative in humans.

Inflammation seems to be associated with MDD and may indeed play a role in the aetiology of MDD, at least in a "cohort" of vulnerable individuals - for example, those from high socioeconomic deprivation or those with a medical condition. Inflammation in these situations, may not only act as a precipitating factor that pushes a person into depression, but also a perpetuating factor that may pose an obstacle to recovery. In Figure 8-1 I have summarised potential pathways through which inflammation may play a role in the etiopathogenesis of major depression. Inflammatory pathways are activated in response to a stressor – endogenous (medical illness) or exogenous (psychological/ medication/ socioeconomic status) as shown in Figure 8-1 (1). This inflammatory load, acts as a precipitating factor in those who are vulnerable (predisposed). This relationship is perhaps bidirectional; for example, the inflammation may worsen a physical morbidity. It is known that psychological and physical stressors can worsen clinical outcomes in a number of physical conditions. There are a number of potential pathways through which high inflammation may precipitate a depressive episode (Figure 8-1- (2)). These have been detailed in the introductory chapter, and discussions throughout the thesis. However, the key mechanisms that link circulating inflammatory markers to central inflammation in the brain in humans, remain to be established. It should be noted that there are perhaps other pathways that mediate the relationship between stressors and depressed mood. These need to be accounted for when modelling causal mechanistic associations between stressors and depression (Figure 8-1 -(3)). There are also pathways through which depression may worsen a pre-existing medical condition (or the ability to cope with other stressors) For example, the presence of depression itself may increase inflammation (Figure 8-1-(4)). The raised inflammation may further perpetuate and maintain the major depressive phenotype by preventing recovery (Figure 8-1 (5)and (6)).

Inflammatory markers may be potential biomarkers, aiding diagnosis or even helping to predict prognosis in major depressive illness. Future work will focus on cementing the precise role of inflammation in depressive illness, through more sophisticated animal models and clinical and cognitive neuroscience, and

will hopefully result in beneficial treatments for what remains a significantly disabling psychiatric illness.

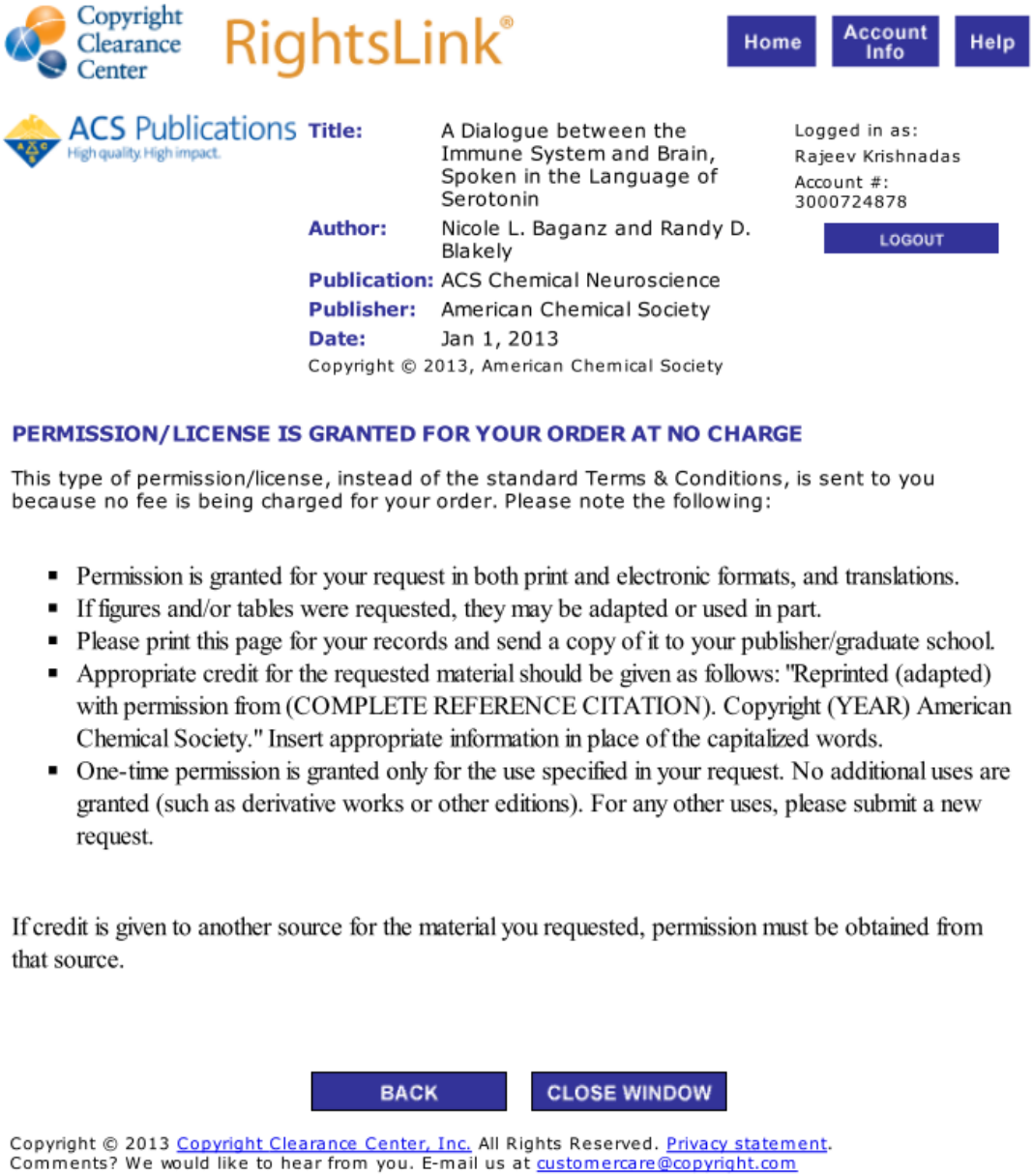
Figure 8-1: Potential pathways through which inflammation may play a part in the pathogenesis of major depression.



Reused with permission from(Krishnadas and Cavanagh, 2012)

Appendices

9.1 Appendix 1: Copyrights and permissions



Copyright Clearance Center RightsLink®

Home | Account Info | Help

ACS Publications High quality. High impact.

Title: A Dialogue between the Immune System and Brain, Spoken in the Language of Serotonin

Author: Nicole L. Baganz and Randy D. Blakely

Publication: ACS Chemical Neuroscience

Publisher: American Chemical Society

Date: Jan 1, 2013

Copyright © 2013, American Chemical Society

Logged in as:
Rajeev Krishnadas
Account #:
3000724878

LOGOUT

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.

BACK | CLOSE WINDOW

Copyright © 2013 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

Thank you very much for your order.

This is a License Agreement between Rajeev Krishnadas ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

Get the printable license.

License Number	3278040230519
License date	Nov 29, 2013
Licensed content publisher	Elsevier
Licensed content publication	Pharmacology & Therapeutics
Licensed content title	Immune system to brain signaling: Neuropsychopharmacological implications
Licensed content author	Lucile Capuron,Andrew H. Miller
Licensed content date	May 2011
Licensed content volume number	130
Licensed content issue number	2
Number of pages	13
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	2
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title of your thesis/dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size (number of pages)	300
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	**0.00 USD**

Back

Copyright Clearance Center

Confirmation Number: 11147513
Order Date: 12/30/2013

Customer Information

Customer: Rajeev Krishnadas
Account Number: 3000724878
Organization: Rajeev Krishnadas
Email: rajeev.krishnadas@glasgow.ac.uk
Phone: +44 7508740010
Payment Method: Invoice

This not an invoice

Order Details

Biology of depression : from novel insights to therapeutic strategies

Billing Status: **N/A**

Order detail ID:	64244041
ISBN:	978-3-527-61967-2
Publication Type:	e-Book
Volume:	
Issue:	
Start page:	
Publisher:	Wiley-VCH
Author/Editor:	Licinio, J. ; Wong, Ma-Li ; Wiley InterScience (Online service)

Permission Status: **Granted**
Permission type: Republish or display content
Type of use: Thesis/Dissertation
Order License Id: 3298990051907

Requestor type	Author of requested content
Format	Print, Electronic
Portion	chart/graph/table/figure
Number of charts/graphs/tables/ figures	1
Title or numeric reference of the portion(s)	Chapter 1, Table 1.1
Title of the article or chapter the portion is from	Chapter 1 Classification of Depression: Research and Diagnostic Criteria: DSM-IV and ICD-10
Editor of portion(s)	Julio Licinio, Ma-Li Wong
Author of portion(s)	Alan M. Gruenberg, Reed D. Goldstein and Harold Alan Pincus
Volume of serial or monograph	N/A
Issue, if republishing an article from a serial	N/A
Page range of portion	7
Publication date of portion	2005
Rights for	Main product
Duration of use	Current edition and up to 10 years
Creation of copies for the disabled	no
With minor editing privileges	no
For distribution to	Worldwide
In the following language(s)	Original language of publication
With incidental promotional use	no
Lifetime unit quantity of new product	500 to 999

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

Thank you very much for your order.

This is a License Agreement between Rajeev Krishnadas ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

Get the printable license.

License Number	3278030185444
License date	Nov 29, 2013
Licensed content publisher	Elsevier
Licensed content publication	Neuroscience
Licensed content title	Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications
Licensed content author	J.C. Felger,F.E. Lotrich
Licensed content date	29 August 2013
Licensed content volume number	246
Number of pages	31
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title of your thesis/dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size (number of pages)	300
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 GBP
VAT/Local Sales Tax	0.00 GBP / 0.00 GBP
Total	**0.00 GBP**

Back

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

This is a License Agreement between Rajeev Krishnadas ("You") and John Wiley and Sons ("John Wiley and Sons"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

Get the printable license.

License Number	3290740834751
License date	Dec 16, 2013
Licensed content publisher	John Wiley and Sons
Licensed content publication	Acta Psychiatrica Scandinavica
Licensed content title	Effectiveness and tolerance of anti-inflammatory drugs' add-on therapy in major mental disorders: a systematic qualitative review
Licensed copyright line	© 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd
Licensed content author	G. Fond,N. Hamdani,F. Kapczinski,W. Boukouaci,N. Drancourt,A. Dargel,J. Oliveira,E. Le Guen,E. Marlinge,R. Tamouza,M. Leboyer
Licensed content date	Nov 11, 2013
Start page	n/a
End page	n/a
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	Table 2
Will you be translating?	No
Total	**0.00 USD**

Back

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

This is a License Agreement between Rajeev Krishnadas ("You") and BMJ Publishing Group Ltd. ("BMJ Publishing Group Ltd."). The license consists of your order details, the terms and conditions provided by BMJ Publishing Group Ltd., and the payment terms and conditions.

Get the printable license.

License Number	3278030570777
License date	Nov 29, 2013
Licensed content publisher	BMJ Publishing Group Ltd.
Licensed content publication	Journal of Neurology, Neurosurgery and Psychiatry
Licensed content title	Depression: an inflammatory illness?
Licensed content author	Rajeev Krishnadas, Jonathan Cavanagh
Licensed content date	May 1, 2012
Volume number	83
Issue number	5
Type of Use	Thesis/Dissertation
Requestor type	Author of this article
Format	Print and electronic
Portion	Figure/table/extract
Number of figure/table/extracts	1
Will you be translating?	No
Circulation/distribution	5
Title of your thesis / dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size(pages)	300
BMJ VAT number	674738491
Billing Type	Invoice
Billing address	Sackler Institute Institute of Health and Wellbeing Glasgow, None G51 4TF United Kingdom
Permissions price	0.00 USD
VAT (0.00%)	0.00 USD
Total	**0.00 USD**

Back

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

Thank you very much for your order.

This is a License Agreement between Rajeev Krishnadas ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

Get the printable license.

License Number	3284770917513
License date	Dec 09, 2013
Licensed content publisher	Elsevier
Licensed content publication	NeuroImage: Clinical
Licensed content title	Cardio-metabolic risk factors and cortical thickness in a neurologically healthy male population: Results from the psychological, social and biological determinants of ill health (pSoBid) study
Licensed content author	Rajeev Krishnadas,John McLean,David G. Batty,Harry Burns,Kevin A. Deans,Ian Ford,Alex McConnachie,Agnes McGinty,Jennifer S. McLean,Keith Millar,Naveed Sattar,Paul G. Shiels,Yoga N. Velupillai,Chris J. Packard,Jonathan Cavanagh
Licensed content date	2013
Licensed content volume number	2
Number of pages	12
Type of Use	reuse in a thesis/dissertation
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Title of your thesis/dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size (number of pages)	300
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 GBP
VAT/Local Sales Tax	0.00 GBP / 0.00 GBP
Total	**0.00 GBP**

Back

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

This is a License Agreement between Rajeev Krishnadas ("You") and Wolters Kluwer Health ("Wolters Kluwer Health"). The license consists of your order details, the terms and conditions provided by Wolters Kluwer Health, and the payment terms and conditions.

Get the printable license.

License Number	3278030641836
License date	Nov 29, 2013
Licensed content publisher	Wolters Kluwer Health
Licensed content publication	Psychosomatic Medicine
Licensed content title	Socioeconomic Deprivation and Cortical Morphology: Psychological, Social, and Biological Determinants of Ill Health Study
Licensed content author	Rajeev Krishnadas, John McLean, G. David Batty, Harry Burns, Kevin A. Deans, Ian Ford, Alex McConnachie, Jennifer S. McLean, Keith Millar, Naveed Sattar, Paul G. Shiels, Carol Tannahill, Yoga N. Velupillai, Chris J. Packard, Jonathan Cavanagh
Licensed content date	Sep 1, 2013
Volume number	75
Issue Number	7
Type of Use	Dissertation/Thesis
Requestor type	Individual Account
Author of this Wolters Kluwer article	Yes
Title of your thesis / dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size(pages)	300
Total	**0.00 USD**

Back

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

This is a License Agreement between Rajeev Krishnadas ("You") and Nature Publishing Group ("Nature Publishing Group"). The license consists of your order details, the terms and conditions provided by Nature Publishing Group, and the payment terms and conditions.

Get the printable license.

License Number	3278050153180
License date	Nov 29, 2013
Licensed content publisher	Nature Publishing Group
Licensed content publication	Nature Neuroscience
Licensed content title	Long story short: the serotonin transporter in emotion regulation and social cognition
Licensed content author	Turhan CanliandKlaus-Peter Lesch
Licensed content date	Sep 1, 2007
Type of Use	reuse in a dissertation / thesis
Volume number	10
Issue number	9
Requestor type	academic/educational
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no
Figures	SERT
Author of this NPG article	no
Your reference number	None
Title of your thesis / dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size (number of pages)	300
Total	**0.00 USD**

Back

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

Thank you very much for your order.

This is a License Agreement between Rajeev Krishnadas ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

Get the printable license.

License Number	3278030262472
License date	Nov 29, 2013
Licensed content publisher	Elsevier
Licensed content publication	Journal of Affective Disorders
Licensed content title	Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression
Licensed content author	Yang Liu,Roger Chun-Man Ho,Anselm Mak
Licensed content date	August 2012
Licensed content volume number	139
Licensed content issue number	3
Number of pages	10
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title of your thesis/dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size (number of pages)	300
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 USD
VAT/Local Sales Tax	0.00 USD / 0.00 GBP
Total	**0.00 USD**

Back

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

This is a License Agreement between Rajeev Krishnadas ("You") and John Wiley and Sons ("John Wiley and Sons"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

Get the printable license.

License Number	3278770644529
License date	Nov 30, 2013
Licensed content publisher	John Wiley and Sons
Licensed content publication	International Journal of Dermatology
Licensed content title	Psoriasis, a model of dermatologic psychosomatic disease: psychiatric implications and treatments
Licensed copyright line	© 2012 The International Society of Dermatology
Licensed content author	Evan Rieder,Francisco Tausk
Licensed content date	Dec 20, 2011
Start page	12
End page	26
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	2
Original Wiley figure/table number(s)	Table 1 and Table 3
Will you be translating?	No
Total	**0.00 USD**

Back

Copyright Clearance Center RightsLink®

My Orders My Library My Profile Welcome rajeev.krishnadas@glasgow.ac.uk Log out | Help

My Orders > Orders > All Orders

License Details

Thank you very much for your order.

This is a License Agreement between Rajeev Krishnadas ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

Get the printable license.

License Number	3287570539065
License date	Dec 14, 2013
Licensed content publisher	Elsevier
Licensed content publication	Journal of Affective Disorders
Licensed content title	CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies
Licensed content author	Vyara Valkanova,Klaus P. Ebmeier,Charlotte L. Allan
Licensed content date	25 September 2013
Licensed content volume number	150
Licensed content issue number	3
Number of pages	9
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title of your thesis/dissertation	Exploring the relationship between circulating inflammatory markers and the brain.
Expected completion date	Dec 2013
Estimated size (number of pages)	300
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 GBP
VAT/Local Sales Tax	0.00 GBP / 0.00 GBP
Total	**0.00 GBP**

Back

List of references

ABBASI, S. H., HOSSEINI, F., MODABBERNIA, A., ASHRAFI, M. & AKHONDZADEH, S. 2012. Effect of celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: randomized double-blind placebo-controlled study. *J Affect Disord,* 141**,** 308-14.

ACHARD, S. & BULLMORE, E. 2007. Efficiency and cost of economical brain functional networks. *PLoS Comput Biol,* 3**,** e17.

AKHONDZADEH, S., JAFARI, S., RAISI, F., NASEHI, A. A., GHOREISHI, A., SALEHI, B., MOHEBBI-RASA, S., RAZNAHAN, M. & KAMALIPOUR, A. 2009. Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. *Depress Anxiety,* 26**,** 607-11.

ALBENSI, B. C. & MATTSON, M. P. 2000. Evidence for the involvement of TNF and NF-kappaB in hippocampal synaptic plasticity. *Synapse,* 35**,** 151-9.

ALEXANDER-BLOCH, A., GIEDD, J. N. & BULLMORE, E. 2013. Imaging structural co-variance between human brain regions. *Nat Rev Neurosci,* 14**,** 322-36.

ALLEY, D. E., SEEMAN, T. E., KI KIM, J., KARLAMANGLA, A., HU, P. & CRIMMINS, E. M. 2006. Socioeconomic status and C-reactive protein levels in the US population: NHANES IV. *Brain Behav Immun,* 20**,** 498-504.

ALPSOY, E., OZCAN, E., CETIN, L., OZGUR, O., ER, H., YILMAZ, E. & KARAMAN, T. 1998. Is the efficacy of topical corticosteroid therapy for psoriasis vulgaris enhanced by concurrent moclobemide therapy? A double-blind, placebo-controlled study. *J Am Acad Dermatol,* 38**,** 197-200.

ANACKER, C., ZUNSZAIN, P. A., CATTANEO, A., CARVALHO, L. A., GARABEDIAN, M. J., THURET, S., PRICE, J. & PARIANTE, C. M. 2011. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Mol Psychiatry,* 16**,** 738-50.

ANESHENSEL, C. S. 2009. Toward explaining mental health disparities. *J Health Soc Behav,* 50**,** 377-94.

BAGANZ, N. L. & BLAKELY, R. D. 2013. A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chem Neurosci,* 4**,** 48-63.

BALDWIN, R. M., ZEA-PONCE, Y., ZOGHBI, S. S., LAURELLE, M., AL-TIKRITI, M. S., SYBIRSKA, E. H., MALISON, R. T., NEUMEYER, J. L., MILIUS, R. A., WANG, S. & ET AL. 1993. Evaluation of the monoamine uptake site ligand [123I]methyl 3 beta-(4-iodophenyl)-tropane-2 beta-carboxylate ([123I]beta-CIT) in non-human primates: pharmacokinetics, biodistribution and SPECT brain imaging coregistered with MRI. *Nucl Med Biol,* 20**,** 597-606.

BASSETT, D. S., BULLMORE, E., VERCHINSKI, B. A., MATTAY, V. S., WEINBERGER, D. R. & MEYER-LINDENBERG, A. 2008. Hierarchical organization of human cortical networks in health and schizophrenia. *J Neurosci,* 28**,** 9239-48.

BAUM, A., GAROFALO, J. P. & YALI, A. M. 1999. Socioeconomic status and chronic stress. Does stress account for SES effects on health? *Ann N Y Acad Sci,* 896**,** 131-44.

BAUNE, B. T., DANNLOWSKI, U., DOMSCHKE, K., JANSSEN, D. G., JORDAN, M. A., OHRMANN, P., BAUER, J., BIROS, E., AROLT, V., KUGEL, H., BAXTER, A. G. & SUSLOW, T. 2009. The interleukin 1 beta (IL1B) gene is associated with

failure to achieve remission and impaired emotion processing in major depression. *Biol Psychiatry,* 67**,** 543-9.

BAYRAMGURLER, D., KARSON, A., OZER, C. & UTKAN, T. 2013. Effects of long-term etanercept treatment on anxiety- and depression-like neurobehaviors in rats. *Physiol Behav,* 119**,** 145-8.

BECK, A. T., STEER, R. A., BALL, R. & RANIERI, W. 1996. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess,* 67**,** 588-97.

BELARBI, K., ARELLANO, C., FERGUSON, R., JOPSON, T. & ROSI, S. 2012. Chronic neuroinflammation impacts the recruitment of adult-born neurons into behaviorally relevant hippocampal networks. *Brain Behav Immun,* 26**,** 18-23.

BENDER, R. & LANGE, S. 2001. Adjusting for multiple testing--when and how? *J Clin Epidemiol,* 54**,** 343-9.

BENJAMINI, Y. & HOCHBERG, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of Royal Statistical Society. Series B,* 57**,** 289 - 300.

BERNARDINO, L., XAPELLI, S., SILVA, A. P., JAKOBSEN, B., POULSEN, F. R., OLIVEIRA, C. R., VEZZANI, A., MALVA, J. O. & ZIMMER, J. 2005. Modulator effects of interleukin-1beta and tumor necrosis factor-alpha on AMPA-induced excitotoxicity in mouse organotypic hippocampal slice cultures. *J Neurosci,* 25**,** 6734-44.

BERTHOLD-LOSLEBEN, M. & HIMMERICH, H. 2008. The TNF- System: Functional Aspects in Depression, Narcolepsy and Psychopharmacology *Current Neuropharmacology,* 6**,** 193-202.

BEYGI, S., LAJEVARDI, V. & ABEDINI, R. 2013. C-reactive protein in psoriasis: a review of the literature. *J Eur Acad Dermatol Venereol*.

BHATIA, A. & KAST, R. E. 2007. Tumor necrosis factor (TNF) can paradoxically increase on etanercept treatment, occasionally contributing to TNF-mediated disease. *J Rheumatol,* 34**,** 447-9; author reply 449-50.

BIERHAUS, A., WOLF, J., ANDRASSY, M., ROHLEDER, N., HUMPERT, P. M., PETROV, D., FERSTL, R., VON EYNATTEN, M., WENDT, T., RUDOFSKY, G., JOSWIG, M., MORCOS, M., SCHWANINGER, M., MCEWEN, B., KIRSCHBAUM, C. & NAWROTH, P. P. 2003. A mechanism converting psychosocial stress into mononuclear cell activation. *Proc Natl Acad Sci U S A,* 100**,** 1920-5.

BORNSTEIN, S. R., SCHUPPENIES, A., WONG, M. L. & LICINIO, J. 2006. Approaching the shared biology of obesity and depression: the stress axis as the locus of gene-environment interactions. *Mol Psychiatry,* 11**,** 892-902.

BRESSLER, S. L. & MENON, V. 2010. Large-scale brain networks in cognition: emerging methods and principles. *Trends in Cognitive Sciences,* 14**,** 277-290.

BRET-DIBAT, J. L., BLUTHE, R. M., KENT, S., KELLEY, K. W. & DANTZER, R. 1995. Lipopolysaccharide and interleukin-1 depress food-motivated behavior in mice by a vagal-mediated mechanism. *Brain Behav Immun,* 9**,** 242-6.

BROYLES, S. T., STAIANO, A. E., DRAZBA, K. T., GUPTA, A. K., SOTHERN, M. & KATZMARZYK, P. T. 2012. Elevated C-reactive protein in children from risky neighborhoods: evidence for a stress pathway linking neighborhoods and inflammation in children. *PLoS One,* 7**,** e45419.

BRUCE, B. & FRIES, J. F. 2003a. The Stanford Health Assessment Questionnaire: a review of its history, issues, progress, and documentation. *J Rheumatol,* 30**,** 167-78.

BRUCE, B. & FRIES, J. F. 2003b. The Stanford Health Assessment Questionnaire: dimensions and practical applications. *Health Qual Life Outcomes,* 1**,** 20.

BRUCE, M. L., TAKEUCHI, D. T. & LEAF, P. J. 1991. Poverty and psychiatric status. Longitudinal evidence from the New Haven Epidemiologic Catchment Area study. *Arch Gen Psychiatry,* 48**,** 470-4.

BRUCKE, T., KORNHUBER, J., ANGELBERGER, P., ASENBAUM, S., FRASSINE, H. & PODREKA, I. 1993. SPECT imaging of dopamine and serotonin transporters with [123I]beta-CIT. Binding kinetics in the human brain. *J Neural Transm Gen Sect,* 94**,** 137-46.

BRUNNER, E. 1997. Stress and the biology of inequality. *BMJ,* 314**,** 1472-6.

BRYDON, L., HARRISON, N. A., WALKER, C., STEPTOE, A. & CRITCHLEY, H. D. 2008. Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biol Psychiatry,* 63**,** 1022-9.

BULL, S. J., HUEZO-DIAZ, P., BINDER, E. B., CUBELLS, J. F., RANJITH, G., MADDOCK, C., MIYAZAKI, C., ALEXANDER, N., HOTOPF, M., CLEARE, A. J., NORRIS, S., CASSIDY, E., AITCHISON, K. J., MILLER, A. H. & PARIANTE, C. M. 2009. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. *Mol Psychiatry,* 14**,** 1095-104.

BULLMORE, E. & SPORNS, O. 2009. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci,* 10**,** 186-98.

BULLMORE, E. & SPORNS, O. 2012. The economy of brain network organization. *Nat Rev Neurosci,* 13**,** 336-49.

BUSH, G., LUU, P. & POSNER, M. I. 2000. Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences,* 4**,** 215-222.

BUSINELLE, M. S., MILLS, B. A., CHARTIER, K. G., KENDZOR, D. E., REINGLE, J. M. & SHUVAL, K. 2013. Do stressful events account for the link between socioeconomic status and mental health? *J Public Health (Oxf).*

BUTTERWORTH, P., CHERBUIN, N., SACHDEV, P. & ANSTEY, K. J. 2011. The association between financial hardship and amygdala and hippocampal volumes: results from the PATH through life project. *Social Cognitive and Affective Neuroscience,* 7**,** 548-556.

CANLI, T. & LESCH, K.-P. 2007. Long story short: the serotonin transporter in emotion regulation and social cognition. *Nat Neurosci,* 10**,** 1103-1109.

CAPURON, L., FORNWALT, F. B., KNIGHT, B. T., HARVEY, P. D., NINAN, P. T. & MILLER, A. H. 2009. Does cytokine-induced depression differ from idiopathic major depression in medically healthy individuals? *J Affect Disord,* 119**,** 181-5.

CAPURON, L., GUMNICK, J. F., MUSSELMAN, D. L., LAWSON, D. H., REEMSNYDER, A., NEMEROFF, C. B. & MILLER, A. H. 2002. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. *Neuropsychopharmacology,* 26**,** 643-52.

CAPURON, L. & MILLER, A. H. 2011. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacol Ther,* 130**,** 226-38.

CAPURON, L., PAGNONI, G., DRAKE, D. F., WOOLWINE, B. J., SPIVEY, J. R., CROWE, R. J., VOTAW, J. R., GOODMAN, M. M. & MILLER, A. H. 2012. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiatry,* 69**,** 1044-53.

CARDENAS, V. A., REED, B., CHAO, L. L., CHUI, H., SANOSSIAN, N., DECARLI, C. C., MACK, W., KRAMER, J., HODIS, H. N., YAN, M., BUONOCORE, M. H.,

CARMICHAEL, O., JAGUST, W. J. & WEINER, M. W. 2012. Associations Among Vascular Risk Factors, Carotid Atherosclerosis, and Cortical Volume and Thickness in Older Adults. *Stroke*.

CARROLL, J. E., COHEN, S. & MARSLAND, A. L. 2011. Early childhood socioeconomic status is associated with circulating interleukin-6 among mid-life adults. *Brain Behav Immun,* 25**,** 1468-74.

CAVANAGH, J., PATERSON, C., MCLEAN, J., PIMLOTT, S., MCDONALD, M., PATTERSON, J., WYPER, D. & MCINNES, I. 2010. Tumour necrosis factor blockade mediates altered serotonin transporter availability in rheumatoid arthritis: a clinical, proof-of-concept study. *Ann Rheum Dis,* 69**,** 1251-2.

CAVANAGH, J., PATTERSON, J., PIMLOTT, S., DEWAR, D., EERSELS, J., DEMPSEY, M. F. & WYPER, D. 2006. Serotonin transporter residual availability during long-term antidepressant therapy does not differentiate responder and nonresponder unipolar patients. *Biol Psychiatry,* 59**,** 301-8.

CELANO, C. M. & HUFFMAN, J. C. 2011. Depression and cardiac disease: a review. *Cardiol Rev,* 19**,** 130-42.

CHANDRAN, V., BHELLA, S., SCHENTAG, C. & GLADMAN, D. D. 2007. Functional assessment of chronic illness therapy-fatigue scale is valid in patients with psoriatic arthritis. *Ann Rheum Dis,* 66**,** 936-9.

CHAUDHURI, A. & BEHAN, P. O. 2000. Fatigue and basal ganglia. *J Neurol Sci,* 179**,** 34-42.

CHEN, G. & GOEDDEL, D. V. 2002. TNF-R1 signaling: a beautiful pathway. *Science,* 296**,** 1634-5.

CHEN, Y., BENDER, R. A., BRUNSON, K. L., POMPER, J. K., GRIGORIADIS, D. E., WURST, W. & BARAM, T. Z. 2004. Modulation of dendritic differentiation by corticotropin-releasing factor in the developing hippocampus. *Proc Natl Acad Sci U S A,* 101**,** 15782-7.

CHEN, Z. J., HE, Y., ROSA-NETO, P., GERMANN, J. & EVANS, A. C. 2008. Revealing Modular Architecture of Human Brain Structural Networks by Using Cortical Thickness from MRI. *Cerebral Cortex,* 18**,** 2374-2381.

CHEN, Z. J., HE, Y., ROSA-NETO, P., GONG, G. & EVANS, A. C. 2011. Age-related alterations in the modular organization of structural cortical network by using cortical thickness from MRI. *Neuroimage,* 56**,** 235-45.

CHRISTMAS, D. M., POTOKAR, J. & DAVIES, S. J. 2011. A biological pathway linking inflammation and depression: activation of indoleamine 2,3-dioxygenase. *Neuropsychiatr Dis Treat,* 7**,** 431-9.

CLEMENT, H. W., BUSCHMANN, J., REX, S., GROTE, C., OPPER, C., GEMSA, D. & WESEMANN, W. 1997. Effects of interferon-gamma, interleukin-1 beta, and tumor necrosis factor-alpha on the serotonin metabolism in the nucleus raphe dorsalis of the rat. *J Neural Transm,* 104**,** 981-91.

COIMBRA, S., FIGUEIREDO, A., CASTRO, E., ROCHA-PEREIRA, P. & SANTOS-SILVA, A. 2012. The roles of cells and cytokines in the pathogenesis of psoriasis. *International journal of dermatology,* 51**,** 389-398.

COLLINS, P. Y., PATEL, V., JOESTL, S. S., MARCH, D., INSEL, T. R., DAAR, A. S., ANDERSON, W., DHANSAY, M. A., PHILLIPS, A., SHURIN, S., WALPORT, M., EWART, W., SAVILL, S. J., BORDIN, I. A., COSTELLO, E. J., DURKIN, M., FAIRBURN, C., GLASS, R. I., HALL, W., HUANG, Y., HYMAN, S. E., JAMISON, K., KAAYA, S., KAPUR, S., KLEINMAN, A., OGUNNIYI, A., OTERO-OJEDA, A., POO, M. M., RAVINDRANATH, V., SAHAKIAN, B. J., SAXENA, S., SINGER, P. A. & STEIN, D. J. 2011. Grand challenges in global mental health. *Nature,* 475**,** 27-30.

CONSOLI, S. M., ROLHION, S., MARTIN, C., RUEL, K., CAMBAZARD, F., PELLET, J. & MISERY, L. 2006. Low levels of emotional awareness predict a better response to dermatological treatment in patients with psoriasis. *Dermatology,* 212**,** 128-36.

COTEL, F., EXLEY, R., CRAGG, S. J. & PERRIER, J. F. 2013. Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. *Proc Natl Acad Sci U S A,* 110**,** 4774-9.

COUCH, Y., ANTHONY, D. C., DOLGOV, O., REVISCHIN, A., FESTOFF, B., SANTOS, A. I., STEINBUSCH, H. W. & STREKALOVA, T. 2013. Microglial activation, increased TNF and SERT expression in the prefrontal cortex define stress-altered behaviour in mice susceptible to anhedonia. *Brain Behav Immun,* 29**,** 136-46.

CRAIG, A. D. 2009. How do you feel--now? The anterior insula and human awareness. *Nat Rev Neurosci,* 10**,** 59-70.

CRITCHLEY, H. D. & HARRISON, N. A. 2013. Visceral influences on brain and behavior. *Neuron,* 77**,** 624-38.

DALE, A. M., FISCHL, B. & SERENO, M. I. 1999. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage,* 9**,** 179-94.

DANESH, J., KAPTOGE, S., MANN, A. G., SARWAR, N., WOOD, A., ANGLEMAN, S. B., WENSLEY, F., HIGGINS, J. P., LENNON, L., EIRIKSDOTTIR, G., RUMLEY, A., WHINCUP, P. H., LOWE, G. D. & GUDNASON, V. 2008. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med,* 5**,** e78.

DANTZER, R., O'CONNOR, J. C., FREUND, G. G., JOHNSON, R. W. & KELLEY, K. W. 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci,* 9**,** 46-56.

DAWS, L. C. & GOULD, G. G. 2011. Ontogeny and regulation of the serotonin transporter: providing insights into human disorders. *Pharmacol Ther,* 131**,** 61-79.

DE GRAAF, R., BIJL, R. V., SMIT, F., VOLLEBERGH, W. A. & SPIJKER, J. 2002. Risk factors for 12-month comorbidity of mood, anxiety, and substance use disorders: findings from the Netherlands Mental Health Survey and Incidence Study. *Am J Psychiatry,* 159**,** 620-9.

DE JONGE, P. & ROEST, A. M. 2012. Depression and cardiovascular disease: the end of simple models. *Br J Psychiatry,* 201**,** 337-8.

DE WIN, M. M., HABRAKEN, J. B., RENEMAN, L., VAN DEN BRINK, W., DEN HEETEN, G. J. & BOOIJ, J. 2005. Validation of [(123)I]beta-CIT SPECT to assess serotonin transporters in vivo in humans: a double-blind, placebo-controlled, crossover study with the selective serotonin reuptake inhibitor citalopram. *Neuropsychopharmacology,* 30**,** 996-1005.

DEAN, B., TAWADROS, N., SCARR, E. & GIBBONS, A. S. 2010. Regionally-specific changes in levels of tumour necrosis factor in the dorsolateral prefrontal cortex obtained postmortem from subjects with major depressive disorder. *J Affect Disord,* 120**,** 245-8.

DEANS, K. A., BEZLYAK, V., FORD, I., BATTY, G. D., BURNS, H., CAVANAGH, J., DE GROOT, E., MCGINTY, A., MILLAR, K., SHIELS, P. G., TANNAHILL, C., VELUPILLAI, Y. N., SATTAR, N. & PACKARD, C. J. 2009. Differences in atherosclerosis according to area level socioeconomic deprivation: cross sectional, population based study. *BMJ,* 339**,** b4170.

DENKE, M. A., SEMPOS, C. T. & GRUNDY, S. M. 1994. Excess body weight. An under-recognized contributor to dyslipidemia in white American women. *Archives of internal medicine,* 154**,** 401-10.

DESIKAN, R. S., SEGONNE, F., FISCHL, B., QUINN, B. T., DICKERSON, B. C., BLACKER, D., BUCKNER, R. L., DALE, A. M., MAGUIRE, R. P., HYMAN, B. T., ALBERT, M. S. & KILLIANY, R. J. 2006a. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage,* 31**,** 968-80.

DESIKAN, R. S., SÉGONNE, F., FISCHL, B., QUINN, B. T., DICKERSON, B. C., BLACKER, D., BUCKNER, R. L., DALE, A. M., MAGUIRE, R. P., HYMAN, B. T., ALBERT, M. S. & KILLIANY, R. J. 2006b. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage,* 31**,** 968-980.

DESTRIEUX, C., FISCHL, B., DALE, A. & HALGREN, E. 2010. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. *Neuroimage,* 53**,** 1-15.

DICKENS, C., MCGOWAN, L., CLARK-CARTER, D. & CREED, F. 2002. Depression in rheumatoid arthritis: a systematic review of the literature with meta-analysis. *Psychosom Med,* 64**,** 52-60.

DIETRICH, J. 2002. The adhesion molecule ICAM-1 and its regulation in relation with the blood–brain barrier. *Journal of Neuroimmunology,* 128**,** 58-68.

DIEZ ROUX, A. V. 2001. Investigating neighborhood and area effects on health. *Am J Public Health,* 91**,** 1783-9.

DIEZ ROUX, A. V. & MAIR, C. 2010. Neighborhoods and health. *Annals of the New York Academy of Sciences,* 1186**,** 125-145.

DOWLATI, Y., HERRMANN, N., SWARDFAGER, W., LIU, H., SHAM, L., REIM, E. K. & LANCTOT, K. L. 2010. A meta-analysis of cytokines in major depression. *Biol Psychiatry,* 67**,** 446-57.

DOWLATSHAHI, E. A., VAN DER VOORT, E. A., ARENDS, L. R. & NIJSTEN, T. 2013. Markers of systemic inflammation in psoriasis: a systematic review and meta-analysis. *Br J Dermatol,* 169**,** 266-82.

DRAKE, C., BOUTIN, H., JONES, M. S., DENES, A., MCCOLL, B. W., SELVARAJAH, J. R., HULME, S., GEORGIOU, R. F., HINZ, R., GERHARD, A., VAIL, A., PRENANT, C., JULYAN, P., MAROY, R., BROWN, G., SMIGOVA, A., HERHOLZ, K., KASSIOU, M., CROSSMAN, D., FRANCIS, S., PROCTOR, S. D., RUSSELL, J. C., HOPKINS, S. J., TYRRELL, P. J., ROTHWELL, N. J. & ALLAN, S. M. 2011. Brain inflammation is induced by co-morbidities and risk factors for stroke. *Brain Behav Immun,* 25**,** 1113-22.

DREVETS, W. C., SAVITZ, J. & TRIMBLE, M. 2008. The Subgenual Anterior Cingulate Cortex in Mood Disorders. *CNS Spectrum,* 13**,** 663-681.

DUIVIS, H. E., VOGELZANGS, N., KUPPER, N., DE JONGE, P. & PENNINX, B. W. 2013. Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: findings from the Netherlands Study of Depression and Anxiety (NESDA). *Psychoneuroendocrinology,* 38**,** 1573-85.

ECHTERMEYER, C., HAN, C. E., ROTARSKA-JAGIELA, A., MOHR, H., UHLHAAS, P. J. & KAISER, M. 2011. Integrating temporal and spatial scales: human structural network motifs across age and region of interest size. *Front Neuroinform,* 5**,** 10.

EHRLICH, S., BRAUNS, S., YENDIKI, A., HO, B. C., CALHOUN, V., SCHULZ, S. C., GOLLUB, R. L. & SPONHEIM, S. R. 2012. Associations of cortical thickness and cognition in patients with schizophrenia and healthy controls. *Schizophr Bull,* 38**,** 1050-62.

EK, M., KUROSAWA, M., LUNDEBERG, T. & ERICSSON, A. 1998. Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins. *J Neurosci,* 18**,** 9471-9.

EKDAHL, C. T., CLAASEN, J. H., BONDE, S., KOKAIA, Z. & LINDVALL, O. 2003. Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A,* 100**,** 13632-7.

ELIAS, M. F., ELIAS, P. K., SULLIVAN, L. M., WOLF, P. A. & D'AGOSTINO, R. B. 2005a. Obesity, diabetes and cognitive deficit: The Framingham Heart Study. *Neurobiology of aging,* 26 Suppl 1**,** 11-6.

ELIAS, P. K., ELIAS, M. F., D'AGOSTINO, R. B., SULLIVAN, L. M. & WOLF, P. A. 2005b. Serum cholesterol and cognitive performance in the Framingham Heart Study. *Psychosomatic medicine,* 67**,** 24-30.

ELLER, T., VASAR, V., SHLIK, J. & MARON, E. 2008. Pro-inflammatory cytokines and treatment response to escitalopram in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry,* 32**,** 445-50.

ENERBACK, C. 2011. Soluble biomarkers in psoriasis. *Eur J Dermatol,* 21**,** 844-50.

ERIKSSON, P. S., PERFILIEVA, E., BJORK-ERIKSSON, T., ALBORN, A. M., NORDBORG, C., PETERSON, D. A. & GAGE, F. H. 1998. Neurogenesis in the adult human hippocampus. *Nat Med,* 4**,** 1313-7.

ERRITZOE, D., HOLST, K., FROKJAER, V. G., LICHT, C. L., KALBITZER, J., NIELSEN, F. A., SVARER, C., MADSEN, J. & KNUDSEN, G. 2010. A nonlinear relationship between cerebral serotonin transporter and 5-HT(2A) receptor binding: an in vivo molecular imaging study in humans. *J Neurosci,* 30**,** 3391-7.

EUSTON, D. R., GRUBER, A. J. & MCNAUGHTON, B. L. 2012. The role of medial prefrontal cortex in memory and decision making. *Neuron,* 76**,** 1057-70.

EVANS, D. L. & NEMEROFF, C. B. 1987. The clinical use of the dexamethasone suppression test in DSM-III affective disorders: correlation with the severe depressive subtypes of melancholia and psychosis. *J Psychiatr Res,* 21**,** 185-94.

EVANS, G. W. & KIM, P. 2012. Childhood poverty and young adults' allostatic load: the mediating role of childhood cumulative risk exposure. *Psychol Sci,* 23**,** 979-83.

EVANS, G. W. & SCHAMBERG, M. A. 2009. Childhood poverty, chronic stress, and adult working memory. *Proc Natl Acad Sci U S A,* 106**,** 6545-9.

FANTUZZI, F., DEL GIGLIO, M., GISONDI, P. & GIROLOMONI, G. 2008. Targeting tumor necrosis factor alpha in psoriasis and psoriatic arthritis *Expert opinions in therapeutic targets,* 12**,** 1085-1096.

FARAH, M. J., SHERA, D. M., SAVAGE, J. H., BETANCOURT, L., GIANNETTA, J. M., BRODSKY, N. L., MALMUD, E. K. & HURT, H. 2006. Childhood poverty: specific associations with neurocognitive development. *Brain Res,* 1110**,** 166-74.

FELDMANN, M. & MAINI, R. N. 2001. Anti-TNF alpha therapy of rheumatoid arthritis: What have we learned? *Annual review of Immunology,* 19.

FELDMANN, M. & MAINI, R. N. 2003. TNF defined as a therapeutic target for rheumatoid arthritis and autoimmune diseases. *Nature Medicine,* 9**,** 1245-1250.

FELDMANN, M. & MAINI, R. N. 2010. Anti-TNF therapy, from rationale to standard of care: what lessons has it taught us? *J Immunol,* 185**,** 791-4.

FELDMANN, M., WILLIAMS, R. O. & PALEOLOG, E. 2010. What have we learnt from targeted anti-TNF therapy? *Ann Rheum Dis,* 69 Suppl 1**,** i97-99.

FELGER, J. C. & LOTRICH, F. E. 2013. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience,* 246**,** 199-229.

FELGER, J. C., MUN, J., KIMMEL, H. L., NYE, J. A., DRAKE, D. F., HERNANDEZ, C. R., FREEMAN, A. A., RYE, D. B., GOODMAN, M. M., HOWELL, L. L. &

MILLER, A. H. 2013. Chronic interferon-alpha decreases dopamine 2 receptor binding and striatal dopamine release in association with anhedonia-like behavior in nonhuman primates. *Neuropsychopharmacology,* 38**,** 2179-87.

FISCHL, B. & DALE, A. M. 2000a. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Sciences,* 97**,** 11050-11055.

FISCHL, B. & DALE, A. M. 2000b. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A,* 97**,** 11050-5.

FISCHL, B., LIU, A. & DALE, A. M. 2001. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans Med Imaging,* 20**,** 70-80.

FISCHL, B., RAJENDRAN, N., BUSA, E., AUGUSTINACK, J., HINDS, O., YEO, B. T. T., MOHLBERG, H., AMUNTS, K. & ZILLES, K. 2008. Cortical folding patterns and predicting cytoarchitecture. *Cerebral cortex (New York, N Y : 1991),* 18**,** 1973-80.

FISCHL, B., SALAT, D. H., BUSA, E., ALBERT, M., DIETERICH, M., HASELGROVE, C., VAN DER KOUWE, A., KILLIANY, R., KENNEDY, D., KLAVENESS, S., MONTILLO, A., MAKRIS, N., ROSEN, B. & DALE, A. M. 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron,* 33**,** 341-55.

FISCHL, B., SALAT, D. H., VAN DER KOUWE, A. J., MAKRIS, N., SEGONNE, F., QUINN, B. T. & DALE, A. M. 2004a. Sequence-independent segmentation of magnetic resonance images. *Neuroimage,* 23 Suppl 1**,** S69-84.

FISCHL, B., SERENO, M. I. & DALE, A. M. 1999a. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *NeuroImage,* 9**,** 195-207.

FISCHL, B., SERENO, M. I., TOOTELL, R. B. & DALE, A. M. 1999b. High-resolution intersubject averaging and a coordinate system for the cortical surface. *Human Brain Mapping,* 8**,** 272-284.

FISCHL, B., VAN DER KOUWE, A., DESTRIEUX, C., HALGREN, E., SEGONNE, F., SALAT, D. H., BUSA, E., SEIDMAN, L. J., GOLDSTEIN, J., KENNEDY, D., CAVINESS, V., MAKRIS, N., ROSEN, B. & DALE, A. M. 2004b. Automatically parcellating the human cerebral cortex. *Cerebral Cortex,* 14.

FITZGERALD, O. & CHANDRAN, V. 2012. Update on biomarkers in psoriatic arthritis: a report from the GRAPPA 2010 annual meeting. *J Rheumatol,* 39**,** 427-30.

FOND, G., HAMDANI, N., KAPCZINSKI, F., BOUKOUACI, W., DRANCOURT, N., DARGEL, A., OLIVEIRA, J., LE GUEN, E., MARLINGE, E., TAMOUZA, R. & LEBOYER, M. 2013. Effectiveness and tolerance of anti-inflammatory drugs' add-on therapy in major mental disorders: a systematic qualitative review. *Acta Psychiatr Scand.*

FRANK, P. G. & LISANTI, M. P. 2008. ICAM-1: role in inflammation and in the regulation of vascular permeability. *Am J Physiol Heart Circ Physiol,* 295**,** H926-H927.

FREIRE, M., RODRIGUEZ, J., MOLLER, I., VALCARCEL, A., TORNERO, C., DIAZ, G., ARMENDARIZ, Y. & PAREDES, S. 2011. [Prevalence of symptoms of anxiety and depression in patients with psoriatic arthritis attending rheumatology clinics]. *Reumatol Clin,* 7**,** 20-6.

FRIES, J. F., SPITZ, P. W. & YOUNG, D. Y. 1982. The dimensions of health outcomes: the health assessment questionnaire, disability and pain scales. *J Rheumatol,* 9**,** 789-93.

FRISTON, K. 2012. Ten ironic rules for non-statistical reviewers. *NeuroImage,* 61**,** 1300-10.

GALLACHER, J., BAYER, A., LOWE, G., FISH, M., PICKERING, J., PEDRO, S., DUNSTAN, F., WHITE, J., YARNELL, J. & BEN-SHLOMO, Y. 2010. Is sticky blood bad for the brain?: Hemostatic and inflammatory systems and dementia in the Caerphilly Prospective Study. *Arterioscler Thromb Vasc Biol,* 30**,** 599-604.

GAUR, U. & AGGARWAL, B. B. 2003. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. *Biochem Pharmacol,* 66**,** 1403-8.

GELFAND, J. M., KIMBALL, A. B., MOSTOW, E. N., CHIOU, C. F., PATEL, V., XIA, H. A., FREUNDLICH, B. & STEVENS, S. R. 2008. Patient-reported outcomes and health-care resource utilization in patients with psoriasis treated with etanercept: continuous versus interrupted treatment. *Value Health,* 11**,** 400-7.

GENOVESE, C. R., LAZAR, N. A. & NICHOLS, T. 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage,* 15**,** 870-8.

GERONIMUS, A. T. & BOUND, J. 1998. Use of census-based aggregate variables to proxy for socioeconomic group: evidence from national samples. *Am J Epidemiol,* 148**,** 475-86.

GIANAROS, P. J. & HACKMAN, D. A. 2013. Contributions of neuroscience to the study of socioeconomic health disparities. *Psychosom Med,* 75**,** 610-5.

GIANAROS, P. J., HORENSTEIN, J. A., COHEN, S., MATTHEWS, K. A., BROWN, S. M., FLORY, J. D., CRITCHLEY, H. D., MANUCK, S. B. & HARIRI, A. R. 2007a. Perigenual anterior cingulate morphology covaries with perceived social standing. *Soc Cogn Affect Neurosci,* 2**,** 161-73.

GIANAROS, P. J., HORENSTEIN, J. A., HARIRI, A. R., SHEU, L. K., MANUCK, S. B., MATTHEWS, K. A. & COHEN, S. 2008. Potential neural embedding of parental social standing. *Social Cognitive and Affective Neuroscience,* 3**,** 91-96.

GIANAROS, P. J., JENNINGS, J. R., SHEU, L. K., GREER, P. J., KULLER, L. H. & MATTHEWS, K. A. 2007b. Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus. *Neuroimage,* 35**,** 795-803.

GIANAROS, P. J., MANUCK, S. B., SHEU, L. K., KUAN, D. C. H., VOTRUBA-DRZAL, E., CRAIG, A. E. & HARIRI, A. R. 2010. Parental Education Predicts Corticostriatal Functionality in Adulthood. *Cerebral Cortex,* 21**,** 896-910.

GIANAROS, P. J., MARSLAND, A. L., SHEU, L. K., ERICKSON, K. I. & VERSTYNEN, T. D. 2012. Inflammatory Pathways Link Socioeconomic Inequalities to White Matter Architecture. *Cereb Cortex.*

GIMENO, D., KIVIMAKI, M., BRUNNER, E. J., ELOVAINIO, M., DE VOGLI, R., STEPTOE, A., KUMARI, M., LOWE, G. D., RUMLEY, A., MARMOT, M. G. & FERRIE, J. E. 2009. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychol Med,* 39**,** 413-23.

GIMENO, D., MARMOT, M. G. & SINGH-MANOUX, A. 2008. Inflammatory markers and cognitive function in middle-aged adults: The Whitehall II study. *Psychoneuroendocrinology,* 33**,** 1322-1334.

GIRVAN, M. & NEWMAN, M. E. 2002. Community structure in social and biological networks. *Proc Natl Acad Sci U S A,* 99**,** 7821-7826.

GLADMAN, D. D. 1998. Psoriatic arthritis. *Rheum Dis Clin North Am,* 24**,** 829-844.

GLADMAN, D. D. 2012. Early psoriatic arthritis. *Rheum Dis Clin North Am,* 38**,** 373-86.

GOEHLER, L. E., RELTON, J. K., DRIPPS, D., KIECHLE, R., TARTAGLIA, N., MAIER, S. F. & WATKINS, L. R. 1997. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull,* 43**,** 357-64.

GOLD, S. M., DZIOBEK, I., SWEAT, V., TIRSI, A., ROGERS, K., BRUEHL, H., TSUI, W., RICHARDSON, S., JAVIER, E. & CONVIT, A. 2007. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia,* 50**,** 711-9.

GONZÁLEZ, S., QUEIRO, R. & BALLINA, J. 2012. Update on the pathogenesis of psoriatic arthritis. *Reumatología Clínica,* 8**,** S1-S6.

GOTTLIEB, A. B., CHAO, C. & DANN, F. 2008. Psoriasis comorbidities. *Journal of Dermatological Treatment,* 19**,** 5-21.

GOULD, E., MCEWEN, B. S., TANAPAT, P., GALEA, L. A. & FUCHS, E. 1997. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J Neurosci,* 17**,** 2492-8.

GRAFF, L. A., WALKER, J. R. & BERNSTEIN, C. N. 2009. Depression and anxiety in inflammatory bowel disease: a review of comorbidity and management. *Inflamm Bowel Dis,* 15**,** 1105-18.

GREICIUS, M. D., FLORES, B., MENON, V., GLOVER, G. H., SOLVASON, H., KENNA, H., REISS, A. L. & SCHATZBERG, A. F. 2007. Resting-State Functional Connectivity in Major Depression: Abnormally Increased Contributions from Subgenual Cingulate Cortex and Thalamus. *Biological Psychiatry,* 62**,** 429 - 437.

GRIVENNIKOV, S. I., GRETEN, F. R. & KARIN, M. 2010. Immunity, inflammation, and cancer. *Cell,* 140**,** 883-99.

GRUENBERG, A., GOLDSTEIN, R. & PINCUS, H. A. 2005. Classification of Depression: Research and Diagnostic Criteria: DSM-IV and ICD-10. *In:* LICINIO, J. & WONG, M.-L. (eds.) *Biology of depression : from novel insights to therapeutic strategies.* Weinheim ; [Great Britain] : Wiley-VCH, 2005.

GRUENEWALD, T. L., COHEN, S., MATTHEWS, K. A., TRACY, R. & SEEMAN, T. E. 2009. Association of socioeconomic status with inflammation markers in black and white men and women in the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Soc Sci Med,* 69**,** 451-9.

GRUENEWALD, T. L., KARLAMANGLA, A. S., HU, P., STEIN-MERKIN, S., CRANDALL, C., KORETZ, B. & SEEMAN, T. E. 2012. History of socioeconomic disadvantage and allostatic load in later life. *Soc Sci Med,* 74**,** 75-83.

GUIJARRO, A., LAVIANO, A. & MEGUID, M. M. 2006. Hypothalamic integration of immune function and metabolism. *Prog Brain Res,* 153**,** 367-405.

GUNSTAD, J., PAUL, R. H., COHEN, R. A., TATE, D. F., SPITZNAGEL, M. B., GRIEVE, S. & GORDON, E. 2008. Relationship between body mass index and brain volume in healthy adults. *The International journal of neuroscience,* 118**,** 1582-93.

HAAN, M., KAPLAN, G. A. & CAMACHO, T. 1987. Poverty and health. Prospective evidence from the Alameda County Study. *Am J Epidemiol,* 125**,** 989-98.

HABETS, P., MARCELIS, M., GRONENSCHILD, E., DRUKKER, M. & VAN OS, J. 2011. Reduced Cortical Thickness as an Outcome of Differential Sensitivity to Environmental Risks in Schizophrenia. *Biological Psychiatry,* 69**,** 487-494.

HACKETT, M. L. & ANDERSON, C. S. 2005. Predictors of depression after stroke: a systematic review of observational studies. *Stroke,* 36**,** 2296-301.

HACKMAN, D. A. & FARAH, M. J. 2009. Socioeconomic status and the developing brain. *Trends in Cognitive Sciences,* 13**,** 65-73.

HACKMAN, D. A., FARAH, M. J. & MEANEY, M. J. 2010. Socioeconomic status and the brain: mechanistic insights from human and animal research. *Nature Reviews Neuroscience,* 11**,** 651-659.

HAGLER, D. J., JR., SAYGIN, A. P. & SERENO, M. I. 2006. Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. *NeuroImage,* 33**,** 1093-103.

HAMILTON, J. P., ETKIN, A., FURMAN, D. J., LEMUS, M. G., JOHNSON, R. F. & GOTLIB, I. H. 2012. Functional neuroimaging of major depressive disorder: a meta-analysis and new integration of base line activation and neural response data. *Am J Psychiatry,* 169**,** 693-703.

HAN, X., JOVICICH, J., SALAT, D., VAN DER KOUWE, A., QUINN, B., CZANNER, S., BUSA, E., PACHECO, J., ALBERT, M., KILLIANY, R., MAGUIRE, P., ROSAS, D., MAKRIS, N., DALE, A., DICKERSON, B. & FISCHL, B. 2006. Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer. *NeuroImage,* 32**,** 180-194.

HANGGI, J., WOTRUBA, D. & JANCKE, L. 2011. Globally altered structural brain network topology in grapheme-color synesthesia. *J Neurosci,* 31**,** 5816-28.

HANNESTAD, J., DELLAGIOIA, N. & BLOCH, M. 2011. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology,* 36**,** 2452-9.

HANNESTAD, J., DELLAGIOIA, N., GALLEZOT, J. D., LIM, K., NABULSI, N., ESTERLIS, I., PITTMAN, B., LEE, J. Y., O'CONNOR, K. C., PELLETIER, D. & CARSON, R. E. 2013. The neuroinflammation marker translocator protein is not elevated in individuals with mild-to-moderate depression: a [(1)(1)C]PBR28 PET study. *Brain Behav Immun,* 33**,** 131-8.

HANNESTAD, J., GALLEZOT, J. D., SCHAFBAUER, T., LIM, K., KLOCZYNSKI, T., MORRIS, E. D., CARSON, R. E., DING, Y. S. & COSGROVE, K. P. 2012a. Endotoxin-induced systemic inflammation activates microglia: [(1)(1)C]PBR28 positron emission tomography in nonhuman primates. *Neuroimage,* 63**,** 232-9.

HANNESTAD, J., SUBRAMANYAM, K., DELLAGIOIA, N., PLANETA-WILSON, B., WEINZIMMER, D., PITTMAN, B. & CARSON, R. E. 2012b. Glucose metabolism in the insula and cingulate is affected by systemic inflammation in humans. *J Nucl Med,* 53**,** 601-7.

HANSON, J., CHANDRA, A., WOLFE, B. & POLLAK, S. 2011. Association between Income and the Hippocampus. *PLOS One,* 6**,** e18712.

HARLEY, J., LUTY, S., CARTER, J., MULDER, R. & JOYCE, P. 2010. Elevated C-reactive protein in depression: a predictor of good long-term outcome with antidepressants and poor outcome with psychotherapy. *J Psychopharmacol,* 24**,** 625-6.

HAROON, E., WILSON, A. E., UDELSON, H., CHEN, X., WOOLWINE, B. J., PAREKH, S., HU, X., SPIVEY, J. R. & MILLER, A. H. 2013. Inflammation induced changes in Anterior Cingulate Cortex Glutamate is associated with Depression and Fatigue. *Brain, Behavior, and Immunity,* 32**,** e33.

HARRISON, N. A., BRYDON, L., WALKER, C., GRAY, M. A., STEPTOE, A. & CRITCHLEY, H. D. 2009a. Inflammation Causes Mood Changes Through Alterations in Subgenual Cingulate Activity and Mesolimbic Connectivity. *Biological Psychiatry,* 66**,** 407-414.

HARRISON, N. A., BRYDON, L., WALKER, C., GRAY, M. A., STEPTOE, A., DOLAN, R. J. & CRITCHLEY, H. D. 2009b. Neural origins of human sickness in interoceptive responses to inflammation. *Biol Psychiatry,* 66**,** 415-22.

HARTWELL, L. H., HOPFIELD, J. J., LEIBLER, S. & MURRAY, A. W. 1999. From molecular to modular cell biology. *Nature,* 402**,** C47-52.

HARTWIGSEN, G. & SIEBNER, H. R. 2012. Probing the involvement of the right hemisphere in language processing with online transcranial magnetic stimulation in healthy volunteers. *Aphasiology,* 26**,** 1131-1152.

HATCH, S. L. & DOHRENWEND, B. P. 2007. Distribution of traumatic and other stressful life events by race/ethnicity, gender, SES and age: a review of the research. *Am J Community Psychol,* 40**,** 313-32.

HAYES, J. & KOO, J. 2010. Psoriasis: depression, anxiety, smoking and drinking habits. *Dermatologic therapy,* 23**,** 174-180.

HE, Y., CHEN, Z. J. & EVANS, A. C. 2007. Small-world anatomical networks in the human brain revealed by cortical thickness from MRI. *Cereb Cortex,* 17**,** 2407-19.

HE, Y., DAGHER, A., CHEN, Z., CHARIL, A., ZIJDENBOS, A., WORSLEY, K. & EVANS, A. 2009. Impaired small-world efficiency in structural cortical networks in multiple sclerosis associated with white matter lesion load. *Brain,* 132**,** 3366-79.

HELFAND, M., BUCKLEY, D. I., FREEMAN, M., FU, R., ROGERS, K., FLEMING, C. & HUMPHREY, L. L. 2009. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. *Ann Intern Med,* 151**,** 496-507.

HERINGA, S., VAN DEN BERG, E., REIJMER, Y. D., NIJPELS, G., STEHOUWER, C. D., SCHALKWIJK, C. G., TEERLINK, T., SCHEFFER, P. G., VAN DEN HURK, K., KAPPELLE, L. J., DEKKER, J. M. & BIESSELS, G. J. 2014. Markers of low-grade inflammation and endothelial dysfunction are related to reduced information processing speed and executive functioning in an older population – the Hoorn Study. *Psychoneuroendocrinology,* 40**,** 108-118.

HESS, A., AXMANN, R., RECH, J., FINZEL, S., HEINDL, C., KREITZ, S., SERGEEVA, M., SAAKE, M., GARCIA, M., KOLLIAS, G., STRAUB, R. H., SPORNS, O., DOERFLER, A., BRUNE, K. & SCHETT, G. 2011. Blockade of TNF-alpha rapidly inhibits pain responses in the central nervous system. *Proc Natl Acad Sci U S A,* 108**,** 3731-6.

HINTZE, A. & ADAMI, C. 2008. Evolution of complex modular biological networks. *PLoS Comput Biol,* 4**,** e23.

HOGSTROM, L. J., WESTLYE, L. T., WALHOVD, K. B. & FJELL, A. M. 2012. The Structure of the Cerebral Cortex Across Adult Life: Age-Related Patterns of Surface Area, Thickness, and Gyrification. *Cerebral Cortex*.

HOOGENDAM, Y. Y., VAN DER GEEST, J. N., VAN DER LIJN, F., VAN DER LUGT, A., NIESSEN, W. J., KRESTIN, G. P., HOFMAN, A., VERNOOIJ, M. W., BRETELER, M. M. & IKRAM, M. A. 2012. Determinants of cerebellar and cerebral volume in the general elderly population. *Neurobiol Aging,* 33**,** 2774-81.

HORNUNG, J. 2010. The Neuronatomy of the Serotonergic System *In:* MULLER, C. P. & JACOBS, B. (eds.) *Handbook of Behavioural neurobiology of Serotonin.* Elsevier B.V.

HOSSEINI, S. M., HOEFT, F. & KESLER, S. R. 2012a. GAT: a graph-theoretical analysis toolbox for analyzing between-group differences in large-scale structural and functional brain networks. *PLoS One,* 7**,** e40709.

HOSSEINI, S. M., KOOVAKKATTU, D. & KESLER, S. R. 2012b. Altered small-world properties of gray matter networks in breast cancer. *BMC Neurol,* 12**,** 28.

HOWREN, M. B., LAMKIN, D. M. & SULS, J. 2009. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med,* 71**,** 171-86.

HU, S., SHENG, W. S., EHRLICH, L. C., PETERSON, P. K. & CHAO, C. C. 2000. Cytokine effects on glutamate uptake by human astrocytes. *Neuroimmunomodulation,* 7**,** 153-9.

HUBER, J., CAMPOS, C., MARK, K. & DAVIS, T. 2006. Alterations in blood-brain barrier ICAM-1 expression and brain microglial activation after lambda -carrageenan-induced inflammatory pain. *American Journal of Heart and Circulation Physiology,* 290.

HUDSON, C. G. 2005. Socioeconomic status and mental illness: tests of the social causation and selection hypotheses. *Am J Orthopsychiatry,* 75**,** 3-18.

IM, K., LEE, J.-M., YOON, U., SHIN, Y.-W., HONG, S. B., KIM, I. Y., KWON, J. S. & KIM, S. I. 2006. Fractal dimension in human cortical surface: Multiple regression analysis with cortical thickness, sulcal depth, and folding area. *Human Brain Mapping,* 27**,** 994-1003.

IOSIF, R. E., EKDAHL, C. T., AHLENIUS, H., PRONK, C. J., BONDE, S., KOKAIA, Z., JACOBSEN, S. E. & LINDVALL, O. 2006. Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. *J Neurosci,* 26**,** 9703-12.

IRWIN, M. R., OLMSTEAD, R., VALLADARES, E. M., BREEN, E. C. & EHLERS, C. L. 2009. Tumor necrosis factor antagonism normalizes rapid eye movement sleep in alcohol dependence. *Biol Psychiatry,* 66**,** 191-5.

ISAAC, V., SIM, S., ZHENG, H., ZAGORODNOV, V., TAI, E. S. & CHEE, M. 2011. Adverse Associations between Visceral Adiposity, Brain Structure, and Cognitive Performance in Healthy Elderly. *Frontiers in aging neuroscience,* 3**,** 12.

JANSSEN, D. G., CANIATO, R. N., VERSTER, J. C. & BAUNE, B. T. 2010. A psychoneuroimmunological review on cytokines involved in antidepressant treatment response. *Hum Psychopharmacol,* 25**,** 201-15.

JEDNORÓG, K., FRASCH, M. G., ALTARELLI, I., MONZALVO, K., FLUSS, J., DUBOIS, J., BILLARD, C., DEHAENE-LAMBERTZ, G. & RAMUS, F. 2012. The Influence of Socioeconomic Status on Children's Brain Structure. *PLOS One,* 7**,** e42486.

JEFFERSON, A. L., MASSARO, J. M., WOLF, P. A., SESHADRI, S., AU, R., VASAN, R. S., LARSON, M. G., MEIGS, J. B., KEANEY, J. F., LIPINSKA, I., KATHIRESAN, S., BENJAMIN, E. J. & DECARLI, C. 2007. Inflammatory biomarkers are associated with total brain volume: The Framingham Heart Study. *Neurology,* 68**,** 1032-1038.

JURADO, M. B. & ROSSELLI, M. 2007. The Elusive Nature of Executive Functions: A Review of our Current Understanding. *Neuropsychology Review,* 17**,** 213-233.

JUSTER, R. P., MCEWEN, B. S. & LUPIEN, S. J. 2010. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci Biobehav Rev,* 35**,** 2-16.

KAISER, M. 2007. Brain architecture: a design for natural computation. *Philos Transact A Math Phys Eng Sci,* 365**,** 3033-45.

KANEKO, N., KUDO, K., MABUCHI, T., TAKEMOTO, K., FUJIMAKI, K., WATI, H., IGUCHI, H., TEZUKA, H. & KANBA, S. 2006. Suppression of cell proliferation by interferon-alpha through interleukin-1 production in adult rat dentate gyrus. *Neuropsychopharmacology,* 31**,** 2619-26.

KARG, K., BURMEISTER, M., SHEDDEN, K. & SEN, S. 2011. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. *Arch Gen Psychiatry,* 68**,** 444-54.

KARSON, A., DEMIRTAS, T., BAYRAMGURLER, D., BALCI, F. & UTKAN, T. 2013. Chronic administration of infliximab (TNF-alpha inhibitor) decreases depression and anxiety-like behaviour in rat model of chronic mild stress. *Basic Clin Pharmacol Toxicol,* 112**,** 335-40.

KATAFUCHI, T., KONDO, T., TAKE, S. & YOSHIMURA, M. 2006. Brain cytokines and the 5-HT system during poly I:C-induced fatigue. *Ann N Y Acad Sci,* 1088**,** 230-7.

KELLEY, K. W., BLUTHE, R., DANTZER, R., ZHOU, J., SHEN, W., JOHNSON, R. W. & BROUSSARD, S. 2003. Cytokine-induced sickness behaviour. *Brain Behavior Immunity,* 17**,** S112-S118.

KESSLER, R. C., CHIU, W. T., DEMLER, O., MERIKANGAS, K. R. & WALTERS, E. E. 2005. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry,* 62**,** 617-27.

KESSLER, R. C., MCGONAGLE, K. A., ZHAO, S., NELSON, C. B., HUGHES, M., ESHLEMAN, S., WITTCHEN, H. U. & KENDLER, K. S. 1994. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry,* 51**,** 8-19.

KIM, P., EVANS, G. W., ANGSTADT, M., HO, S. S., SRIPADA, C. S., SWAIN, J. E., LIBERZON, I. & PHAN, K. L. 2013. Effects of childhood poverty and chronic stress on emotion regulatory brain function in adulthood. *Proc Natl Acad Sci U S A,* 110**,** 18442-7.

KIN, T., YAMANO, S., SAKURAI, R., KAJITANI, M., OKAHASHI, Y., NISHIURA, N., SAITO, Y. & UENO, S. 2007. Carotid atherosclerosis is associated with brain atrophy in Japanese elders. *Gerontology,* 53**,** 1-6.

KNOX, S., WELSH, P., BEZLYAK, V., MCCONNACHIE, A., BOULTON, E., DEANS, K. A., FORD, I., DAVID BATTY, G., BURNS, H., CAVANAGH, J., MILLAR, K., MCINNES, I. B., MCLEAN, J., VELUPILLAI, Y., SHIELS, P., TANNAHILL, C., PACKARD, C. J., MICHAEL WALLACE, A. & SATTAR, N. 2012. 25-Hydroxyvitamin D is lower in deprived groups, but is not associated with carotid intima media thickness or plaques: Results from pSoBid. *Atherosclerosis,* 223**,** 437-41.

KOHMAN, R. A. & RHODES, J. S. 2013. Neurogenesis, inflammation and behavior. *Brain Behav Immun,* 27**,** 22-32.

KONSMAN, J., PARNET, P. & DANTZER, R. 2002. Cytokine induced sickness behaviour: mechanisms and implications. *Trends Neurosci,* 25**,** 154-159.

KOO, J. W. & DUMAN, R. S. 2008. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U S A,* 105**,** 751-6.

KOSTER, A., BOSMA, H., PENNINX, B. W., NEWMAN, A. B., HARRIS, T. B., VAN EIJK, J. T., KEMPEN, G. I., SIMONSICK, E. M., JOHNSON, K. C., ROOKS, R. N., AYONAYON, H. N., RUBIN, S. M. & KRITCHEVSKY, S. B. 2006. Association of inflammatory markers with socioeconomic status. *J Gerontol A Biol Sci Med Sci,* 61**,** 284-90.

KOTSIS, K., VOULGARI, P. V., TSIFETAKI, N., MACHADO, M. O., CARVALHO, A. F., CREED, F., DROSOS, A. A. & HYPHANTIS, T. 2012. Anxiety and depressive symptoms and illness perceptions in psoriatic arthritis and associations

with physical health-related quality of life. *Arthritis Care Res (Hoboken),* 64**,** 1593-601.

KRAUS, M. R., AL-TAIE, O., SCHAFER, A., PFERSDORFF, M., LESCH, K. P. & SCHEURLEN, M. 2007. Serotonin-1A receptor gene HTR1A variation predicts interferon-induced depression in chronic hepatitis C. *Gastroenterology,* 132**,** 1279-86.

KRAUSS, R. M., WINSTON, M., FLETCHER, R. N. & GRUNDY, S. M. 1998. Obesity: impact of cardiovascular disease. *Circulation,* 98**,** 1472-6.

KRISHNADAS, R. 2010. Etanercept for sleep in patients with alcohol use disorder--mechanisms need to be elucidated. *Biol Psychiatry,* 67**,** e1; author reply e3.

KRISHNADAS, R. & CAVANAGH, J. 2011. Sustained remission of rheumatoid arthritis with a specific serotonin reuptake inhibitor antidepressant: a case report and review of the literature. *J Med Case Reports,* 5**,** 112.

KRISHNADAS, R. & CAVANAGH, J. 2012. Depression: an inflammatory illness? *J Neurol Neurosurg Psychiatry,* 83**,** 495-502.

KRISHNADAS, R., KIM, J., MCLEAN, J., BATTY, G. D., MCLEAN, J. S., MILLAR, K., PACKARD, C. J. & CAVANAGH, J. 2013a. The envirome and the connectome: exploring the structural noise in the human brain associated with socioeconomic deprivation. *Front Hum Neurosci,* 7**,** 722.

KRISHNADAS, R., MALLON, V., MCINNES, I. & CAVANAGH, J. 2011. Correlates of depression and quality of life in patients with inflammatory arthritides. *European Psychiatry,* 26**,** 383.

KRISHNADAS, R., MCLEAN, J., BATTY, D., BURNS, H., DEANS, K., FORD, I., MCCONNACHIE, A., MCGINTY, A., MCLEAN, J. S., MILLAR, K., SATTAR, N., SHIELS, P., TANNAHILL, C., VELUPILLAI, Y., PACKARD, C. & CAVANAGH, J. 2013b. Cardio-metabolic risk factors and cortical thickness in a neurologically healthy male population: results from the psychological, social and biological determinants of ill health (pSoBid) study. *NeuroImage: Clinical,* 2**,** 646-657.

KRISHNADAS, R., MCLEAN, J., BATTY, G. D., BURNS, H., DEANS, K. A., FORD, I., MCCONNACHIE, A., MCLEAN, J. S., MILLAR, K., SATTAR, N., SHIELS, P. G., TANNAHILL, C., VELUPILLAI, Y. N., PACKARD, C. J. & CAVANAGH, J. 2013c. Socioeconomic deprivation and cortical morphology: psychological, social, and biological determinants of ill health study. *Psychosom Med,* 75**,** 616-23.

KRISHNAN, V. & NESTLER, E. J. 2010. Linking Molecules to Mood: New Insight Into the Biology of Depression. *American Journal of Psychiatry,* 167**,** 1305-1320.

KRUGEL, U., FISCHER, J., RADICKE, S., SACK, U. & HIMMERICH, H. 2013. Antidepressant effects of TNF-alpha blockade in an animal model of depression. *J Psychiatr Res,* 47**,** 611-6.

KUHN, S., SCHUBERT, F. & GALLINAT, J. 2010. Reduced thickness of medial orbitofrontal cortex in smokers. *Biological Psychiatry,* 68**,** 1061-5.

KUPERBERG, G. R., BROOME, M. R., MCGUIRE, P. K., DAVID, A. S., EDDY, M., OZAWA, F., GOFF, D., WEST, W. C., WILLIAMS, S. C., VAN DER KOUWE, A. J., SALAT, D. H., DALE, A. M. & FISCHL, B. 2003. Regionally localized thinning of the cerebral cortex in schizophrenia. *Arch Gen Psychiatry,* 60**,** 878-88.

KURD, S. K., TROXEL, A. B., CRITS-CHRISTOPH, P. & GELFAND, J. M. 2010. The risk of depression, anxiety, and suicidality in patients with psoriasis: a population-based cohort study. *Arch Dermatol,* 146**,** 891-5.

LAKOSKI, S. G., CUSHMAN, M., CRIQUI, M., RUNDEK, T., BLUMENTHAL, R. S., D'AGOSTINO, R. B., JR. & HERRINGTON, D. M. 2006. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. *Am Heart J,* 152**,** 593-8.

LAMPA, J., WESTMAN, M., KADETOFF, D., AGREUS, A. N., LE MAITRE, E., GILLIS-HAEGERSTRAND, C., ANDERSSON, M., KHADEMI, M., CORR, M., CHRISTIANSON, C. A., DELANEY, A., YAKSH, T. L., KOSEK, E. & SVENSSON, C. I. 2012. Peripheral inflammatory disease associated with centrally activated IL-1 system in humans and mice. *Proc Natl Acad Sci U S A,* 109**,** 12728-33.

LANGLEY, R. G., FELDMAN, S. R., HAN, C., SCHENKEL, B., SZAPARY, P., HSU, M. C., ORTONNE, J. P., GORDON, K. B. & KIMBALL, A. B. 2010. Ustekinumab significantly improves symptoms of anxiety, depression, and skin-related quality of life in patients with moderate-to-severe psoriasis: Results from a randomized, double-blind, placebo-controlled phase III trial. *J Am Acad Dermatol,* 63**,** 457-65.

LANGLEY, R. G., PALLER, A. S., HEBERT, A. A., CREAMER, K., WENG, H. H., JAHREIS, A., GLOBE, D., PATEL, V. & ORLOW, S. J. 2011. Patient-reported outcomes in pediatric patients with psoriasis undergoing etanercept treatment: 12-week results from a phase III randomized controlled trial. *J Am Acad Dermatol,* 64**,** 64-70.

LANQUILLON, S., KRIEG, J. C., BENING-ABU-SHACH, U. & VEDDER, H. 2000. Cytokine production and treatment response in major depressive disorder. *Neuropsychopharmacology,* 22**,** 370-9.

LEE, A. L., OGLE, W. O. & SAPOLSKY, R. M. 2002. Stress and depression: possible links to neuron death in the hippocampus. *Bipolar Disord,* 4**,** 117-28.

LEKAKIS, J., IKONOMIDIS, I., PAPOUTSI, Z., MOUTSATSOU, P., NIKOLAOU, M., PARISSIS, J. & KREMASTINOS, D. T. 2010. Selective serotonin re-uptake inhibitors decrease the cytokine-induced endothelial adhesion molecule expression, the endothelial adhesiveness to monocytes and the circulating levels of vascular adhesion molecules. *International Journal of Cardiology,* 139**,** 150-158.

LERCH, J. P., WORSLEY, K., SHAW, W. P., GREENSTEIN, D. K., LENROOT, R. K., GIEDD, J. & EVANS, A. C. 2006. Mapping anatomical correlations across cerebral cortex (MACACC) using cortical thickness from MRI. *Neuroimage,* 31**,** 993-1003.

LERITZ, E. C., SALAT, D. H., WILLIAMS, V. J., SCHNYER, D. M., RUDOLPH, J. L., LIPSITZ, L., FISCHL, B., MCGLINCHEY, R. E. & MILBERG, W. P. 2011. Thickness of the human cerebral cortex is associated with metrics of cerebrovascular health in a normative sample of community dwelling older adults. *NeuroImage,* 54**,** 2659-2671.

LEVINE, J., BARAK, Y., CHENGAPPA, K. N., RAPOPORT, A., REBEY, M. & BARAK, V. 1999. Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiology,* 40**,** 171-6.

LI, Y., LIU, Y., LI, J., QIN, W., LI, K., YU, C. & JIANG, T. 2009. Brain anatomical network and intelligence. *PLoS Comput Biol,* 5**,** e1000395.

LINDEN, D. E. 2012. The challenges and promise of neuroimaging in psychiatry. *Neuron,* 73**,** 8-22.

LINDQVIST, D., JANELIDZE, S., HAGELL, P., ERHARDT, S., SAMUELSSON, M., MINTHON, L., HANSSON, O., BJORKQVIST, M., TRASKMAN-BENDZ, L. & BRUNDIN, L. 2009. Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. *Biol Psychiatry,* 66**,** 287-92.

LISTON, C., MILLER, M. M., GOLDWATER, D. S., RADLEY, J. J., ROCHER, A. B., HOF, P. R., MORRISON, J. H. & MCEWEN, B. S. 2006. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J Neurosci,* 26**,** 7870-4.

LIU, J.-P., TANG, Y., ZHOU, S., TOH, B. H., MCLEAN, C. & LI, H. 2010. Cholesterol involvement in the pathogenesis of neurodegenerative diseases. *Molecular and cellular neurosciences,* 43**,** 33-42.

LIU, Y., HO, R. C. & MAK, A. 2012a. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-alpha) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *J Affect Disord,* 139**,** 230-9.

LIU, Y., JULKUNEN, V., PAAJANEN, T., WESTMAN, E., WAHLUND, L. O., AITKEN, A., SOBOW, T., MECOCCI, P., TSOLAKI, M., VELLAS, B., MUEHLBOECK, S., SPENGER, C., LOVESTONE, S., SIMMONS, A. & SOININEN, H. 2012b. Education increases reserve against Alzheimer's disease--evidence from structural MRI analysis. *Neuroradiology,* 54**,** 929-38.

LO FERMO, S., BARONE, R., PATTI, F., LAISA, P., CAVALLARO, T. L., NICOLETTI, A. & ZAPPIA, M. Outcome of psychiatric symptoms presenting at onset of multiple sclerosis: a retrospective study. *Mult Scler,* 16**,** 742-8.

LOFTIS, J. M., PATTERSON, A. L., WILHELM, C. J., MCNETT, H., MORASCO, B. J., HUCKANS, M., MORGAN, T., SAPERSTEIN, S., ASGHAR, A. & HAUSER, P. 2013. Vulnerability to somatic symptoms of depression during interferon-alpha therapy for hepatitis C: a 16-week prospective study. *J Psychosom Res,* 74**,** 57-63.

LOGAN, A. C. 2004. Omega-3 fatty acids and major depression: a primer for the mental health professional. *Lipids Health Dis,* 3**,** 25.

LOPEZ-LARSON, M. P., BOGORODZKI, P., ROGOWSKA, J., MCGLADE, E., KING, J. B., TERRY, J. & YURGELUN-TODD, D. 2011. Altered prefrontal and insular cortical thickness in adolescent marijuana users. *Behavioural Brain Research,* 220**,** 164-172.

LORANT, V., DELIEGE, D., EATON, W., ROBERT, A., PHILIPPOT, P. & ANSSEAU, M. 2003. Socioeconomic inequalities in depression: a meta-analysis. *Am J Epidemiol,* 157**,** 98-112.

LORENZ, M. W., MARKUS, H. S., BOTS, M. L., ROSVALL, M. & SITZER, M. 2007. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation,* 115**,** 459-67.

LOTRICH, F. E., FERRELL, R. E., RABINOVITZ, M. & POLLOCK, B. G. 2009. Risk for depression during interferon-alpha treatment is affected by the serotonin transporter polymorphism. *Biol Psychiatry,* 65**,** 344-8.

LUC, G., ARVEILER, D., EVANS, A., AMOUYEL, P., FERRIERES, J., BARD, J. M., ELKHALIL, L., FRUCHART, J. C. & DUCIMETIERE, P. 2003. Circulating soluble adhesion molecules ICAM-1 and VCAM-1 and incident coronary heart disease: the PRIME Study. *Atherosclerosis,* 170**,** 169-76.

MADHUSUDAN, S., FOSTER, M., MUTHURAMALINGAM, S. R., BRAYBROOKE, J. P., WILNER, S., KAUR, K., HAN, C., HOARE, S., BALKWILL, F., TALBOT, D. C., GANESAN, T. S. & HARRIS, A. L. 2004. A phase II study of etanercept (Enbrel), a tumor necrosis factor alpha inhibitor in patients with metastatic breast cancer. *Clin Cancer Res,* 10**,** 6528-34.

MAES, M. 2008. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. *Neuro Endocrinol Lett,* 29**,** 287-91.

MAES, M., LEONARD, B. E., MYINT, A. M., KUBERA, M. & VERKERK, R. 2011. The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiatry,* 35**,** 702-21.

MAGIN, P., ADAMS, J., HEADING, G., POND, D. & SMITH, W. 2009. The psychological sequelae of psoriasis: results of a qualitative study. *Psychol Health Med,* 14**,** 150-61.

MALPASS, K. 2011. Neurodegenerative disease: the kynurenine pathway--promising new targets and therapies for neurodegenerative disease. *Nat Rev Neurol,* 7**,** 417.

MALYNN, S., CAMPOS-TORRES, A., MOYNAGH, P. & HAASE, J. 2013. The pro-inflammatory cytokine TNF-alpha regulates the activity and expression of the serotonin transporter (SERT) in astrocytes. *Neurochem Res,* 38**,** 694-704.

MARGULIES, D. S., KELLY, A. M. C., UDDIN, L. Q., BISWAL, B. B., CASTELLANOS, F. X. & MILHAM, M. P. 2007. Mapping the functional connectivity of anterior cingulate cortex. *NeuroImage,* 37**,** 579-588.

MARK, L. P., PROST, R. W., ULMER, J. L., SMITH, M. M., DANIELS, D. L., STROTTMANN, J. M., BROWN, W. D. & HACEIN-BEY, L. 2001. Pictorial review of glutamate excitotoxicity: fundamental concepts for neuroimaging. *AJNR Am J Neuroradiol,* 22**,** 1813-24.

MARSLAND, A. L., GIANAROS, P. J., ABRAMOWITCH, S. M., MANUCK, S. B. & HARIRI, A. R. 2008. Interleukin-6 Covaries Inversely with Hippocampal Grey Matter Volume in Middle-Aged Adults. *Biological Psychiatry,* 64**,** 484-490.

MARTINEZ, J. M., GARAKANI, A., YEHUDA, R. & GORMAN, J. M. 2012. Proinflammatory and "resiliency" proteins in the CSF of patients with major depression. *Depress Anxiety,* 29**,** 32-8.

MAXIMINO, C. 2012. Serotonin in the Nervous System of Vertebrates. 15-36.

MAYBERG, H. 1997. Limbic-Cortical Dysregulation: A proposed model of depression. *The Journal of Neuropsychiatry and Clinical Neurosciences,* 9**,** 471 - 481.

MAYBERG, H. S., LIOTTI, M., BRANNAN, S. K., MCGINNIS, S., MAHURIN, R. K., JERABEK, P. A., SILVA, J. A., TEKELL, J. L., MARTIN, C. C., LANCASTER, J. L. & FOX, P. T. 1999. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *Am J Psychiatry,* 156**,** 675-82.

MCAFOOSE, J. & BAUNE, B. T. 2009. Evidence for a cytokine model of cognitive function. *Neurosci Biobehav Rev,* 33**,** 355-66.

MCCRORY, E., DE BRITO, S. A. & VIDING, E. 2010. Research review: the neurobiology and genetics of maltreatment and adversity. *J Child Psychol Psychiatry,* 51**,** 1079-95.

MCEWEN, B. S. 1998. Stress, adaptation, and disease. Allostasis and allostatic load. *Ann N Y Acad Sci,* 840**,** 33-44.

MCEWEN, B. S. & GIANAROS, P. J. 2010. Central role of the brain in stress and adaptation: Links to socioeconomic status, health, and disease. *Annals of the New York Academy of Sciences,* 1186**,** 190-222.

MCEWEN, B. S. & SEEMAN, T. 1999. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. *Ann N Y Acad Sci,* 896**,** 30-47.

MCEWEN, B. S. & STELLAR, E. 1993. Stress and the individual. Mechanisms leading to disease. *Arch Intern Med,* 153**,** 2093-101.

MCGRATH, C. L., KELLEY, M. E., HOLTZHEIMER, P. E., DUNLOP, B. W., CRAIGHEAD, W. E., FRANCO, A. R., CRADDOCK, R. C. & MAYBERG, H. S. 2013. Toward a neuroimaging treatment selection biomarker for major depressive disorder. *JAMA Psychiatry,* 70**,** 821-9.

MCGUINNESS, D., MCGLYNN, L. M., JOHNSON, P. C., MACINTYRE, A., BATTY, G. D., BURNS, H., CAVANAGH, J., DEANS, K. A., FORD, I., MCCONNACHIE, A., MCGINTY, A., MCLEAN, J. S., MILLAR, K., PACKARD, C. J., SATTAR, N. A., TANNAHILL, C., VELUPILLAI, Y. N. & SHIELS, P. G. 2012. Socio-economic status is associated with epigenetic differences in the pSoBid cohort. *Int J Epidemiol,* 41**,** 151-60.

MCKITTRICK, C. R., MAGARINOS, A. M., BLANCHARD, D. C., BLANCHARD, R. J., MCEWEN, B. S. & SAKAI, R. R. 2000. Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. *Synapse,* 36**,** 85-94.

MCLEAN, J., KRISHNADAS, R., BATTY, G. D., BURNS, H., DEANS, K. A., FORD, I., MCCONNACHIE, A., MCGINTY, A., MCLEAN, J. S., MILLAR, K., SATTAR, N., SHIELS, P. G., TANNAHILL, C., VELUPILLAI, Y. N., PACKARD, C. J., CONDON, B. R., HADLEY, D. M. & CAVANAGH, J. 2012. Early life socioeconomic status, chronic physiological stress and hippocampal N-acetyl aspartate concentrations. *Behavioural Brain Research,* 235**,** 225-230.

MENDLEWICZ, J., KRIWIN, P., OSWALD, P., SOUERY, D., ALBONI, S. & BRUNELLO, N. 2006. Shortened onset of action of antidepressants in major depression using acetylsalicylic acid augmentation: a pilot open-label study. *Int Clin Psychopharmacol,* 21**,** 227-31.

MESULAM, M. 1990. Large-scale neurocognitive networks and distributed processing for attention, language and memory. *Annals of Neurology,* 28**,** 597 - 613.

MEYER, J. H. 2008. Applying neuroimaging ligands to study major depressive disorder. *Semin Nucl Med,* 38**,** 287-304.

MEYER, J. H. 2012. Neuroimaging markers of cellular function in major depressive disorder: implications for therapeutics, personalized medicine, and prevention. *Clin Pharmacol Ther,* 91**,** 201-14.

MIECH, R., CASPI, A., MOFFITT, T., ENTNER WRIGHT, B. & SILVA, P. 1999. Low Socioeconomic Status and Mental Disorders: A Longitudinal Study of Selection and Causation During Young Adulthood. *American Journal of Sociology,* 104**,** 1096-1131.

MIKOCKA-WALUS, A. A., TURNBULL, D. A., MOULDING, N. T., WILSON, I. G., ANDREWS, J. M. & HOLTMANN, G. J. 2006. Antidepressants and inflammatory bowel disease: a systematic review. *Clin Pract Epidemiol Ment Health,* 2**,** 24.

MILLER, B. J., BUCKLEY, P., SEABOLT, W., MELLOR, A. & KIRKPATRICK, B. 2011. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biol Psychiatry,* 70**,** 663-71.

MILLER, G. A. & CHAPMAN, J. P. 2001. Misunderstanding analysis of covariance. *J Abnorm Psychol,* 110**,** 40-8.

MIYAOKA, T., WAKE, R., FURUYA, M., LIAURY, K., IEDA, M., KAWAKAMI, K., TSUCHIE, K., TAKI, M., ISHIHARA, K., ARAKI, T. & HORIGUCHI, J. 2012. Minocycline as adjunctive therapy for patients with unipolar psychotic depression: an open-label study. *Prog Neuropsychopharmacol Biol Psychiatry,* 37**,** 222-6.

MODELL, J. G., BOYCE, S., TAYLOR, E. & KATHOLI, C. 2002. Treatment of atopic dermatitis and psoriasis vulgaris with bupropion-SR: a pilot study. *Psychosom Med,* 64**,** 835-40.

MOMENAN, R., STECKLER, L. E., SAAD, Z. S., VAN RAFELGHEM, S., KERICH, M. J. & HOMMER, D. W. 2012. Effects of alcohol dependence on cortical thickness as determined by magnetic resonance imaging. *Psychiatry Research: Neuroimaging,* 204**,** 101-111.

MONJE, M. L., TODA, H. & PALMER, T. D. 2003. Inflammatory blockade restores adult hippocampal neurogenesis. *Science,* 302**,** 1760-5.

MONTESANO, R., SOULIE, P., EBLE, J. A. & CARROZZINO, F. 2005. Tumour necrosis factor alpha confers an invasive, transformed phenotype on mammary epithelial cells. *J Cell Sci,* 118**,** 3487-500.

MOREY, R. A., PETTY, C. M., XU, Y., PANNU HAYES, J., WAGNER, H. R., LEWIS, D. V., LABAR, K. S., STYNER, M. & MCCARTHY, G. 2009. A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *NeuroImage,* 45**,** 855-866.

MORIKAWA, O., SAKAI, N., OBARA, H. & SAITO, N. 1998. Effects of interferon-alpha, interferon-gamma and cAMP on the transcriptional regulation of the serotonin transporter. *Eur J Pharmacol,* 349**,** 317-24.

MORON, J. A., ZAKHAROVA, I., FERRER, J. V., MERRILL, G. A., HOPE, B., LAFER, E. M., LIN, Z. C., WANG, J. B., JAVITCH, J. A., GALLI, A. & SHIPPENBERG, T. S. 2003. Mitogen-activated protein kinase regulates dopamine transporter surface expression and dopamine transport capacity. *J Neurosci,* 23**,** 8480-8.

MOSSNER, R., HEILS, A., STOBER, G., OKLADNOVA, O., DANIEL, S. & LESH, K. 1998. Enhancement of serotonin transporter function by tumor necrosis factor alpha but not by interleukin-6. *Neurochemistry International,* 33**,** 251-254.

MULLER, N., SCHWARZ, M. J., DEHNING, S., DOUHE, A., CEROVECKI, A., GOLDSTEIN-MULLER, B., SPELLMANN, I., HETZEL, G., MAINO, K., KLEINDIENST, N., MOLLER, H. J., AROLT, V. & RIEDEL, M. 2006. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol Psychiatry,* 11**,** 680-4.

MURMU, M. S., SALOMON, S., BIALA, Y., WEINSTOCK, M., BRAUN, K. & BOCK, J. 2006. Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy. *Eur J Neurosci,* 24**,** 1477-87.

MYINT, A. M., SCHWARZ, M. J., STEINBUSCH, H. W. & LEONARD, B. E. 2009. Neuropsychiatric disorders related to interferon and interleukins treatment. *Metab Brain Dis,* 24**,** 55-68.

NA, K. S., LEE, K. J., LEE, J. S., CHO, Y. S. & JUNG, H. Y. 2014. Efficacy of adjunctive celecoxib treatment for patients with major depressive disorder: A meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry,* 48**,** 79-85.

NAUDE, P. J., EISEL, U. L., COMIJS, H. C., GROENEWOLD, N. A., DE DEYN, P. P., BOSKER, F. J., LUITEN, P. G., DEN BOER, J. A. & OUDE VOSHAAR, R. C. 2013. Neutrophil gelatinase-associated lipocalin: a novel inflammatory marker associated with late-life depression. *J Psychosom Res,* 75**,** 444-50.

NAZMI, A., DIEZ ROUX, A., RANJIT, N., SEEMAN, T. E. & JENNY, N. S. 2010. Cross-sectional and longitudinal associations of neighborhood characteristics with inflammatory markers: findings from the multi-ethnic study of atherosclerosis. *Health Place,* 16**,** 1104-12.

NEE, D. E., BROWN, J. W., ASKREN, M. K., BERMAN, M. G., DEMIRALP, E., KRAWITZ, A. & JONIDES, J. 2013. A meta-analysis of executive components of working memory. *Cereb Cortex,* 23**,** 264-82.

NESTLE, F., KAPLAN, D. & BARKER, J. 2009. Psoriasis. *The New England Journal of Medicine,* 361**,** 496-509.

NEWMAN, M. E. 2006. Modularity and community structure in networks. *Proc Natl Acad Sci U S A,* 103**,** 8577-82.

NICHOLLS, S. J., TUZCU, E. M., SIPAHI, I., SCHOENHAGEN, P., HAZEN, S. L., NTANIOS, F., WUN, C.-C. & NISSEN, S. E. 2006. Effects of obesity on lipid-lowering, anti-inflammatory, and antiatherosclerotic benefits of atorvastatin or pravastatin in patients with coronary artery disease (from the REVERSAL Study). *The American journal of cardiology,* 97**,** 1553-7.

NICOL, A., KRISHNADAS, R., CHAMPION, S., TAMAGNAN, G., STEHOUWER, J. S., GOODMAN, M. M., HADLEY, D. M. & PIMLOTT, S. L. 2012. Biodistribution and dosimetry of (1)(2)(3)I-mZIENT: a novel ligand for imaging serotonin transporters. *Eur J Nucl Med Mol Imaging,* 39**,** 786-91.

NOBLE, K. G., HOUSTON, S. M., KAN, E. & SOWELL, E. R. 2012. Neural correlates of socioeconomic status in the developing human brain. *Developmental Science,* 15**,** 516-527.

NOGUCHI, T. 1999. Soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 concentrations, and leukocyte count in smokers. *Environ Health Prev Med,* 4**,** 71-4.

NOONAN, M. P., KOLLING, N., WALTON, M. E. & RUSHWORTH, M. F. 2012. Re-evaluating the role of the orbitofrontal cortex in reward and reinforcement. *Eur J Neurosci,* 35**,** 997-1010.

NOWLAN, M. L., DREWE, E., BULSARA, H., ESPOSITO, N., ROBINS, R. A., TIGHE, P. J., POWELL, R. J. & TODD, I. 2006. Systemic cytokine levels and the effects of etanercept in TNF receptor-associated periodic syndrome (TRAPS) involving a C33Y mutation in TNFRSF1A. *Rheumatology (Oxford),* 45**,** 31-7.

O'BRIEN, S. M., SCOTT, L. V. & DINAN, T. G. 2006. Antidepressant therapy and C-reactive protein levels. *Br J Psychiatry,* 188**,** 449-52.

O'BRIEN, S. M., SCULLY, P., FITZGERALD, P., SCOTT, L. V. & DINAN, T. G. 2007. Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. *J Psychiatr Res,* 41**,** 326-31.

OAKES, J. M. 2004. The (mis)estimation of neighborhood effects: causal inference for a practicable social epidemiology. *Soc Sci Med,* 58**,** 1929-52.

OLOFSSON, P. S., ROSAS-BALLINA, M., LEVINE, Y. A. & TRACEY, K. J. 2012. Rethinking inflammation: neural circuits in the regulation of immunity. *Immunol Rev,* 248**,** 188-204.

PACE, T. W., HU, F. & MILLER, A. H. 2007. Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. *Brain Behav Immun,* 21**,** 9-19.

PACE, T. W. & MILLER, A. H. 2009. Cytokines and glucocorticoid receptor signaling. Relevance to major depression. *Ann N Y Acad Sci,* 1179**,** 86-105.

PACKARD, C. J., BEZLYAK, V., MCLEAN, J. S., BATTY, G. D., FORD, I., BURNS, H., CAVANAGH, J., DEANS, K. A., HENDERSON, M., MCGINTY, A., MILLAR, K., SATTAR, N., SHIELS, P. G., VELUPILLAI, Y. N. & TANNAHILL, C. 2011. Early life socioeconomic adversity is associated in adult life with chronic inflammation, carotid atherosclerosis, poorer lung function and decreased

cognitive performance: a cross-sectional, population-based study. *BMC Public Health,* 11**,** 42.

PADOA-SCHIOPPA, C. & CAI, X. 2011. The orbitofrontal cortex and the computation of subjective value: consolidated concepts and new perspectives. *Ann N Y Acad Sci,* 1239**,** 130-7.

PALANIYAPPAN, L. & LIDDLE, P. F. 2012. Differential effects of surface area, gyrification and cortical thickness on voxel based morphometric deficits in schizophrenia. *NeuroImage,* 60**,** 693-9.

PANIZZON, M. S., FENNEMA-NOTESTINE, C., EYLER, L. T., JERNIGAN, T. L., PROM-WORMLEY, E., NEALE, M., JACOBSON, K., LYONS, M. J., GRANT, M. D., FRANZ, C. E., XIAN, H., TSUANG, M., FISCHL, B., SEIDMAN, L., DALE, A. & KREMEN, W. S. 2009. Distinct Genetic Influences on Cortical Surface Area and Cortical Thickness. *Cerebral Cortex,* 19**,** 2728-2735.

PARIANTE, C. M. 2003. Depression, stress and the adrenal axis. *J Neuroendocrinol,* 15**,** 811-2.

PARK, H.-J., LEE, J. D., KIM, E. Y., PARK, B., OH, M.-K., LEE, S. & KIM, J.-J. 2009. Morphological alterations in the congenital blind based on the analysis of cortical thickness and surface area. *NeuroImage,* 47**,** 98-106.

PASCOE, M. C., CREWTHER, S. G., CAREY, L. M. & CREWTHER, D. P. 2011. Inflammation and depression: why poststroke depression may be the norm and not the exception. *Int J Stroke,* 6**,** 128-35.

PATERSON, L. M., KORNUM, B. R., NUTT, D. J., PIKE, V. W. & KNUDSEN, G. M. 2013. 5-HT radioligands for human brain imaging with PET and SPECT. *Med Res Rev,* 33**,** 54-111.

PATERSON, L. M., TYACKE, R. J., NUTT, D. J. & KNUDSEN, G. M. 2010. Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. *J Cereb Blood Flow Metab,* 30**,** 1682-706.

PAWLAK, R., RAO, B. S., MELCHOR, J. P., CHATTARJI, S., MCEWEN, B. & STRICKLAND, S. 2005. Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. *Proc Natl Acad Sci U S A,* 102**,** 18201-6.

PERSOONS, P., VERMEIRE, S., DEMYTTENAERE, K., FISCHLER, B., VANDENBERGHE, J., VAN OUDENHOVE, L., PIERIK, M., HLAVATY, T., VAN ASSCHE, G., NOMAN, M. & RUTGEERTS, P. 2005. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. *Aliment Pharmacol Ther,* 22**,** 101-10.

PETERSEN, K. L., MARSLAND, A. L., FLORY, J., VOTRUBA-DRZAL, E., MULDOON, M. F. & MANUCK, S. B. 2008. Community socioeconomic status is associated with circulating interleukin-6 and C-reactive protein. *Psychosom Med,* 70**,** 646-52.

PHILLIPS, A. C., BATTY, G. D., VAN ZANTEN, J. J. C. S. V., MORTENSEN, L. H., DEARY, I. J., CALVIN, C. M. & CARROLL, D. 2011. Cognitive ability in early adulthood is associated with systemic inflammation in middle age: The Vietnam experience study. *Brain, Behavior, and Immunity,* 25**,** 298-301.

PICARDI, A., MAZZOTTI, E. & PASQUINI, P. 2006. Prevalence and correlates of suicidal ideation among patients with skin disease. *J Am Acad Dermatol,* 54**,** 420-6.

PICKERING, M., CUMISKEY, D. & O'CONNOR, J. J. 2005. Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. *Exp Physiol,* 90**,** 663-70.

PICKETT, K. E. & PEARL, M. 2001. Multilevel analyses of neighbourhood socioeconomic context and health outcomes: a critical review. *J Epidemiol Community Health,* 55**,** 111-22.

PIERUCCI-LAGHA, A., COVAULT, J., BONKOVSKY, H. L., FEINN, R., ABREU, C., STERLING, R. K., FONTANA, R. J. & KRANZLER, H. R. 2010. A functional serotonin transporter gene polymorphism and depressive effects associated with interferon-alpha treatment. *Psychosomatics,* 51**,** 137-48.

PILETZ, J. E., HALARIS, A., IQBAL, O., HOPPENSTEADT, D., FAREED, J., ZHU, H., SINACORE, J. & DEVANE, C. L. 2009. Pro-inflammatory biomakers in depression: treatment with venlafaxine. *World J Biol Psychiatry,* 10**,** 313-23.

PIRAS, F., SALANI, F., BOSSU, P., CALTAGIRONE, C. & SPALLETTA, G. 2012. High serum levels of transforming growth factor beta1 are associated with increased cortical thickness in cingulate and right frontal areas in healthy subjects. *Journal of Neuroinflammation,* 9**,** 42.

POLDRACK, R. A., MUMFORD, J. A. & NICHOLS, T. E. 2011. *Handbook of functional MRI data analysis,* Cambridge, Cambridge University Press.

POLLITT, R. A., KAUFMAN, J. S., ROSE, K. M., DIEZ-ROUX, A. V., ZENG, D. & HEISS, G. 2007. Early-life and adult socioeconomic status and inflammatory risk markers in adulthood. *Eur J Epidemiol,* 22**,** 55-66.

POLLITT, R. A., KAUFMAN, J. S., ROSE, K. M., DIEZ-ROUX, A. V., ZENG, D. & HEISS, G. 2008. Cumulative life course and adult socioeconomic status and markers of inflammation in adulthood. *J Epidemiol Community Health,* 62**,** 484-91.

PREACHER, K. J. & HAYES, A. F. 2008. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods,* 40**,** 879-91.

PRESKORN, S. H. 2012. The use of biomarkers in psychiatric research: how serotonin transporter occupancy explains the dose-response curves of SSRIs. *J Psychiatr Pract,* 18**,** 38-45.

PULKKI-RABACK, L., AHOLA, K., ELOVAINIO, M., KIVIMAKI, M., HINTSANEN, M., ISOMETSA, E., LONNQVIST, J. & VIRTANEN, M. 2012. Socio-economic position and mental disorders in a working-age Finnish population: the health 2000 study. *Eur J Public Health,* 22**,** 327-32.

QUINN, T. J., GALLACHER, J., DEARY, I. J., LOWE, G. D., FENTON, C. & STOTT, D. J. 2011. Association between circulating hemostatic measures and dementia or cognitive impairment: systematic review and meta-analyzes. *J Thromb Haemost,* 9**,** 1475-82.

RAISON, C. L., BORISOV, A. S., MAJER, M., DRAKE, D. F., PAGNONI, G., WOOLWINE, B. J., VOGT, G. J., MASSUNG, B. & MILLER, A. H. 2009. Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. *Biol Psychiatry,* 65**,** 296-303.

RAISON, C. L., CAPURON, L. & MILLER, A. H. 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol,* 27**,** 24-31.

RAISON, C. L., DANTZER, R., KELLEY, K. W., LAWSON, M. A., WOOLWINE, B. J., VOGT, G., SPIVEY, J. R., SAITO, K. & MILLER, A. H. 2010. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry,* 15**,** 393-403.

RAISON, C. L. & MILLER, A. H. 2011. Is Depression an Inflammatory Disorder? *Curr Psychiatry Rep.*

RAISON, C. L., RUTHERFORD, R. E., WOOLWINE, B. J., SHUO, C., SCHETTLER, P., DRAKE, D. F., HAROON, E. & MILLER, A. H. 2013. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-

resistant depression: the role of baseline inflammatory biomarkers. *JAMA Psychiatry,* 70**,** 31-41.
RAJ, A., MUELLER, S. G., YOUNG, K., LAXER, K. D. & WEINER, M. 2010. Network-level analysis of cortical thickness of the epileptic brain. *Neuroimage,* 52**,** 1302-13.
RAJI, C. A., HO, A. J., PARIKSHAK, N. N., BECKER, J. T., LOPEZ, O. L., KULLER, L. H., HUA, X., LEOW, A. D., TOGA, A. W. & THOMPSON, P. M. 2010. Brain structure and obesity. *Human brain mapping,* 31**,** 353-64.
RAKIC, P. 2007. The radial edifice of cortical architecture: from neuronal silhouettes to genetic engineering. *Brain Res Rev,* 55**,** 204-19.
RAKIC, P. 2009. Evolution of the neocortex: a perspective from developmental biology. *Nature Reviews Neuroscience,* 10**,** 724-735.
RAMAMOORTHY, S., RAMAMOORTHY, J., PRASAD, P., BHAT, K., MAHESH, V., LEIBACH, F. & GANAPATHY, V. 1995. Regulation of the human serotonin transporter by interleukin 1B. *Biochemical and biophysical research communications,* 216**,** 560-567.
RANGEL, A., CAMERER, C. & MONTAGUE, P. R. 2008. A framework for studying the neurobiology of value-based decision making. *Nat Rev Neurosci,* 9**,** 545-56.
RATHBONE, R., COUNSELL, S. J., KAPELLOU, O., DYET, L., KENNEA, N., HAJNAL, J., ALLSOP, J. M., COWAN, F. & EDWARDS, A. D. 2011. Perinatal cortical growth and childhood neurocognitive abilities. *Neurology,* 77**,** 1510-7.
RAZNAHAN, A., SHAW, P., LALONDE, F., STOCKMAN, M., WALLACE, G. L., GREENSTEIN, D., CLASEN, L., GOGTAY, N. & GIEDD, J. N. 2011. How Does Your Cortex Grow? *Journal of Neuroscience,* 31**,** 7174-7177.
REDDI, D. & CURRAN, N. 2013. *An introduction to pain pathways and mechanisms* [Online]. Available: http://www.ucl.ac.uk/anaesthesia/StudentsandTrainees/PainPathwaysIntroduction [Accessed November 2013.
REUTER, M., ROSAS, H. D. & FISCHL, B. 2010. Highly accurate inverse consistent registration: a robust approach. *Neuroimage,* 53**,** 1181-96.
REUTER, M., SCHMANSKY, N. J., ROSAS, H. D. & FISCHL, B. 2012. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage,* 61**,** 1402-18.
RICHARDS, B. L., WHITTLE, S. L. & BUCHBINDER, R. 2011. Antidepressants for pain management in rheumatoid arthritis. *Cochrane Database Syst Rev,* 11**,** CD008920.
RIEDER, E. & TAUSK, F. 2013. Psoriasis, a model of dermatologic psychosomatic disease:

psychiatric implications and treatments. *International journal of dermatology,* 51**,** 12-26.
ROETERS VAN LENNEP, J. E., WESTERVELD, H. T., ERKELENS, D. W. & VAN DER WALL, E. E. 2002. Risk factors for coronary heart disease: implications of gender. *Cardiovasc Res,* 53**,** 538-49.
ROMERO-GARCIA, R., ATIENZA, M., CLEMMENSEN, L. H. & CANTERO, J. L. 2012. Effects of network resolution on topological properties of human neocortex. *Neuroimage,* 59**,** 3522-32.
ROSAS, H. D., LIU, A. K., HERSCH, S., GLESSNER, M., FERRANTE, R. J., SALAT, D. H., VAN DER KOUWE, A., JENKINS, B. G., DALE, A. M. & FISCHL, B. 2002. Regional and progressive thinning of the cortical ribbon in Huntington's disease. *Neurology,* 58**,** 695-701.

RUHE, H. G., BOOIJ, J., REITSMA, J. B. & SCHENE, A. H. 2009. Serotonin transporter binding with [123I]beta-CIT SPECT in major depressive disorder versus controls: effect of season and gender. *Eur J Nucl Med Mol Imaging,* 36**,** 841-9.

RUIT, K. G. & NEAFSEY, E. J. 1990. Hippocampal input to a "visceral motor" corticobulbar pathway: an anatomical and electrophysiological study in the rat. *Exp Brain Res,* 82**,** 606-16.

SAARTO, T. & WIFFEN, P. J. 2007. Antidepressants for neuropathic pain: a Cochrane review. *J Neurol Neurosurg Psychiatry,* 81**,** 1372-3.

SAHA, R. N., LIU, X. & PAHAN, K. 2006. Up-regulation of BDNF in astrocytes by TNF-alpha: a case for the neuroprotective role of cytokine. *J Neuroimmune Pharmacol,* 1**,** 212-22.

SAHAY, A. & HEN, R. 2007. Adult hippocampal neurogenesis in depression. *Nat Neurosci,* 10**,** 1110-5.

SALAT, D. H., BUCKNER, R. L., SNYDER, A. Z., GREVE, D. N., DESIKAN, R. S., BUSA, E., MORRIS, J. C., DALE, A. M. & FISCHL, B. 2004. Thinning of the cerebral cortex in aging. *Cereb Cortex,* 14**,** 721-30.

SALINAS, J., MILLS, E. D., CONRAD, A. L., KOSCIK, T., ANDREASEN, N. C. & NOPOULOS, P. 2012. Sex Differences in Parietal Lobe Structure and Development. *Gender Medicine,* 9**,** 44-55.

SAMPOGNA, F., TABOLLI, S. & ABENI, D. 2007. The impact of changes in clinical severity on psychiatric morbidity in patients with psoriasis: a follow-up study. *Br J Dermatol,* 157**,** 508-13.

SAMUVEL, D. J. 2005. A Role for p38 Mitogen-Activated Protein Kinase in the Regulation of the Serotonin Transporter: Evidence for Distinct Cellular Mechanisms Involved in Transporter Surface Expression. *Journal of Neuroscience,* 25**,** 29-41.

SANABRIA-DIAZ, G., MELIE-GARCÍA, L., ITURRIA-MEDINA, Y., ALEMÁN-GÓMEZ, Y., HERNÁNDEZ-GONZÁLEZ, G., VALDÉS-URRUTIA, L., GALÁN, L. & VALDÉS-SOSA, P. 2010. Surface area and cortical thickness descriptors reveal different attributes of the structural human brain networks. *NeuroImage,* 50**,** 1497-1510.

SAVITZ, J. B. & DREVETS, W. C. 2013. Neuroreceptor imaging in depression. *Neurobiol Dis,* 52**,** 49-65.

SCHABERG, T., RAU, M., OERTER, R., LIEBERS, U., RAHN, W., KAISER, D., WITT, C. & LODE, H. 1996. Expression of adhesion molecules in peripheral pulmonary vessels from smokers and nonsmokers. *Lung,* 174**,** 71-81.

SCHAFER, A., SCHEURLEN, M., SEUFERT, J., KEICHER, C., WEISSBRICH, B., RIEGER, P. & KRAUS, M. R. 2010. Platelet serotonin (5-HT) levels in interferon-treated patients with hepatitis C and its possible association with interferon-induced depression. *J Hepatol,* 52**,** 10-5.

SCHILDKRAUT, J. J. 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am J Psychiatry,* 122**,** 509-22.

SCHMIDT, H. D., SHELTON, R. C. & DUMAN, R. S. 2011. Functional biomarkers of depression: diagnosis, treatment, and pathophysiology. *Neuropsychopharmacology,* 36**,** 2375-94.

SCHMITT, J. & FORD, D. E. 2010. Psoriasis is independently associated with psychiatric morbidity and adverse cardiovascular risk factors, but not with cardiovascular events in a population-based sample. *J Eur Acad Dermatol Venereol,* 24**,** 885-92.

SCHMITT, J. M. & FORD, D. E. 2007. Role of depression in quality of life for patients with psoriasis. *Dermatology,* 215**,** 17-27.

SCHULZ, A. J., MENTZ, G., LACHANCE, L., JOHNSON, J., GAINES, C. & ISRAEL, B. A. 2012. Associations between socioeconomic status and allostatic load: effects of neighborhood poverty and tests of mediating pathways. *Am J Public Health,* 102**,** 1706-14.

SCHWARTZ, S., SUSSER, E. & SUSSER, M. 1999. A future for epidemiology? *Annu Rev Public Health,* 20**,** 15-33.

SEELEY, W. W., MENON, V., SCHATZBERG, A. F., KELLER, J., GLOVER, G. H., KENNA, H., REISS, A. L. & GREICIUS, M. D. 2007. Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive Control. *Journal of Neuroscience,* 27**,** 2349-2356.

SEGONNE, F., DALE, A. M., BUSA, E., GLESSNER, M., SALAT, D., HAHN, H. K. & FISCHL, B. 2004. A hybrid approach to the skull stripping problem in MRI. *Neuroimage,* 22**,** 1060-75.

SEGONNE, F., PACHECO, J. & FISCHL, B. 2007. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging,* 26**,** 518-29.

SEO, S. W., LEE, J. M., IM, K., PARK, J. S., KIM, S. H., KIM, S. T., AHN, H. J., CHIN, J., CHEONG, H. K., WEINER, M. W. & NA, D. L. 2012a. Cortical thinning related to periventricular and deep white matter hyperintensities. *Neurobiol Aging,* 33**,** 1156-67.

SEO, S. W., LEE, J. M., IM, K., PARK, J. S., KIM, S. H., KIM, S. T., AHN, J. H., KIM, M. J., KIM, G. H., KIM, J. H., ROH, J. H., CHEONG, H. K. & NA, D. L. 2012b. Cardiovascular risk factors cause cortical thinning in cognitively impaired patients: relationships among cardiovascular risk factors, white matter hyperintensities, and cortical atrophy. *Alzheimer Dis Assoc Disord,* 26**,** 106-12.

SGHENDO, L. & MIFSUD, J. 2012. Understanding the molecular pharmacology of the serotonergic system: using fluoxetine as a model. *J Pharm Pharmacol,* 64**,** 317-25.

SHAMAI, L., LURIX, E., SHEN, M., NOVARO, G. M., SZOMSTEIN, S., ROSENTHAL, R., HERNANDEZ, A. V. & ASHER, C. R. 2011. Association of body mass index and lipid profiles: evaluation of a broad spectrum of body mass index patients including the morbidly obese. *Obesity surgery,* 21**,** 42-7.

SHELTON, R. C., CLAIBORNE, J., SIDORYK-WEGRZYNOWICZ, M., REDDY, R., ASCHNER, M., LEWIS, D. A. & MIRNICS, K. 2011. Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol Psychiatry,* 16**,** 751-62.

SHIELS, P. G., MCGLYNN, L. M., MACINTYRE, A., JOHNSON, P. C., BATTY, G. D., BURNS, H., CAVANAGH, J., DEANS, K. A., FORD, I., MCCONNACHIE, A., MCGINTY, A., MCLEAN, J. S., MILLAR, K., SATTAR, N., TANNAHILL, C., VELUPILLAI, Y. N. & PACKARD, C. J. 2011. Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid cohort. *PLOS One,* 6**,** e22521.

ŠIDÁK, Z. K. 1967. Rectangular Confidence Regions for the Means of Multivariate Normal Distributions. *Journal of the American Statistical Association,* 62**,** 626–633.

SKAPINAKIS, P., WEICH, S., LEWIS, G., SINGLETON, N. & ARAYA, R. 2006. Socio-economic position and common mental disorders. Longitudinal study in the general population in the UK. *Br J Psychiatry,* 189**,** 109-17.

SLED, J. G., ZIJDENBOS, A. P. & EVANS, A. C. 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging,* 17**,** 87-97.

SMITH, A., PATTERSON, C., YARNELL, J., RUMLEY, A., BEN-SHLOMO, Y. & LOWE, G. 2005. Which hemostatic markers add to the predictive value of conventional risk factors for coronary heart disease and ischemic stroke? The Caerphilly Study. *Circulation,* 112**,** 3080-7.

SMITH, D. J., MUIR, W. J. & BLACKWOOD, D. H. 2006. Neurocognitive impairment in euthymic young adults with bipolar spectrum disorder and recurrent major depressive disorder. *Bipolar Disord,* 8**,** 40-6.

SMOTHERMAN, W. P., KOLP, L. A., COYLE, S. & LEVINE, S. 1981. Hippocampal lesion effects on conditioned taste aversion and pituitary-adrenal activity in rats. *Behav Brain Res,* 2**,** 33-48.

SOCKALINGAM, S., LINKS, P. S. & ABBEY, S. E. Suicide risk in hepatitis C and during interferon-alpha therapy: a review and clinical update. *J Viral Hepat,* 18**,** 153-60.

SODHI, M. & SANDERS-BUSH, E. 2004. Serotonin and brain development. *International review of neurobiology,* 59**,** 111-174.

SOLOMON, A., LEONI, V., KIVIPELTO, M., BESGA, A., ÖKSENGÅRD, A. R., JULIN, P., SVENSSON, L., WAHLUND, L.-O., ANDREASEN, N., WINBLAD, B., SOININEN, H. & BJÖRKHEM, I. 2009. Plasma levels of 24S-hydroxycholesterol reflect brain volumes in patients without objective cognitive impairment but not in those with Alzheimer's disease. *Neuroscience Letters,* 462**,** 89-93.

SONTY, S. P., MESULAM, M. M., WEINTRAUB, S., JOHNSON, N. A., PARRISH, T. B. & GITELMAN, D. R. 2007. Altered Effective Connectivity within the Language Network in Primary Progressive Aphasia. *Journal of Neuroscience,* 27**,** 1334-1345.

SOUSA, N., LUKOYANOV, N. V., MADEIRA, M. D., ALMEIDA, O. F. & PAULA-BARBOSA, M. M. 2000. Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience,* 97**,** 253-66.

SOWELL, E. R., PETERSON, B. S., KAN, E., WOODS, R. P., YOSHII, J., BANSAL, R., XU, D., ZHU, H., THOMPSON, P. M. & TOGA, A. W. 2007. Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age. *Cereb Cortex,* 17**,** 1550-60.

SPALDING, K. L., BERGMANN, O., ALKASS, K., BERNARD, S., SALEHPOUR, M., HUTTNER, H. B., BOSTROM, E., WESTERLUND, I., VIAL, C., BUCHHOLZ, B. A., POSSNERT, G., MASH, D. C., DRUID, H. & FRISEN, J. 2013. Dynamics of hippocampal neurogenesis in adult humans. *Cell,* 153**,** 1219-27.

SPITZER, C., BARNOW, S., VOLZKE, H., WALLASCHOFSKI, H., JOHN, U., FREYBERGER, H. J., LOWE, B. & GRABE, H. J. 2010. Association of posttraumatic stress disorder with low-grade elevation of C-reactive protein: evidence from the general population. *J Psychiatr Res,* 44**,** 15-21.

SPORNS, O. 2011. The human connectome: a complex network. *Annals of the New York Academy of Sciences,* 1224**,** 109-125.

SQUIRE, L. R., STARK, C. E. & CLARK, R. E. 2004. The medial temporal lobe. *Annu Rev Neurosci,* 27**,** 279-306.

SQUIRE, L. R. & ZOLA-MORGAN, S. 1991. The medial temporal lobe memory system. *Science,* 253**,** 1380-6.

SRIREDDY, P., AGNIHOTRI, A., PARK, J., TAYLOR, J., CONNOLLY, M. & KRISHNADAS, R. 2012. Ethnicity, Deprivation and Psychosis: the Glasgow experience. *Epidemiology and Psychiatric Sciences,* In press.

STAFF, R. T., MURRAY, A. D., AHEARN, T. S., MUSTAFA, N., FOX, H. C. & WHALLEY, L. J. 2012. Childhood socioeconomic status and adult brain

size: Childhood socioeconomic status influences adult hippocampal size. *Annals of Neurology,* 71**,** 653-660.

STAFFORD, M. & MARMOT, M. 2003. Neighbourhood deprivation and health: does it affect us all equally? *Int J Epidemiol,* 32**,** 357-66.

STEIN, J. H., KORCARZ, C. E., HURST, R. T., LONN, E., KENDALL, C. B., MOHLER, E. R., NAJJAR, S. S., REMBOLD, C. M., POST, W. S. & AMERICAN SOCIETY OF ECHOCARDIOGRAPHY CAROTID INTIMA-MEDIA THICKNESS TASK, F. 2008. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr,* 21**,** 93-111; quiz 189-90.

STEINER, J., BIELAU, H., BRISCH, R., DANOS, P., ULLRICH, O., MAWRIN, C., BERNSTEIN, H. G. & BOGERTS, B. 2008. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J Psychiatr Res,* 42**,** 151-7.

STEINER, J., WALTER, M., GOS, T., GUILLEMIN, G. J., BERNSTEIN, H. G., SARNYAI, Z., MAWRIN, C., BRISCH, R., BIELAU, H., MEYER ZU SCHWABEDISSEN, L., BOGERTS, B. & MYINT, A. M. 2011. Severe depression is associated with increased microglial quinolinic acid in subregions of the anterior cingulate gyrus: evidence for an immune-modulated glutamatergic neurotransmission? *J Neuroinflammation,* 8**,** 94.

STELLWAGEN, D., BEATTIE, E. C., SEO, J. Y. & MALENKA, R. C. 2005. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. *J Neurosci,* 25**,** 3219-28.

STEPTOE, A. & FELDMAN, P. 2001. Neighborhood Problems as Sources of Chronic Stress: Development of a Measure of Neighborhood Problems, and Associations With Socioeconomic Status and Health. *Annals of Behavioural Medicine,* 23**,** 177-185.

STEPTOE, A. & MARMOT, M. 2002. The role of psychobiological pathways in socio-economic inequalities in cardiovascular disease risk. *Eur Heart J,* 23**,** 13-25.

STEWART, J. C., RAND, K. L., MULDOON, M. F. & KAMARCK, T. W. 2009. A prospective evaluation of the directionality of the depression-inflammation relationship. *Brain Behav Immun,* 23**,** 936-44.

STRIKE, P. C., WARDLE, J. & STEPTOE, A. 2004. Mild acute inflammatory stimulation induces transient negative mood. *J Psychosom Res,* 57**,** 189-94.

STRINGHINI, S., BATTY, G. D., BOVET, P., SHIPLEY, M. J., MARMOT, M. G., KUMARI, M., TABAK, A. G. & KIVIMAKI, M. 2013. Association of lifecourse socioeconomic status with chronic inflammation and type 2 diabetes risk: the Whitehall II prospective cohort study. *PLoS Med,* 10**,** e1001479.

STROBER, B., TELLER, C., YAMAUCHI, P., MILLER, J. L., HOOPER, M., YANG, Y. C. & DANN, F. 2008. Effects of etanercept on C-reactive protein levels in psoriasis and psoriatic arthritis. *British Journal of Dermatology,* 159**,** 322-330.

SU, K. P., HUANG, S. Y., PENG, C. Y., LAI, H. C., HUANG, C. L., CHEN, Y. C., AITCHISON, K. J. & PARIANTE, C. M. 2010. Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-alpha-induced depression by regulating polyunsaturated fatty acids levels. *Biol Psychiatry,* 67**,** 550-7.

SUFFREDINI, A. F., REDA, D., BANKS, S. M., TROPEA, M., AGOSTI, J. M. & MILLER, R. 1995. Effects of recombinant dimeric TNF receptor on human

inflammatory responses following intravenous endotoxin administration. *J Immunol,* 155**,** 5038-45.
SULLIVAN, G. M., APERGIS, J., BUSH, D. E., JOHNSON, L. R., HOU, M. & LEDOUX, J. E. 2004. Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. *Neuroscience,* 128**,** 7-14.
SULLIVAN, R. M. & GRATTON, A. 1999. Lateralized effects of medial prefrontal cortex lesions on neuroendocrine and autonomic stress responses in rats. *J Neurosci,* 19**,** 2834-40.
SULLIVAN, R. M. & GRATTON, A. 2002. Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: side matters. *Psychoneuroendocrinology,* 27**,** 99-114.
SUSSER, M. 1994. The logic in ecological: I. The logic of analysis. *Am J Public Health,* 84**,** 825-9.
TAKI, Y., THYREAU, B., KINOMURA, S., SATO, K., GOTO, R., WU, K., KAKIZAKI, M., TSUJI, I., KAWASHIMA, R. & FUKUDA, H. 2012. Correlation between high-sensitivity C-reactive protein and brain gray matter volume in healthy elderly subjects. *Human Brain Mapping***,** n/a-n/a.
TALAIRACH J & P, T. 1988. *Co-planar stereotaxic atlas of the human brain,* New York, Thieme
TAN, J. K., APHALE, A., MALAVIYA, R., SUN, Y. & GOTTLIEB, A. B. 2007. Mechanisms of action of etanercept in psoriasis. *J Investig Dermatol Symp Proc,* 12**,** 38-45.
TEICHER, M. H., ANDERSON, C. M. & POLCARI, A. 2012. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. *Proceedings of the National Academy of Sciences,* 109**,** E563-E572.
TEUNISSEN, C. E., VAN BOXTEL, M. P., BOSMA, H., BOSMANS, E., DELANGHE, J., DE BRUIJN, C., WAUTERS, A., MAES, M., JOLLES, J., STEINBUSCH, H. W. & DE VENTE, J. 2003. Inflammation markers in relation to cognition in a healthy aging population. *J Neuroimmunol,* 134**,** 142-50.
THESCOTTISHGOVERNMENT. 2004. *Scottish index of multiple deprivation 2004 : summary technical report* [Online]. Edinburgh: Scottish Executive. Available: http://www.scotland.gov.uk/Topics/Statistics/SIMD.
THORSLUND, K., AMATYA, B., DUFVA, A. E. & NORDLIND, K. 2013. The expression of serotonin transporter protein correlates with the severity of psoriasis and chronic stress. *Arch Dermatol Res,* 305**,** 99-104.
TILI, E., MICHAILLE, J. J., WERNICKE, D., ALDER, H., COSTINEAN, S., VOLINIA, S. & CROCE, C. M. 2011. Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer. *Proc Natl Acad Sci U S A,* 108**,** 4908-13.
TOBINICK, E. 2009. Perispinal etanercept for neuroinflammatory disorders. *Drug Discov Today,* 14**,** 168-77.
TOBINICK, E. 2010. Perispinal etanercept: a new therapeutic paradigm in neurology. *Expert Rev Neurother,* 10**,** 985-1002.
TOBINICK, E. L. & GROSS, H. 2008. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. *J Neuroinflammation,* 5**,** 2.
TOMASI, D. & VOLKOW, N. D. 2012. Resting functional connectivity of language networks: characterization and reproducibility. *Mol Psychiatry,* 17**,** 841-54.
TOTH, P. P. 2004. High-density lipoprotein and cardiovascular risk. *Circulation,* 109**,** 1809-12.

TSAO, C. W., LIN, Y. S., CHENG, J. T., LIN, C. F., WU, H. T., WU, S. R. & TSAI, W. H. 2008. Interferon-alpha-induced serotonin uptake in Jurkat T cells via mitogen-activated protein kinase and transcriptional regulation of the serotonin transporter. *J Psychopharmacol,* 22**,** 753-60.

TSIMBERIDOU, A. M., WADDELOW, T., KANTARJIAN, H. M., ALBITAR, M. & GILES, F. J. 2003. Pilot study of recombinant human soluble tumor necrosis factor (TNF) receptor (p75) fusion protein (TNFR:Fc; Enbrel) in patients with refractory multiple myeloma: increase in plasma TNF alpha levels during treatment. *Leuk Res,* 27**,** 375-80.

TSUKASAKI, K., MILLER, C. W., KUBOTA, T., TAKEUCHI, S., FUJIMOTO, T., IKEDA, S., TOMONAGA, M. & KOEFFLER, H. P. 2001. Tumor necrosis factor alpha polymorphism associated with increased susceptibility to development of adult T-cell leukemia/lymphoma in human T-lymphotropic virus type 1 carriers. *Cancer Res,* 61**,** 3770-4.

TYRING, S., GOTTLIEB, A., PAPP, K., GORDON, K., LEONARDI, C., WANG, A., LALLA, D., WOOLLEY, M., JAHREIS, A., ZITNIK, R., CELLA, D. & KRISHNAN, R. 2006. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet,* 367**,** 29-35.

ULRICH-LAI, Y. M. & HERMAN, J. P. 2009. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci,* 10**,** 397-409.

UMEMURA, T., KAWAMURA, T., UMEGAKI, H., MASHITA, S., KANAI, A., SAKAKIBARA, T., HOTTA, N. & SOBUE, G. 2011. Endothelial and inflammatory markers in relation to progression of ischaemic cerebral small-vessel disease and cognitive impairment: a 6-year longitudinal study in patients with type 2 diabetes mellitus. *J Neurol Neurosurg Psychiatry,* 82**,** 1186-94.

UNDURRAGA, J. & BALDESSARINI, R. J. 2012. Randomized, placebo-controlled trials of antidepressants for acute major depression: thirty-year meta-analytic review. *Neuropsychopharmacology,* 37**,** 851-64.

VAESSEN, M. J., BRAAKMAN, H. M., HEERINK, J. S., JANSEN, J. F., DEBEIJ-VAN HALL, M. H., HOFMAN, P. A., ALDENKAMP, A. P. & BACKES, W. H. 2012. Abnormal Modular Organization of Functional Networks in Cognitively Impaired Children with Frontal Lobe Epilepsy. *Cereb Cortex*.

VALKANOVA, V., EBMEIER, K. P. & ALLAN, C. L. 2013. CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. *Journal of Affective Disorders,* 150**,** 736–744.

VAN DEN HEUVEL, M. P., STAM, C. J., KAHN, R. S. & HULSHOFF POL, H. E. 2009. Efficiency of functional brain networks and intellectual performance. *J Neurosci,* 29**,** 7619-24.

VAN DER MEER, I. M., BOTS, M. L., HOFMAN, A., DEL SOL, A. I., VAN DER KUIP, D. A. & WITTEMAN, J. C. 2004. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. *Circulation,* 109**,** 1089-94.

VAN WIJK, B., STAM, C. & DAFFERTSHOFER, A. 2010. Comparing brain networks of different size and connectivity density using graph theory. *PloS one,* 5.

VELUPILLAI, Y. N., PACKARD, C. J., BATTY, G. D., BEZLYAK, V., BURNS, H., CAVANAGH, J., DEANS, K., FORD, I., MCGINTY, A., MILLAR, K., SATTAR, N., SHIELS, P. & TANNAHILL, C. 2008. Psychological, social and biological determinants of ill health (pSoBid): Study Protocol of a population-based study. *BMC Public Health,* 8**,** 126.

VEZZANI, A., BALOSSO, S. & RAVIZZA, T. 2008a. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun,* 22**,** 797-803.

VEZZANI, A., FRENCH, J., BARTFAI, T. & BARAM, T. Z. 2011. The role of inflammation in epilepsy. *Nat Rev Neurol,* 7**,** 31-40.

VEZZANI, A., RAVIZZA, T., BALOSSO, S. & ARONICA, E. 2008b. Glia as a source of cytokines: implications for neuronal excitability and survival. *Epilepsia,* 49 Suppl 2**,** 24-32.

VIVIANI, B., BARTESAGHI, S., GARDONI, F., VEZZANI, A., BEHRENS, M. M., BARTFAI, T., BINAGLIA, M., CORSINI, E., DI LUCA, M., GALLI, C. L. & MARINOVICH, M. 2003. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci,* 23**,** 8692-700.

VYAS, A., MITRA, R., SHANKARANARAYANA RAO, B. S. & CHATTARJI, S. 2002. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci,* 22**,** 6810-8.

WAJANT, H., PFIZENMAIER, K. & SCHEURICH, P. 2003. Tumor necrosis factor signaling. *Cell Death Differ,* 10**,** 45-65.

WALHOVD, K. B., FJELL, A. M., REINVANG, I., LUNDERVOLD, A., FISCHL, B., SALAT, D., QUINN, B. T., MAKRIS, N. & DALE, A. M. 2005. Cortical volume and speed-of-processing are complementary in prediction of performance intelligence. *Neuropsychologia,* 43**,** 704-13.

WALTHER, K., BIRDSILL, A. C., GLISKY, E. L. & RYAN, L. 2010. Structural brain differences and cognitive functioning related to body mass index in older females. *Human brain mapping,* 31**,** 1052-64.

WALTON, M. E., BEHRENS, T. E., NOONAN, M. P. & RUSHWORTH, M. F. 2011. Giving credit where credit is due: orbitofrontal cortex and valuation in an uncertain world. *Ann N Y Acad Sci,* 1239**,** 14-24.

WAN, W., WETMORE, L., SORENSEN, C. M., GREENBERG, A. H. & NANCE, D. M. 1994. Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. *Brain Res Bull,* 34**,** 7-14.

WANG, J. L., SCHMITZ, N. & DEWA, C. S. 2010. Socioeconomic status and the risk of major depression: the Canadian National Population Health Survey. *J Epidemiol Community Health,* 64**,** 447-52.

WANNAMETHEE, S. G., WHINCUP, P. H., LENNON, L., RUMLEY, A. & LOWE, G. D. 2012. Fibrin D-dimer, tissue-type plasminogen activator, von Willebrand factor, and risk of incident stroke in older men. *Stroke,* 43**,** 1206-11.

WARD, M. A., BENDLIN, B. B., MCLAREN, D. G., HESS, T. M., GALLAGHER, C. L., KASTMAN, E. K., ROWLEY, H. A., ASTHANA, S., CARLSSON, C. M., SAGER, M. A. & JOHNSON, S. C. 2010. Low HDL Cholesterol is Associated with Lower Gray Matter Volume in Cognitively Healthy Adults. *Frontiers in aging neuroscience,* 2.

WARNER-SCHMIDT, J. L., VANOVER, K. E., CHEN, E. Y., MARSHALL, J. J. & GREENGARD, P. 2011. Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by antiinflammatory drugs in mice and humans. *Proc Natl Acad Sci U S A,* 108**,** 9262-7.

WATERS, J. P., POBER, J. S. & BRADLEY, J. R. 2013. Tumour necrosis factor in infectious disease. *J Pathol,* 230**,** 132-47.

WEBSTER, K., CELLA, D. & YOST, K. 2003. The Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System: properties, applications, and interpretation. *Health Qual Life Outcomes,* 1**,** 79.

WERSCHING, H., DUNING, T., LOHMANN, H., MOHAMMADI, S., STEHLING, C., FOBKER, M., CONTY, M., MINNERUP, J., RINGELSTEIN, E. B., BERGER, K., DEPPE, M. & KNECHT, S. 2010. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. *Neurology,* 74**,** 1022-9.

WILLIAMS, V. J., LERITZ, E. C., SHEPEL, J., MCGLINCHEY, R. E., MILBERG, W. P., RUDOLPH, J. L., LIPSITZ, L. A. & SALAT, D. H. 2012. Interindividual variation in serum cholesterol is associated with regional white matter tissue integrity in older adults. *Hum Brain Mapp*.

WILSON, C. J., FINCH, C. E. & COHEN, H. J. 2002. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. *J Am Geriatr Soc,* 50**,** 2041-56.

WINKLER, A. M., KOCHUNOV, P., BLANGERO, J., ALMASY, L., ZILLES, K., FOX, P. T., DUGGIRALA, R. & GLAHN, D. C. 2010. Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *NeuroImage,* 53**,** 1135-1146.

WITTLING, W. 1997. The right hemisphere and the human stress response. *Acta Physiol Scand Suppl,* 640**,** 55-9.

WITTLING, W. & SCHWEIGER, E. 1993a. Alterations of neuroendocrine brain asymmetry: a neural risk factor affecting physical health. *Neuropsychobiology,* 28**,** 25-9.

WITTLING, W. & SCHWEIGER, E. 1993b. Neuroendocrine brain asymmetry and physical complaints. *Neuropsychologia,* 31**,** 591-608.

WORSLEY, K. J., CHEN, J. I., LERCH, J. & EVANS, A. C. 2005. Comparing functional connectivity via thresholding correlations and singular value decomposition. *Philos Trans R Soc Lond B Biol Sci,* 360**,** 913-20.

WRIGHT, C. B., SACCO, R. L., RUNDEK, T. R., DELMAN, J. B., RABBANI, L. E. & ELKIND, M. S. V. 2006. Interleukin-6 Is Associated With Cognitive Function: The Northern Manhattan Study. *Journal of Stroke and Cerebrovascular Diseases,* 15**,** 34-38.

WRIGHT, C. E., STRIKE, P. C., BRYDON, L. & STEPTOE, A. 2005. Acute inflammation and negative mood: mediation by cytokine activation. *Brain Behav Immun,* 19**,** 345-50.

WU, H. Q., RASSOULPOUR, A. & SCHWARCZ, R. 2007. Kynurenic acid leads, dopamine follows: a new case of volume transmission in the brain? *J Neural Transm,* 114**,** 33-41.

WU, Y., SHAGHAGHI, E. K., JACQUOT, C., PALLARDY, M. & GARDIER, A. M. 1999. Synergism between interleukin-6 and interleukin-1beta in hypothalamic serotonin release: a reverse in vivo microdialysis study in F344 rats. *Eur Cytokine Netw,* 10**,** 57-64.

YAFFE, K., LINDQUIST, K., PENNINX, B. W., SIMONSICK, E. M., PAHOR, M., KRITCHEVSKY, S., LAUNER, L., KULLER, L., RUBIN, S. & HARRIS, T. 2003. Inflammatory markers and cognition in well-functioning African-American and white elders. *Neurology,* 61**,** 76-80.

YANG, Y., RAINE, A., JOSHI, A. A., JOSHI, S., CHANG, Y. T., SCHUG, R. A., WHELAND, D., LEAHY, R. & NARR, K. L. 2012. Frontal information flow and connectivity in psychopathy. *Br J Psychiatry,* 201**,** 408-9.

YARKONI, T. 2009. Big Correlations in Little Studies: Inflated fMRI Correlations Reflect Low Statistical Power—Commentary on Vul et al. *Perspectives of Psychoological Science,* 4**,** 294-298.

YOKUM, S., NG, J. & STICE, E. 2012. Relation of regional gray and white matter volumes to current BMI and future increases in BMI: a prospective MRI study. *International journal of obesity (2005),* 36**,** 656-64.

YU, B., BECNEL, J., ZERFAOUI, M., ROHATGI, R., BOULARES, A. H. & NICHOLS, C. D. 2008. Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency. *J Pharmacol Exp Ther,* 327**,** 316-23.

ZALESKY, A., FORNITO, A. & BULLMORE, E. T. 2010. Network-based statistic: identifying differences in brain networks. *Neuroimage,* 53**,** 1197-207.

ZARATE, C., JR., MACHADO-VIEIRA, R., HENTER, I., IBRAHIM, L., DIAZGRANADOS, N. & SALVADORE, G. 2010. Glutamatergic modulators: the future of treating mood disorders? *Harv Rev Psychiatry,* 18**,** 293-303.

ZHANG, J., TERRENI, L., DE SIMONI, M. G. & DUNN, A. J. 2001. Peripheral interleukin-6 administration increases extracellular concentrations of serotonin and the evoked release of serotonin in the rat striatum. *Neurochem Int,* 38**,** 303-8.

ZHAO, Y., LEE, H., KIM, J. & CHO, K. 2011. Low-degree nodes having strong local effects but weak global effects could be drug targets. *The 12th International Conference on Systems Biology*. Heidelberg and Mannheim.

ZHAO, Z. Q., CHIECHIO, S., SUN, Y. G., ZHANG, K. H., ZHAO, C. S., SCOTT, M., JOHNSON, R. L., DENERIS, E. S., RENNER, K. J., GEREAU, R. W. T. & CHEN, Z. F. 2007. Mice lacking central serotonergic neurons show enhanced inflammatory pain and an impaired analgesic response to antidepressant drugs. *J Neurosci,* 27**,** 6045-53.

ZHU, C. B., BLAKELY, R. D. & HEWLETT, W. A. 2006. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology,* 31**,** 2121-31.

ZHU, C. B., LINDLER, K. M., OWENS, A. W., DAWS, L. C., BLAKELY, R. D. & HEWLETT, W. A. 2010. Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. *Neuropsychopharmacology,* 35**,** 2510-20.

ZOU, J., RUDWALEIT, M., BRANDT, J., THIEL, A., BRAUN, J. & SIEPER, J. 2003. Up regulation of the production of tumour necrosis factor alpha and interferon gamma by T cells in ankylosing spondylitis during treatment with etanercept. *Ann Rheum Dis,* 62**,** 561-4.