Neuroinflammation in Major Depressive Disorder and Schizophrenia: a PET study

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

2015

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<td>ACTH</td>
<td>Adrenocorticotrophic hormone</td>
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<tr>
<td>APP</td>
<td>Active Phase Protein</td>
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<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
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<td>BBB</td>
<td>Blood-brain-barrier</td>
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<td>BDI</td>
<td>Beck Depression Inventory</td>
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<td>BDNF</td>
<td>Brain-derived Neurotrophic Factor</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BPND</td>
<td>Binding Potential (non-displaceable)</td>
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<td>CAQ</td>
<td>Childhood Adversity Questionnaire</td>
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<tr>
<td>CBT</td>
<td>Cognitive Behaviour Therapy</td>
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<tr>
<td>CER</td>
<td>Cerebellum</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation (cell surface markers)</td>
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<td>CMHT</td>
<td>Community Mental Health Team</td>
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<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
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<td>CNS</td>
<td>Central Nervous System</td>
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<td>COX-2</td>
<td>Cyclooxygenase-2</td>
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<tr>
<td>CRH</td>
<td>Corticotrophin Releasing Hormone</td>
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<tr>
<td>CRP</td>
<td>C-reactive Protein</td>
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<tr>
<td>CSF</td>
<td>Cerebro-Spinal Fluid</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>DA</td>
<td>Dopamine</td>
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<td>DLPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
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<td>DOI</td>
<td>Depth of Interaction</td>
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<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
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<td>Early Intervention Service</td>
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<td>GC</td>
<td>Glucocorticoid</td>
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<td>Glutamate</td>
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<td>Glucocorticoid Receptor</td>
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<td>GWAS</td>
<td>Genome Wide Association Study</td>
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<td>FEP</td>
<td>First Episode Psychosis</td>
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<td>FGA</td>
<td>First Generation Antipsychotic</td>
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<td>HAB</td>
<td>High-Affinity Binder</td>
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<td>Hamilton Depression Rating Scale</td>
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<td>HDRS</td>
<td>Hamilton Depression Rating Scale</td>
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<td>HPA axis</td>
<td>Hypothalamic-Pituitary-Adrenal axis</td>
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<td>HRRT</td>
<td>High Resolution Research Tomograph</td>
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<tr>
<td>HV</td>
<td>Healthy Volunteer</td>
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<tr>
<td>ICD</td>
<td>International Statistical Classification of Diseases and Related Health Problems</td>
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<td>IDO</td>
<td>Indoleamine 2,3-dioxygenase</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>KYN</td>
<td>Kynurenine</td>
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<td>KYNA</td>
<td>Kynurenic Acid</td>
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<td>LAB</td>
<td>Low-Affinity Binder</td>
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<td>Lipopolysaccharide</td>
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<td>LOR</td>
<td>Line of Response</td>
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<td>MAB</td>
<td>Mixed-Affinity Binder</td>
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<td>MAOI</td>
<td>Monoamine Oxidase Inhibitor</td>
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<td>MADRS</td>
<td>Montgomery-Asberg Depression Rating Scale</td>
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<tr>
<td>MDE</td>
<td>Major Depressive Episode</td>
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<td>MDD</td>
<td>Major Depressive Disorder</td>
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<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<td>MI</td>
<td>Myocardial Infarction</td>
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<tr>
<td>Abbreviation</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<tr>
<td>NMDAr</td>
<td>N-methyl-D-aspartate receptor</td>
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<tr>
<td>O&amp;NS</td>
<td>Oxidative and Nitrosative Stress</td>
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<td>OFC</td>
<td>Orbitofrontal Cortex</td>
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<td>PAMPS</td>
<td>Pathogen-associated molecular patterns</td>
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<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
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<td>Peripheral Benzodiazepine Receptor</td>
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<td>PCC</td>
<td>Posterior Cingulate Cortex</td>
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<td>PET</td>
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<td>Prefrontal Cortex</td>
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<td>Patient Health Questionnaire-9</td>
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<td>PVN</td>
<td>Paraventricular Nucleus</td>
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<td>QA</td>
<td>Quinolinic Acid</td>
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<td>RNS</td>
<td>Reactive Nitrogen Species</td>
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<td>ROI</td>
<td>Region of Interest</td>
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<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<td>SCI</td>
<td>Sleep Condition Indicator</td>
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<td>SGA</td>
<td>Second Generation Antipsychotic</td>
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<td>SRTM</td>
<td>Simplified Reference Tissue Method</td>
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<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
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<td>SVC</td>
<td>Supervised Cluster</td>
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<td>TCA</td>
<td>Tricyclic antidepressant</td>
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<td>TDO</td>
<td>Tryptophan 2,3-dioxygenase</td>
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<td>TGF</td>
<td>Transforming Growth Factor</td>
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<td>TLR</td>
<td>Toll-like receptor</td>
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<td>Tumor Necrosis Factor</td>
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<td>Tryptophan</td>
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<td>TRYCAT</td>
<td>Tryptophan Catabolite</td>
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<td>Translocator Protein</td>
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<td>WMIC</td>
<td>Wolfson Molecular Imaging Centre</td>
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<td>5-HT</td>
<td>Serotonin</td>
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<tr>
<td>2TCM</td>
<td>Two tissue Compartment Model</td>
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Abstract

Neuroinflammation in Major Depressive Disorder and Schizophrenia: A PET Study

A thesis submitted to the University of Manchester for the degree of PhD in the Faculty of Medical and Human Sciences by Sophie E Holmes, September 2015.

Background: Mounting evidence suggests that inflammation is involved in the pathophysiology of both Major Depressive Disorder (MDD) and schizophrenia. The presence of inflammation in the brain, however, is less clear. Microglial activation, a measure of neuroinflammation, can be quantified using PET ligands that bind to the Translocator Protein (TSPO) which is overexpressed by activated microglia. Previous PET studies using TSPO radioligands have shown some evidence for neuroinflammation in both MDD and schizophrenia. However some of these studies have been confounded by antidepressant/antipsychotic medication, low numbers and mild severity. We aimed to address some of these issues and investigate the relationship between neuroinflammation and peripheral inflammation, medication status, symptom severity and cognitive function.

Method: Fourteen patients in a Major Depressive Episode (MDE) of at least moderate severity, sixteen patients with a diagnosis of schizophrenia of at least moderate severity and a total of eighteen age and gender matched healthy volunteers underwent a 60 minute dynamic PET scan with the TSPO radioligand $[\text{11C}]\text{(R)-PK11195}$ on the High Resolution Research Tomograph (HRRT). Parametric maps of binding potential ($\text{BP}_{\text{ND}}$) were generated using the simplified reference tissue model and a grey matter cerebellum input function. All of the MDD patients were antidepressant-free for at least eight months prior to scanning. Of the sixteen schizophrenia patients, eight were antipsychotic-free (for at least twelve months) and eight were on a long-acting injection of risperidone or paliperidone. All patients and healthy volunteers were medically healthy and had drug or alcohol abuse within the previous year.

Results: We found a 26% mean increase in $\text{BP}_{\text{ND}}$ values, indicative of microglial activation, in MDD patients compared to healthy volunteers. Exploratory analysis revealed significantly higher $[\text{11C}]\text{(R)-PK11195}$ binding in the anterior cingulate cortex (ACC). We found no significant correlations between $[\text{11C}]\text{(R)-PK11195}$ binding and peripheral markers of inflammation or with symptom severity. We found no significant correlations between $[\text{11C}]\text{(R)-PK11195}$ and negative symptoms across multiple brain regions. When breaking the cohort down according to medication status, there was no difference between antipsychotic-free patients and healthy volunteers. However, mean $\text{BP}_{\text{ND}}$ values were 30% higher in the ACC. The medicated patients exhibited higher $\text{BP}_{\text{ND}}$ values than healthy volunteers, with a mean increase of 48%. Exploratory t-tests revealed significant increases in dorsolateral prefrontal cortex and ACC.

Conclusions: Our findings are largely consistent with previous PET findings of increased microglial activation in a sample of antidepressant-free patients in a moderate-to-severe MDE, suggesting that neuroinflammation is present in MDD. We also investigated neuroinflammation in antipsychotic-free patients for the first time and found no evidence of microglial activation. However it is likely that the subgroup sample was underpowered. The medicated patients exhibited a 48% increase in $[\text{11C}]\text{(R)-PK11195}$ binding compared to controls, suggesting that either medication or duration of illness might potentiate microglial activation. Our findings also point to an association between neuroinflammation and the negative symptoms of schizophrenia. The PET findings from both cohorts are largely overlapping, suggesting that neuroinflammation is not specific to either disorder but rather a common mechanism. This could reflect a common aetiology and/or an overlap in symptoms. Our findings suggest that inflammation could be used as a potential biomarker as well as a target for novel treatment strategies in both MDD and schizophrenia.
**Declaration**

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Finally, I’d like to express my gratitude to everyone that’s taken part in this study, who did so in the hope that this research will help people in the future. I hope that in some way it does.
Role of the author

I carried out most of the practical aspects of the research as well as contributing intellectually to its design. I handled the overall management of the study, including ethics amendments, R&D approvals and study budget. There were some issues with the initial design of the study concerning the ways in which patients were to be recruited and inclusion/exclusion criteria. In total, I made six substantial amendments and four non-substantial amendments to the study. Amongst these amendments I improved the recruitment strategy, by for example gaining permission to advertise and recruit patients from the community, voluntary organisations and Primary Care. In terms of intellectual contributions, I added a short cognitive testing battery to the protocol, as well as some additional MRI measures allowing us to assess the relationship between inflammation and cognitive function and structural integrity. I also added the assessment of further factors that might affect inflammation to the protocol, including smoking, alcohol use, amount of exercise, sleep quality and childhood adversity. Furthermore, I expanded the inclusion criteria for the schizophrenia patients to include those who were on medication. This significantly improved recruitment rate and enabled a preliminary assessment of medication effects on microglial activation. To complement the investigation of medication effects in the clinical cohort, I set up a collaboration with colleagues (Jo Neill and Mike Harte) in the School of Pharmacy, where we carried out a pilot preclinical PET study investigating TSPO expression in rats treated with risperidone and placebo. Due to funding issues I applied for and was awarded the BMA’s Margaret Temple grant for Schizophrenia research, which covered general study costs such as patient payment, screening blood tests and taxi costs.

I also carried out the recruitment for the study. This involved presenting the study to and making contacts in mental health teams in the Manchester region, attending ‘depot’ clinics, as well as regularly visiting voluntary organisations (such as Manchester’s Self Help Services) to encourage referrals and put up posters so that people could self-refer. I also set up a study website to recruit people with depression, which enabled people to fill in a screening questionnaire (the PHQ-9) online. All potential patients were screened over the phone to check they met the main inclusion/exclusion criteria. I then arranged screening sessions for those who were eligible, where I administered the SCID to all patients and controls, as well as the relevant psychiatric rating scales for the patients in each cohort. The cognitive testing battery was also administered during the screening session. The medical screening was carried out by a registered doctor, but I carried out urinalysis and drug testing. Once eligibility was established I arranged the scanning sessions for each participant (MRI and PET) and was present for both scans for each participant (forty in total) in order to assist the radiographers, explain the procedures to the participants and make them feel comfortable.
After the scans, the list-mode PET data was reconstructed by our reconstruction Physicist. I carried out each image analysis step for each scan from this point (see flowchart in appendix). In short this involved using Linux to create a dynamic PET image, using VINCI software to coregister the T1-weighted MRI image to this PET image, using SPM to segment the MRI into grey and white matter and to normalise the brain atlas into individual space, using Analyze software to edit the PET image for spillover of signal from outside the brain, and using in-house kinetic modelling software (MICK) to generate parametric images of $BP_{ND}$ using the Simplified Reference Tissue Method and both cerebellum and a supervised-cluster derived reference input function. Finally, Analyze was used to apply the normalised brain atlas to the parametric $BP_{ND}$ maps for regional readouts. Statistical tests were carried out using SPSS. Whilst I was unable to handle the animals for the preclinical study, I was involved in its conception and design and shadowed the scans. Post-scanning I cut the brains into sections using a cryostat and carried out immunohistochemistry and autoradiography (results pending). Finally, I have presented preliminary findings from this research at various national and international conferences (oral presentations include Neuroreceptor Mapping: Amsterdam, 2014 and European College for Neuropsychopharmacology: Amsterdam 2015). For the aspects of the study that I was not able to out myself (production of radiotracer, medical screening, cannulation/injection of radiotracer, reconstruction of PET data and the housing, dosing and scanning of the animals) I am grateful to my colleagues for their contribution.
Major depressive disorder (MDD) and schizophrenia are mental disorders that can severely affect quality of life. They have a high prevalence and pose a striking socioeconomic burden. However, current treatments are only partially effective (1). Despite extensive research, their exact pathologies remain unclear and diagnosis is still entirely symptom based. The roles of serotonin in MDD and dopamine in schizophrenia have been studied intensively and it is these neurotransmitter systems that current pharmacological treatments target. However, these treatments are far from perfect. In both disorders, treatment response is highly variable and many patients respond poorly. In MDD, antidepressants lead to a full response in only approximately 50% of patients (2). In schizophrenia, second-generation antipsychotics are limited in both efficacy and tolerability (3). Furthermore, in both disorders, there are often symptoms that remain untreated and there is a high incidence of relapse once medication is withdrawn. This suggests the existence of an underlying pathological process that is unresolved by current antidepressants/antipsychotics. It is vital to identify the causal pathways of depression and schizophrenia to enable the development of more effective treatments for those suffering from these life-limiting disorders. Furthermore, current antidepressants (4) and antipsychotics (5) cause side-effects that can cause significant distress in addition to contributing to an increased risk of morbidity and mortality. Thus it is important to come up with new treatments that are not only more efficacious but also better tolerated. This is the primary motivation for this research, the ultimate goal being to contribute to improving the quality of life of those affected by depression and schizophrenia.

The latest search for more effective treatments in both neurological and psychiatric disorders has largely revolved around inflammation. It has recently been recognised that inflammation is a primary pathological mechanism involved in chronic illness (6). Much of the attention has focused on cardiovascular disease, diabetes and neurodegenerative disorders but mounting evidence suggests that inflammation is also involved in psychiatric disorders such as depression and schizophrenia. The link between inflammation and mental health was first made known by Julius Wagner-Jaureg in 1887, who later became the first ever psychiatrist to win the Nobel Prize (7). However, inflammation as a pathoetiological factor and therapeutic target was largely ignored until more recently. This could be due to the discovery of antidepressants and antipsychotics in the 1950s and a commitment to investigating the serotonergic and dopaminergic pathways, respectively. But as time goes on it is becoming clear that these pathways are being exhausted of their pharmacologic potential. The pressing need to discover, investigate and target alternative pathways is likely to be the driving force behind the renewed interest in the role of inflammation in psychiatry. Indeed there has been
a surge in the amount of publications investigating inflammation in psychiatric disorders over the past few decades and inflammation is now a major funding topic on research agendas (see figure 1.1).

The evidence for a role for inflammation in psychiatric disorders such as depression and schizophrenia is mounting. In both disorders there is evidence of increased peripheral inflammation (8; 9). In line with this, the possibility of using peripheral inflammatory markers as part of a diagnostic blood test is being explored in MDD (10). However, the heterogeneity of mental disorders such as MDD and schizophrenia poses a significant challenge to aetiological research. There may not be one mechanism explaining all the symptoms of depression and schizophrenia. Rather, inflammation could be specific to certain symptoms or symptom profiles. Indeed there is some symptomatic overlap between depression and schizophrenia. There is also a high proportion of comorbid depression in those with schizophrenia, estimated at 50% (11) and it is common for patients to be on both an antipsychotic and an antidepressant. Depression and schizophrenia are rarely considered together. Investigating inflammation in both disorders in parallel could provide valuable insight into potentially shared mechanisms.

The existing research regarding the presence of peripheral inflammation in MDD and schizophrenia is not always consistent. It could be that increased inflammation is only present in a subset of patients. If this is the case, it would be important to determine what is
specific about these patients. For example, they might have a certain symptom profile, or be in a certain stage of the disorder. It could be that a subset of patients with a high inflammatory profile would respond to anti-inflammatory treatments, whereas patients with a ‘normal’ inflammatory profile would not. This gives rise to the idea of personalised medicine where treatments could be selected based on certain biomarkers. Perhaps a more guided treatment selection would result in greater therapeutic efficacy.

The relationship between inflammation and both MDD and schizophrenia has not been fully explored and there are still many unanswered questions. For example the majority of studies have examined the presence of inflammatory markers in the periphery. Few studies have investigated the presence of central inflammation in MDD and schizophrenia. Furthermore, the effects of medication on inflammation are not always taken into account. This thesis aims to address some of the unanswered questions surrounding the involvement of inflammation in these mental disorders. The question at the centre of this research is whether there is increased CNS inflammation in depression and in schizophrenia. We measure neuroinflammation using PET and the radiotracer \([^{11C}](R)-PK11195\). We also explore the relationships between CNS inflammation and peripheral inflammation, symptom severity, cognitive function and antipsychotic treatment. The overarching aim of the thesis is to combine PET, MRI, neuropsychological and preclinical data to provide a comprehensive assessment of the involvement of inflammation in MDD and schizophrenia. This should provide a richer evidence base to inform the development of treatments that target inflammation in these disorders, the ultimate goal being to alleviate the symptoms of those suffering from these disorders more effectively than currently available treatments.

1.1. Thesis outline

This thesis is presented in a traditional format, with ten chapters in total. Chapter one continues with a background to inflammation and neuroimmunology. It describes the ways in which the immune system and the brain communicate and how this can affect our behaviour. It then introduces the salient features of microglial cells and outlines how these can be imaged using PET. Chapters two and three provide a background to depression and schizophrenia, respectively. Within these chapters I define the symptomatology, prevalence and current dominant treatment frameworks for both disorders, before reviewing the literature concerning inflammation and depression/schizophrenia. At the end of each of these chapters I provide the rationale for the study and outline the research questions. The hypotheses are stated at the end of chapter three. In chapter four, the materials and methods, I first give an overview of PET and radiotracer we used. I then outline the methods adopted for this study, giving details of the participants (inclusion/exclusion criteria, recruitment method, demographics), materials used (structured interviews, rating scales, questionnaires,
cognitive tests), the procedure (screening and scanning sessions) and image analysis steps. The results are split across four chapters. In chapter five I present the findings from the MDD cohort. I compare the level of $[^{11}C](R)$-PK11195 binding, representing microglial activation, in MDD patients and controls. I also look at relationships between neuroinflammation and peripheral inflammation, cognitive function and symptom severity. In chapter six I then present findings from the schizophrenia cohort, comparing levels of microglial activation between patients and controls and investigating the relationship between neuroinflammation and other variables. I also present results from the patient subgroups: antipsychotic-free patients and patients on antipsychotics (risperidone/paliperidone). In chapter seven I present findings from a small preclinical study investigating the presence of microglial activation in risperidone-treated vs saline-treated rats using PET and the TSPO radiotracer $[^{18}F]$DPA-714. In the final results chapter (eight) I compare binding potential values obtained from two reference region input functions: a cerebellum and a supervised cluster input function, to determine which is the most appropriate reference region for PET studies investigating the presence of microglial activation in these patient populations. In chapter nine I discuss each of these results chapters in turn and address the research questions posed in the introduction. Finally, in chapter ten I tie all of the findings together, offering an overall conclusion to the thesis. I end with suggestions for future work which would build on this study's contribution to the field.

1.2. Inflammation & cytokines

The first things that come to mind with inflammation tend to be redness, swelling, heat and pain. However, inflammation is governed by a highly complex network of molecular and cellular mechanisms that have far-reaching consequences beyond these classic signs. That inflammatory processes can influence our behaviour and be involved in the pathophysiology of mental disorders are concepts that are receiving increasing attention. In order to give context to the research exploring the role of inflammation in depression and in schizophrenia, an overview of the inflammatory response and of the interplay between the brain and the immune system will first be given. This will be followed by a brief introduction to how neuroinflammation can be measured using Positron Emission Tomography (PET).

Inflammation is an essential immune response to harmful stimuli such as infection and tissue injury (12). It serves a protective function by destroying invading pathogens and initiating cell recovery and is thus vital to our survival. However, there is a distinction between this acute inflammation and chronic inflammation. Systemic chronic inflammation does not appear to be caused by the classic instigators of inflammation: infection and injury. Instead it seems that it is being caused by a homeostatic imbalance that is not directly related to fighting infection or tissue repair (13). Indeed it is this chronic inflammation that is now
recognised as being involved in a wide variety of disease states including neurodegenerative diseases (14), cardiovascular disease (15) and diabetes (16). Mounting evidence (discussed in chapter three) is suggesting that psychiatric disorders such as depression and schizophrenia should also be added to the list of disorders in which inflammation plays a key role.

An effective immune response requires complex interactions between the cells of the immune system. These interactions are coordinated by cytokines – small proteins secreted by immune cells. They coordinate cell-to-cell communication by binding to cell-surface receptors and altering gene expression to modify cell behaviour. There are over 200 known cytokines which include the interleukins (IL-1 through to IL-37) (17), tumor necrosis factors (TNF), interferons (IFN), transforming growth factors (TGF) and chemokines. Cytokines are commonly regarded as either pro- or anti-inflammatory, depending on whether they heighten or dampen the immune response. A balance between pro- and anti-inflammatory cytokines is essential in preventing an excessive inflammatory response. During an infection or injury, release of pro-inflammatory cytokines is usually transient and controlled by anti-inflammatory cytokines. However, if the inflammation becomes chronic or unregulated, then the resultant response may lead to the development of symptoms that may be clinically relevant to psychiatric disorders (discussed in chapter three). Cytokines exert their actions locally at the site of infection/injury, but also at a systemic level. Furthermore, it is now known that cytokines have a role in the CNS; they can gain access to the brain and also be produced centrally. This is of particular relevance to psychiatric disorders (described below).

1.3. Neuroimmunology

The brain has traditionally been viewed as an ‘immune-privileged’ organ due to the existence of the blood-brain-barrier (BBB). The BBB consists of tightly-bound endothelial cells through which infectious agents and antibodies cannot readily pass. Therefore it was thought that the central nervous system (CNS) was inaccessible by the immune system and that the two systems were functionally independent of each other (18). Over recent decades however, this notion has been successfully challenged. Advances in the field of neuroimmunology have revealed that the CNS and the immune system are able to communicate via multiple neuro-immune pathways. It is through these pathways that cytokines are able to access the brain and directly influence our behaviour, for example when we are suffering from a viral or bacterial infection. These infiltrating cytokines can also trigger the activation of the brain's resident immune cells – microglia (described below) and the brain’s own cytokine network (19). The communication is not just one-way however. The brain can also have profound impacts on the immune system. For example in response to an environmental threat, efferent signals can be sent from the brain to the immune system so that it can prepare for injury (20).
Thus there is bi-directional communication between the two systems, based on interactions between neurotransmitters, neuroendocrine hormones, cytokines and their respective receptors (21). Understanding the interactions between brain, behaviour and immunity (psychoneuroimmunology) is central to our understanding of the role of inflammation in psychiatric disorders such as MDD and schizophrenia.

1.4. Immune to brain signalling

The idea that the immune system is capable of sending signals to the brain first came to light in the 1960s as a result of research into fever. This early work revealed that fever is not directly caused by pathogens in the periphery but is in fact triggered centrally by hypothalamic neurons. This was confirmed by Besedovsky (22) who demonstrated that neuronal firing rates in the hypothalamus increased in response to the primary antibody response. Such research led to the notion that the immune system functions as a sensory organ or a 'sixth sense', enabling us to recognise stimuli too small to perceive through sight or touch, such as bacteria and viruses (21). It was argued that the transmission of such information to the CNS allows for a physiological response that helps us fight an infection. This was met with scepticism however, and it was not until compelling evidence from research into 'sickness behaviour' that the notion of immune-to-brain signalling was fully accepted.

Sickness refers to a series of physiological and behavioural changes that occur in response to infection. Anyone who has suffered from a viral or bacterial infection will have experienced sickness behaviours, which include depressed mood, anhedonia (loss of interest/pleasure), social withdrawal, disturbed sleep, fever, loss of appetite and cognitive dysfunction. It is now well established from animal models that these behaviours are caused by cytokines generated from the peripheral immune response gaining access to and exerting their effects on the brain (18). The striking resemblance between these behaviours and the symptoms of depression has led to the hypothesis that cytokines are involved in the pathophysiology of depression. At the clinical level, evidence for immune to brain signalling comes from observations from immunotherapy where pro-inflammatory cytokines are administered to treat cancer and certain viral diseases. Patients receiving this treatment develop behavioural changes including depressed mood, fatigue, anxiety cognitive dysfunction and loss of appetite that are a direct result of actions of the administered cytokine (23). Indeed one study found that 45% of patients receiving IFN alpha developed symptoms severe enough to satisfy criteria for MDD (24). The development of depressive symptoms as a result of immunotherapy, along with the similarity between sickness behaviours and the symptoms of depression, provides compelling evidence for the involvement of cytokines in MDD. Furthermore, the prevalence of MDD is much higher in people suffering from chronic medical
disorders than in the general population (25). It has been suggested that the subjective complaints of patients with a variety of infectious and autoimmune diseases, as well as those with an inflammatory component (e.g. atherosclerosis, obesity, diabetes) might be caused by cytokines acting in the brain (26). The research surrounding immune to brain signalling and MDD will be discussed in more detail in chapter three.

It is clear then, that activation of the immune system results in signalling to the brain. Significant advances in the field of neuroimmunology have been made in the last few decades and we now know that this immune-to-brain signalling can occur via a number of pathways. The first to be discovered was the humoral route. Cytokines are too large, however, to pass freely through the BBB. Instead it is thought that cytokines i) enter through leaky regions of the BBB, such as through circumventricular regions; ii) bind to receptors on perivascular macrophages and endothelial cells which in turn activate secondary messengers (e.g. prostaglandin E2) that are able to enter the brain; iii) are actively transported into the brain through saturable transport systems (27). A neural pathway linking the immune system and brain via the vagus nerve was discovered shortly after (28) (figure 1.2). Thus it seems that thinking of the immune system as a sense organ is more than just an analogy. Once these signals reach the brain, they can cause a cytokine cascade within the CNS where there is a widespread cytokine network. Both glia and neurons express cytokine receptors and can produce cytokines themselves. Cytokines have a vast array of actions on the CNS which affect sleep, behaviour, food intake, cognition and temperature regulation (29). Many of these actions are caused by alterations in neurotransmitter metabolism, neuroendocrine function and neuroplasticity (30). These are discussed in relation to symptoms of depression in the next chapter.

Figure 1.2 Humoral and neural pathways from the immune system to the brain.

**Humoral route:** Pro-inflammatory cytokines released from the peripheral immune response entering the brain through ‘leaky’ regions of the BBB such as the circumventricular organs (CVOs). Once in the brain, cytokines stimulate endothelial cells to release secondary messengers such as prostaglandin E2 (PGE2) and nitrous oxide (NO).

**Neural route:** Cytokines stimulate primary afferent nerve fibres in the vagus nerve. Information is then relayed to brain regions through activation of the nucleus of the solitary tract (NTS).

*Figure adapted from Capuron & Miller, 2011 (594)*
1.5. Brain to immune signalling

The primary physiological role of the CNS is to perceive external conditions in the environment and modulate internal processes to optimally adapt to these external conditions (31). When faced with an environmental threat, the brain responds through activation of either the sympathetic nervous system (SNS) or the hypothalamic-adrenal (HPA) axis. Both of these pathways can have widespread effects on the immune system. This neuroinflammatory link allows the CNS to alert the immune system of environmental changes. It enables the organism to prepare for injury or infection, for example by redistributing immune cells to the site of anticipated insult. This has been shown to enhance post-injury recovery and could therefore be critical to survival (32). It has been suggested that historically this anticipatory host defence system involved environmental danger such as the presence of predatory animals, but that this same innate system can be activated in modern society when faced with adverse conditions that can amount to chronic stress (20). Prolonged activation of these systems through chronic stress is thought to result in an up-regulation of the inflammatory response. Research surrounding stress, depression and schizophrenia, and the mechanisms through which stress can cause a heightened inflammatory state, will be discussed in the next chapter. It should now be clear that the brain and the immune system form a powerful, interconnected network where information can be conveyed in both directions. This is central to our understanding of how inflammation, stress and disease could be related to mental disorders such as depression and schizophrenia.

1.6. Microglia: the immune cells of the brain

Microglia are the resident macrophages of the brain. They play a pivotal role in the immune response of the CNS, acting as a first line of defence to infectious agents and injury. In normal conditions they are in a ‘resting state’, however they are hardly inactive. Their thin, highly branched processes are constantly surveying the surrounding microenvironment for pathological stimuli. They are highly sensitive and respond rapidly to even the subtlest of alterations. In response to pathological stimuli, microglia become activated, proliferate and migrate to the site of injury. Upon activation they transform from a ramified into an amoeboid state (see figure 1.3). In this activated state microglia can destroy invading pathogens, remove debris, and promote tissue repair by secreting growth factors. They thus protect the CNS from potentially fatal damage and facilitate the return to tissue integrity (33).

However, perhaps owing to their presence in almost all neurological diseases, microglia have been portrayed as bearing a dark side. Activated microglia synthesise free radicals such as reactive oxygen (ROS) and reactive nitrogen (RNS) species that are toxic to invading pathogens. Chronic microglial activation can lead to pathological concentrations of free
radicals that can in turn cause neuronal injury and death (34). Activated microglia also release pro-inflammatory cytokines and glutamate, which in turn induce microglial activation, potentially leading to an out of control inflammatory response. An excess of glutamate can lead to excitotoxicity and further neuronal damage. Thus inflammation has been described as a ‘double edged sword’ as the inflammatory process can overstep the threshold of what the brain can tolerate and ultimately do more harm than good. Microglial activation has been found to occur in autoimmune disorders (35), neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (36; 37) and cerebrovascular disorders (38). It is now under investigation in psychiatric disorders such as major depression and schizophrenia too (discussed in Chapter 3). Whether the presence of activated microglia represents an unregulated process with detrimental effects, however, is the subject of ongoing debate. More recently it has been realised that microglia are far from dormant in the absence of immune challenge and that they actually regulate neuronal function and homeostasis. They are required for the development of mature synapses during embryogenesis (39) and have been found to regulate adult neurogenesis (40).

Furthermore, viewing the presence of CNS inflammation as a mediator of neuronal damage is perhaps an oversimplification. It has been suggested that microglia do not comprise a single uniform cell population but rather constitute a family of cells with diverse phenotypes – some that are beneficial and others that cannot be tolerated and thus contribute to the pathology (41). The traditional notion that microglia act in a stereotypical manner regardless of the insult has therefore been challenged (42). The variability in activation phenotypes is thought to be determined by the nature of the stimulus but also by its duration and by prior and subsequent stimuli. For example, microglia exposed to a small amount of IFNγ exhibit properties that are neuroprotective, but microglia exposed to a large amount of IFNγ display a cytotoxic and destructive phenotype (41). Microglia can also become ‘primed’ or sensitised to an enhanced pro-inflammatory response upon secondary stimulation, as has been associated with ageing (43).
It is therefore emerging that the picture surrounding microglial activation is much more complicated than once thought. To view inflammation in the CNS as either detrimental or beneficial is an oversimplification. Instead there seems to be a diverse array of microglial phenotype dependent on an intricate balance of immune-regulated processes. This must be taken into account when investigating neurodegenerative and psychiatric disorders. Attempts to treat Alzheimer’s disease with anti-inflammatories have so far been largely unsuccessful, with some trials even demonstrating a worsening of symptoms (45). Thus activated microglia in AD may not represent a neurotoxic phenotype. Further research is necessary to categorise the various microglial phenotypes and their respective causes. It is particularly important to determine the point at which microglial activation becomes detrimental.

### 1.7. Imaging microglia

Before the advent of neuroimaging modalities such as Positron Emission Tomography (PET), researchers had to rely on autopsy studies to investigate the presence of microglial activation. This was far from ideal as microglial activation can be relatively short-lived and may not be present at autopsy. However, using PET it is now possible to detect microglial activation in-vivo and non-invasively. The radiotracer \([^{11}C](R)-PK11195\) is the most established marker of microglial activation and was chosen for this study. \([^{11}C](R)-PK11195\) selectively binds to the 18-kDa Translocator Protein (TSPO), previously referred to as the peripheral benzodiazepine receptor (PBR), on the outer mitochondrial membrane of microglia (46) (see figure 1.4). TSPO is highly expressed in activated microglia, therefore the high-affinity TSPO ligand PK11195, radiolabelled with \(^{11}C\), has been utilised as a marker of microglial activation and an indirect measure of CNS inflammation.

![Figure 1.4 Increased TSPO expression in outer mitochondrial membrane of activated microglia and binding of PK11195 to the TSPO](image-url)
As such, $[^{11}\text{C}]$(R)-PK11195 has been used to indicate CNS inflammation following ischemic stroke (47) and in neurodegenerative diseases such as Parkinson's disease (37), multiple sclerosis (48) and Alzheimer's disease (49). Whilst $[^{11}\text{C}]$(R)-PK11195 is the most established and consistently used tracer used in PET studies of neuroinflammation, it does display a high level of nonspecific binding which limits its signal-to-noise ratio. During the last several years, over 40 new TSPO radioligands have been reported in the literature (50). Of these second generation TSPO tracers, several have been tested in humans and have demonstrated improved signal to noise ratios. However, these tracers are not without their own limitations. A more detailed review of these radiotracers is given in Chapter four, along with an overview of PET imaging.

This chapter has given an overview of the main aspects of the immune system that are relevant to this thesis. It should now be clear that i) inflammation is an intricate and complex response that is not only present in response to infection or injury; ii) the immune system and the brain are able to communicate via numerous pathways, which is vital for survival; iii) cytokines accessing the brain can lead to changes in behaviour; iv) microglia can have various phenotypes upon activation – some of which are beneficial and others that are detrimental to the CNS; v) microglial activation can be measured in-vivo using PET imaging. This background information gives context to the next chapter, where the research surrounding the involvement of inflammation in depression and in schizophrenia is evaluated.
2.1. Introduction to Major Depressive Disorder

Depression and schizophrenia are distinct disorders with their own symptomatology, prevalence, treatments and presumably causes. This said, there is a high incidence of depression in people with schizophrenia, so there could be some overlap in causality. Still, for the purpose of a coherent structure they are tackled separately, with this chapter (two) focussing on depression and chapter three focussing on schizophrenia. Before discussing the evidence for a role for inflammation in depression I first provide an introduction to the disorder. A discussion of all the theories of depression is beyond the scope of this thesis and so only a brief overview of the dominant research areas is given. The various lines of evidence suggesting a role for inflammation in depression are then reviewed, including an outline of the mechanisms through which inflammation could contribute to its symptoms. The review provides a rationale for the various strands of the current research and highlights the gaps which it aims to fill.

2.1.1 Symptoms, diagnosis & course

The World Health Organisation (WHO) recently identified depression as being the leading cause of disability worldwide (51). This is because of its high prevalence – worldwide it affects an estimated 350 million people – and because it severely affects functioning, often from a young age, resulting in the highest number of years lost due to disability. It is a debilitating disorder which can greatly affect quality of life, medical morbidity and mortality. In its most severe form, depression can lead to suicide. Depression is characterised by persistent low mood, a lack of interest and enjoyment (anhedonia) and a range of associated emotional, physical, cognitive and behavioural symptoms. Merely listing the symptoms of depression, however, does not give an impression of what it might be like to experience it. William Styron, in his Darkness Visible: A memoir of madness, describes living with recurrent severe depression with profound honesty: ‘my brain had begun to endure its familiar siege: panic and dislocation, and a sense that my thought processes were being engulfed by a toxic and unnameable tide that obliterated any enjoyable response to the living world’ (52). Thus it should be clear that depression is distinct from the experience of being sad or feeling low. Sadness is a normal emotion common to all cultures. It is only when this low mood and associated symptoms are persistent and disabling that it is thought of as a mood disorder that reflects a pathological state and requires treatment. It is most commonly diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) (53). Most research has
used DSM-IV for diagnostic criteria up to now. DSM-V has recently been released but the diagnostic criteria for MDD are largely similar. The DSM stipulates that at least five of nine symptoms, one of which has to be depressed mood or anhedonia, must be present for at least two weeks for a diagnosis of depression to be made (figure 2.1). Furthermore, the symptoms must cause clinically significant distress or impairment in social or occupational functioning and cannot be due to the direct physiological effects of a substance, to a general medical condition or to simple bereavement.

The diagnosis of depression is entirely symptom-based and is primarily arrived at through interview. A diagnosis of depression requires assessment of severity, duration and course. Severity is categorised into mild (few, if any, symptoms in excess of the five required for diagnosis, and only minor functional impairment), moderate (symptoms or functional impairment in between mild and severe) and severe (most symptoms are present with marked impairment in functioning). There is substantial variation in the severity, duration and course of depression across patients and it is likely that many subtypes exist. This can prove problematic when researching depression as one homogenous entity.

The course of depression, as with its symptomatology, is varied across individuals. The average age of the first episode of depression occurs in the mid-twenties, however it can occur at any time from childhood or adolescence to old age (54). The average duration for a major depressive disorder is 3 to 5 months and the majority of individuals reach remission within a year. However, after the first episode, at least 50% of people will go on to have at least one more episode and, following the second and third episode, the risk of relapse goes up to 70% and 90% respectively (55). Therefore it seems that with each subsequent episode of depression, the prognosis for full recovery becomes worse and the depression takes a chronic course. In between episodes, the DSM classifies people as being either in partial remission, where they are below the threshold for diagnosis but still displaying symptoms, or in full remission where there are no significant symptoms for the past two months (53).
Recurrent episodes can follow a seasonal pattern (known as Seasonal Affective Disorder) where depression occurs repeatedly at the same time of year – most commonly in the Winter as opposed to the Summer months (56).

2.1.2 Prevalence, disability & mortality

Depression affects a vast amount of people in all nations. Worldwide estimates of the likelihood of developing depression vary widely, but it is thought to range between 4% and 10% (57). This figure may only represent the ‘tip of the iceberg’ though, as a third of people suffering from depression do not consult their GP (58). Depression is therefore a very common disorder. This is problematic in terms of the extent of suffering caused by depression, but also in terms of the wider impact on the health system and on society as a whole. In England alone, the cost of services for depression was approximately £1.7 billion in 2007. When considering the cost of lost employment in addition to service costs, the total rose to £7.5 billion (59).

It is thought by some that the incidence of depression is rising. Systematic epidemiological studies did not begin until the twentieth century and so data from earlier centuries is lacking. However depression or ‘melancholia’ certainly existed in early centuries. There are references to melancholia as a distinct medical condition in Hippocratic writings (60). It seems that it was widespread even in the Seventeenth Century, when Richard Burton in *Anatomy of Melancholy* described it as ‘a universal malady, an epidemical disease, that so often, so much crucifies the body and minds’ (61). Epidemiological studies suggest that depression may be increasing. For example, in cross-sectional retrospective studies, Kessler et al. have reported an increased lifetime risk of mood disorder in younger generations Worldwide (62) and in the US population (63). Longitudinal studies also demonstrate an increase in prevalence of depression over time. For example, prevalence rates for depression in the US rose markedly in every sociodemographic subgroup between 1991 and 2001 (64). There is a wealth of research suggesting that depression is rising and it has even been suggested that we are in the midst of an epidemic of depression (65).

It has been questioned, however, whether there has truly been a rise in depression or whether a misclassification of normal sadness as a mental disorder and an increased readiness to diagnose people with the disorder is responsible for the rise (66). Finding a cut-off point to make the distinction between what is considered normal and what is considered pathological remains a challenge. An inherent weakness of the nosological system is that there is significant overlap between normality and disorder, and between disorders, meaning that they are not necessarily distinct categories. It is likely that symptoms of depression lie on a continuum of severity, ranging from fleeting sadness as a normal reaction to everyday life, to symptoms that are serious, dysfunctional and ongoing that can ultimately result in suicide.
The diagnostic criteria are clear, however, that there has to be significant impairment to functioning and persistent low mood for a diagnosis of MDD to be reached. Numerous factors could contribute to a rise in the incidence of depression. An interesting finding is that 'modernised' countries (as measured by GDP per capita) have higher rates of depression than less developed countries and it has been suggested that certain aspects of modern society contribute to the development of depression. These contributing factors include a rise in obesity (driven by poor diet and physical inactivity) and a social environment that breeds stress, competition, inequality and isolation (65). This is discussed in more detail later.

In terms of demographics, gender, age, marital status and socioeconomic status seem to affect the prevalence of depression. It has been consistently found that there is a higher prevalence of depression amongst women, who have a twofold increased risk of developing depression compared to men (67). Depression rates also vary according to age, though this is different across studies and nations. A UK survey found that prevalence rates were highest for people aged 35-54 (68), though rates are also worryingly high in adolescence, with one US study finding that one fifth of adolescents having experienced a major depressive episode by the age of 19 (69). People who are separated or divorced also show consistently higher rates of depression than people that are married, across countries (54; 70). Socioeconomic factors seem to be associated with depression too. The UK survey found that those who had experienced a depressive episode were more likely to be unemployed, to have no formal educational qualifications, to live in local authority housing and to live in an urban environment (68).

Depression can also have profound impacts on physical health and mortality. Findings from the World Health Surveys indicate that depression is associated with a significantly greater decrement in health than the chronic medical illnesses angina, arthritis, asthma and diabetes (71). Furthermore comorbidity with depression significantly worsens the health state of people with chronic diseases. This is consistent with the first Global Burden of Disease report, which ranked depression as the highest determinant of disability worldwide (72). Given these findings it is not surprising that depression is associated with increased mortality rates. Indeed there is a higher prevalence of depression amongst people with medical illness than in the general population (73). In particular, depression has been associated with Coronary Heart Disease (CHD), with depression significantly increasing the risk of mortality in CHD patients. A meta-analysis revealed that the risk of dying in the two years after initial assessment is two times higher in depressed compared to non-depressed patients (74). The link between depression, comorbidity and mortality is revisited later in relation to inflammation. Suicide also accounts for a significant proportion of the increased mortality in depression, with the risk of suicide in people with depression four times higher than in the general population. This rises to almost 20 times higher in the most severely depressed (75).
It should now be clear that depression is ubiquitous, affecting a vast amount of people worldwide, whose incidence is seemingly rising. The symptoms can have devastating effects on quality of life, often leaving people unable to work or socialise. It is also associated with increased mortality due to medical comorbidity and increased risk of suicide. It is therefore disabling in both a mental and physical sense. This is a big concern not only in terms of those suffering from depression but also in terms of the wider impacts on the health service and economy as a whole. The challenge then is to determine what causes this disorder so that we can work towards treating and eventually preventing its occurrence.

2.1.3 Current theoretical frameworks

There is extensive variation in the symptomatology, course and outcome of depression. There is also a wide variety of theoretical explanations for its aetiology, including genetic, biochemical, endocrine, psychological and social explanations. Such breadth of explanation possibly reflects the heterogenous and complex nature of depression. Indeed it is unlikely that there is just one explanation that encompasses all of depression. Whilst advances in neuroimaging emphasise biological causes, there is also marked awareness of the role of psychological, social and environmental factors in the development of depression. Although the exact aetiology of depression remains unclear, the broad factors that are thought to shape the disorder are becoming increasingly understood. Depression is now thought to develop based on interactions between genes and environment and their subsequent impact on multiple neurobiological mechanisms. Accordingly Clinicians have widely accepted the ‘biopsychosocial’ model as a means of understanding and treating depression and other psychiatric disorders. A review of all the theoretical explanations for depression is beyond the scope of this thesis and I instead give overviews of the dominant and most relevant theories.

2.1.4 Monoamine theory & antidepressants

The first and most widely studied biological theory of depression is the monoamine theory, which was proposed in the 1950s after the accidental discovery of the first antidepressants – the monoamine oxidase inhibitors (MAOIs) and the tricyclic antidepressants (TCAs). Both classes of drugs facilitate monoaminergic transmission. Based on this and their antidepressant effects, it was hypothesised that depression is caused by monoaminergic dysfunction (76). Further research led to a focus on serotonin specifically and in the 1980s the first selective serotonin reuptake inhibitor (SSRI), fluoxetine, was introduced. SSRIs offer clinical advantages over the older antidepressants, in terms of tolerance and safety. However, there remain a series of problems with antidepressant therapy which the monoamine theory seems unable to resolve. For example it cannot explain why the therapeutic effects require
several weeks of drug exposure despite increased serotonin levels within just 1-2 days (77). Perhaps the most important limitation though, is that the efficacy of all currently available antidepressants is far from ideal.

The efficacy of antidepressants has been researched intensively. Evidence clearly indicates that meta-analyses of only published data results in an overestimation of the efficacy of antidepressants due to publication bias (78). Accordingly it has been shown that including unpublished data significantly reduces the already modest effects of antidepressants (79; 80). Such problems led to the introduction of new drugs with different pharmacological properties, such as the serotonin and noradrenaline reuptake inhibitors (SNRIs). However these new drugs still share the common action of facilitating monoaminergic transmission and still show the same limitations in efficacy. For example a recent review indicates that SSRIs are superior to placebo in providing a clinically relevant benefit in only half of depressed patients and that SNRIs are superior to placebo in only a third (2). Even in patients that do respond to antidepressant treatment, they do not necessarily reach remission and there are often residual symptoms that remain untreated (81). Another concern is the safety and tolerability of antidepressants. Although the second-generation antidepressants show better safety profiles than the older MAOIs and TCAs, there are still numerous side effects which can greatly affect quality of life and adverse events are still common. The most common side-effects of second-generation antidepressants are nausea, vomiting, diarrhoea, dry mouth, sweating headache, dizziness, sexual dysfunction and weight gain (82). A particularly serious side effect of antidepressants is suicidality. A large-scale systematic review indicated an association between suicide attempts and the use of SSRIs (83).

Despite all this, it must be noted that current antidepressants can be very effective in alleviating the symptoms of depression and restoring functioning, though this could be partly attributable to the placebo effect (84). They are probably the best available pharmacological treatment option we have. However, they are a far from perfect treatment for depression. That antidepressants are not clinically effective in so many people with depression suggests that there is an underlying pathology which is not being targeted. The monoamine pathway has been researched extensively for the past 60 years and appears to have been fully exhausted of its pharmacological potential (85). Therefore it is clear that we need to explore, identify and target novel pathways so that safer, better tolerated and more effective treatments can be developed to alleviate the suffering caused by depression.

2.1.5 Genetics & environment

According to epidemiological studies, between 40 and 50% of the risk for developing depression is genetic (86). However, a recent large-scale genome-wide association study found that none of the tested polymorphisms reached genome-wide significance levels (87).
The search for depression vulnerability genes may be so challenging because of the complexity of depression and the likelihood that there are many genes involved. The vulnerability to depression is thus only partly genetic and it is now well accepted that depression is caused by an interaction between genetic predisposition and environmental factors such as stress (88). Stress has received the most attention as being an environmental risk factor for depression. Indeed depression is often described as a stress-related disorder, with a wealth of research indicating a connection between adverse life events or stressful circumstances and depression (89). However, stress alone is not sufficient to cause depression as not everyone who experiences stress goes on to develop the disorder. Instead it seems that it is the interaction between a genetic predisposition and stress that can lead to depression. The effects of stress on the brain and the mechanisms through which stress might lead to depression have been studied intensively. Inflammation is closely related to the brain's response to stress and this is discussed in the context of depression shortly.

2.1.6 Insights from neuroimaging

Converging evidence suggests that depression is unlikely to be the result of one neurotransmitter system or one brain region. Rather, it is now being thought of as a systems-level disorder affecting integrated pathways linking certain cortical, limbic and sub-cortical regions (90; 91). Again there is unlikely to be one factor that causes this system dysregulation, but a combination of genetic vulnerability and environmental stressors. Over the past few decades, neuroimaging has given us new insights into the neural substrates of depression. An enormous amount of neuroimaging research has been published and a variety of neurobiological abnormalities have been identified. However, so far none of these abnormalities show sufficient sensitivity or specificity to be used diagnostically. Again, the variability in the identified abnormalities likely reflects the heterogenous nature of depression. I briefly highlight some of the key brain regions that have been identified as playing a role in depression. Due to the core symptoms of depression being pervasive sad mood and negative affect, researchers have investigated the emotional circuitry of the brain, namely the limbic system. MRI studies have shown reduced amygdala volume (92) in depression and functional studies have shown increased amygdala activity both in resting state and in response to a variety of stimuli such as in expectation of negative pictures (93) and negative emotional faces (94). The amygdala is thought to be engaged during the processing of negative stimuli in order to guide the attention to possible sources of danger (95). Accordingly, amygdala hyperactivity has been shown to relate to a negative bias in depression (96).

The hippocampus is another limbic structure that has been studied extensively in depression. MRI studies have consistently shown reduced hippocampal volumes in people with recurrent
depression, as demonstrated by meta-analyses (97; 98). Hippocampal function is thought to influence activity in the prefrontal cortex, amygdala and nucleus accumbens – areas that are associated with emotionality (99). Furthermore, it is closely involved in the regulation of the HPA axis and the stress response, both of which are implicated in depression (100) (discussed in more detail later). Hippocampal dysfunction may also be related to the memory deficits seen in depression (101). Interestingly, antidepressants have been shown to stimulate hippocampal neurogenesis, which may underlie some of their therapeutic benefits (99).

Cortical regions have also been implicated in depression. Decreased cerebral blood flow and metabolism have been found in the dorsolateral/dorsomedial prefrontal cortex (PFC) and in the dorsal anterior cingulate cortex (ACC) using PET (see (102) for a review). A meta-analysis of voxel based morphometry (VBM) studies also revealed grey matter reductions in these regions (103). The PFC is associated with executive functions including planning of goal-directed behaviour and it is thought that underactivation in certain regions of the PFC may result in an inability to override negative thought processes in depressed patients (104). The ACC acts as a bridge between attention and emotion. The ACC is divided into two sections; the ventral section or subgenual, which seems to be involved in affective processes and the dorsal section which plays a role in cognitive processes. It has been hypothesised that hypoactivation of the ventral ACC in depression is related to anhedonia and blunted conscious experience of affect whereas hypoactivation of the dorsal region is associated with impaired attention (105). Deep brain stimulation (DBS) has been used to target the subgenual ACC and has shown promising antidepressant effects in treatment-resistant depressed patients (106). The authors proposed that the effects were due to the disruption of pathological activity in limbo-cortical pathways.

The above neuroimaging research is consistent with the idea that depression is associated with abnormal functioning in two neural systems: the ventral system, including the amygdala, insula, ventral striatum, ventral regions of the prefrontal cortex and anterior cingulate; and the dorsal system, including the dorsal regions of the PFC, the anterior cingulate and the hippocampus (107). The ventral system is important for the identification of the emotional significance of stimuli, the production of affective states and the automatic regulation of affective states. The dorsal system is involved in the effortful regulation of the affective state and executive functioning. Evidence suggests that volume reductions in the amygdala and other components of the ventral system, along with increased activity, may result in the increased negative bias seen in depression. Furthermore, the underactivity in dorsal regions such as the dorsolateral prefrontal cortex (DLPFC) and dorsal ACC, may lead to a reduced top-down regulation of the affective state, which might perpetuate depressed mood and anhedonia (108). Hypoactivity in dorsal regions may also explain the impaired executive
function that is seen in depression (109). Figure 2.2 summarises the key regions implicated in MDD. Evidence for abnormalities in specific brain regions is therefore beginning to explain some of the symptoms seen in depression. There are large variations across studies that may reflect the different clinical characteristics of the subtypes of depression. However, meta-analyses have identified key brain regions that are consistently implicated in depression, providing important insights into its neural substrates.

2.1.7 Cognitive theory

There has been a focus on the biological underpinnings of depression since the discovery of antidepressants in the 1950s and advances in neuroimaging techniques. Alongside this though, there has been a growing emphasis on the value of psychological therapy in treating depression. Before 2006, antidepressant medication was typically the only treatment available to those experiencing depression. However, following recommendations from the National Institute for Clinical Excellence (NICE), the government rolled out the Improving Access to Psychological Therapies (IAPT) initiative, giving people suffering from depression access to psychological therapy in addition to medication. Presently, the NICE guidelines recommend psychological therapy alone as the first line of treatment for mild to moderate depression and in conjunction with antidepressants to treat moderate or severe depression (110).

The NICE guidelines are based on a vast amount of research into the use of psychological therapies in the treatment of depression, which is beyond the scope of this thesis. Instead I will give a brief overview of Cognitive Behavioural Therapy (CBT), which has the largest evidence base for the treatment of depression. CBT was pioneered by Aaron Beck in the 1960s and is largely based on his cognitive theory of psychopathology. According to this
theory it is our perceptions of situations that influence our emotional and behavioural reactions, rather than the situation itself. These perceptions can become distorted based on dysfunctional thought processes or cognitions. In depression, perceptions are distorted by dysfunctional cognitions about the self, the world and the future (known as the cognitive triad). A depressed person may view themselves as useless, the world as unfair and the future as hopeless. In CBT, patients learn to identify such negative ‘automatic thoughts’ and to correct their thought processes to reflect reality rather than their dysfunctional beliefs or schemas. They also learn to identify unhelpful patterns of behaviour which arise from the negative thought patterns and help reinforce positive behaviours (111).

A substantial evidence base indicates that CBT is effective in treating depression. Most studies have shown that CBT is equally as effective as antidepressants in treating depression (112; 113), with some studies indicating superior efficacy to antidepressants (114; 115). Research also suggests that CBT may be more effective in the long-term, with follow-up studies indicating significantly lower relapse rates in CBT treated compared to antidepressant treated groups (116). This is perhaps not surprising considering CBT’s focus on transferring the skills learned in therapy to everyday life (111). There is some evidence that combining CBT with antidepressant medication is superior to either therapy alone (117; 118). Furthermore, people with depression typically prefer psychological treatment to medication, finding it more helpful and free of side effects (119). It must be noted that other non-pharmacological treatments are recommended in the NICE guidelines, including exercise, which has been consistently shown to reduce depressive symptoms (120). This overview of depression should have given a general background to the disorder, key aetiological theories and the dominant treatment strategies. The current research can now be placed in the context of the existing literature. I will now discuss the various lines of evidence suggesting a role for inflammation in the pathophysiology of depression.
2.2 Depression & inflammation

For over two decades evidence for an association between depression and inflammation has been mounting. There is now enough evidence that the association is difficult to deny. However, the relationship seems complex, direction of causality is unclear and the literature is at times contradictory. Also, most of the research has measured the presence of inflammatory markers in peripheral blood, whereas measuring inflammation in the CNS may be essential in unravelling the relationship between inflammation and depression. The various lines of evidence implicating an association between inflammation and depression are now discussed. I start with the literature that first sparked the surge in investigations into inflammation and review the research surrounding peripheral inflammation in depression. This is followed by an overview of the research suggesting that cytokines accessing the brain can contribute to depressive symptoms. I then discuss the medical comorbidity of depression and the possibility that inflammation underlies the association between medical illness and depression. The mechanisms through which inflammation can influence the brain and contribute to symptoms of depression are then reviewed. These include neurotransmitter functioning, the HPA axis, neurodegenerative pathways, neurotrophic factors and stress. I then sum up all this evidence, highlight gaps in the literature and provide a rationale for the study.

2.2.1 Peripheral inflammation

Early research into depression and immunity suggested that depression may be related to immunosuppression (121). Based on this and observations that depressed people were especially vulnerable to certain diseases, Maes et al. began to investigate the immune status of depressed people in-vivo, hypothesising that their immune cells would be downregulated. They in fact found the opposite. A series of experiments published in the early 1990s indicated that depression was associated with immune activation as opposed to suppression. These experiments showed that people with depression exhibited increased numbers of circulating lymphocytes and phagocytic cells, an increased ratio of T helper to T suppressor cells, upregulated levels of indicators of activated immune cells (such as neopterin, PGE₂, soluble IL-2 receptors), higher plasma levels of positive acute phase proteins (APPs) (such as C-reactive protein (CRP) and haptoglobin) along with lower levels of negative APPs (transferrin, albumin) and increased release of pro-inflammatory cytokines such as IL-1, IL-2 and IL-6 in response to activated macrophages and T cells (see (122) for review). This laid the foundations for the theory that inflammation and cell-mediated immunity are key factors in depression, first known as the ‘inflammatory response system (IRS) activation theory’ (123).
Since these initial findings, a cascade of studies has been published indicating a heightened immune response in depression. It must be noted, however, that some of the earlier studies did not control for factors which may influence the levels of peripheral markers of inflammation. For example, higher BMI is associated with increased inflammation (124), inflammation increases with age (125), antidepressants can reduce inflammation (126) and gender can influence inflammation partly due to inflammatory fluctuations through the menstrual cycle (127). Accordingly Haack et al. found that age, gender, BMI, smoking, infectious diseases and psychotropic medication had distinct influences on cytokine levels, whereas psychiatric diagnosis accounted for only a small part of the variance. After taking all the confounding factors into account, there were no significant immunological differences between depressed patients and controls (128). However, a number of meta-analyses have now confirmed the involvement of inflammation in at least a subset of people with depression. The first meta-analysis of inflammatory markers in MDD reported increases in neutrophils, lymphocytes and monocytes (129). In a large meta-analysis of 61 studies, Howren et al. found significant associations between depression and CRP, IL-6 and IL-1 even when adjusting for age, gender, BMI and antidepressant medication (130). The magnitude of the associations was, however, attenuated when adjusting for BMI, suggesting that it does contribute to the association between depression and inflammation. Indeed it is well known that adipose tissue produces pro-inflammatory cytokines (124) and depression is often associated with a sedentary lifestyle which could facilitate weight gain and promote further inflammation. A second meta-analysis of cytokines in major depression across twenty four studies indicated significantly higher concentrations of TNFα and IL-6 compared to controls, adding further evidence to the hypothesis that depression is associated with activation of the inflammatory response system (8). A recent cumulative meta-analysis of 58 studies has confirmed that MDD is associated with increases in IL-6 and CRP (131).

Despite substantial evidence for an association between inflammation and depression there is a considerable amount of contradiction in the literature. Findings between studies are highly variable and there is substantial variation across subjects in each study. One explanation for this is that the patient samples used in most studies are heterogenous in terms of their clinical characteristics and, in all likelihood, their underlying pathology. With the exception of a few, the reviewed studies have included clinically heterogenous samples of depressed patients who vary in symptom severity, symptom profiles, course, duration and treatment. This is not surprising given the scope for variation in clinical characteristics that fall under the single diagnosis of MDD. It is likely that subsets of patients exist within MDD and that these subsets have distinct pathologies, and possibly different immunological profiles.
Indeed in many of the studies there is often a subgroup of depressed patients whose inflammatory markers exceed the rest, which may account for the overall difference between depressed and non-depressed individuals. For example, in a study investigating inflammatory markers in women who were in remission, a subset of five stand out with serum CRP levels that exceed 5mg/L (132). Such findings, along with contradictions in the literature, suggest that inflammation may be associated with some but not all cases of depression. This was summed up nicely in an editorial headed ‘Where there is depression, there is inflammation….Sometimes!’ (133). Further research needs to determine why inflammation is more prominent in some cases of depression than others. It is unlikely that there is one common mechanism, whether it be immunological or molecular, underlying all of depression. Thus there may not be a ‘final common pathway’ which can be treated with one class of drugs. Rather, there may be various causes of depression, one being an elevated inflammatory response. The challenge would then be to identify this subset of depression and treat them accordingly.

One subtype of depression according to DSM-IV is ‘melancholic’ depression, which is characterised by prominent vegetative symptoms and non-reactive mood. Rothermundt et al. compared the immunological profiles of melancholic vs non-melancholic patients and found distinct differences, with evidence of an inflammatory state in non-melancholic but not melancholic patients (134). Some studies have shown correlations between immune parameters and severity of depression (122; 135–137), suggesting that inflammation markers vary according to severity of symptoms. Others have looked at immune parameters in people who had attempted suicide. One study found increased concentrations of plasma soluble interleukin-2 receptor (sIL-2R), an indicator of T-cell activation, in medication-free suicide attempters with mood disorders (138). A more recent study found increased levels of plasma IL-6 and TNFα and decreased IL-2 concentrations in suicide attempters compared to non-suicidal depressed patients and healthy controls (139).

An important question which is yet to be fully addressed is whether a heightened inflammatory response comes before or after the onset of depression. Most studies investigating inflammation and depression have been cross-sectional, making it impossible to infer which came first – the inflammation or the depression? However, a number of longitudinal studies have attempted to tackle this question. Khandaker et al.’s recent study measured serum IL-6 and CRP in participants at age 6 and assessed the presence of depression at age 18. Their findings demonstrate a longitudinal dose-response association between childhood IL-6 levels and future risk for depression, an association which remained after adjusting for sex, BMI, social class, past psychological and behavioural problems (140). Indeed a meta-analysis of longitudinal studies reported elevated CRP and IL-6 preceded the
onset of depression (141), consistent with inflammation playing a causal role in the development of depression.

Further evidence for this comes from research into early life stress and depression. The association between early life stress and the later development of depression has been firmly established. Accumulating evidence suggests that inflammation may mediate this link. Neuroendocrine-immunological abnormalities arising from childhood adversity are thought to result in a pro-inflammatory phenotype in adulthood (142). Accordingly it has been demonstrated that depressed adults who have experienced early life stress exhibit an exaggerated inflammatory response to acute psychosocial stress compared to controls (143; 144). Further research indicates associations between childhood adversity, raised levels of inflammatory markers and depression (145). One study found significantly raised peripheral inflammation in subjects with a history of maltreatment and recent depression, whereas those with depression alone were indistinguishable from controls (146). Indeed, those who have experienced childhood adversity may form a subgroup of depression in which inflammation plays a key role. The relationship between stress and depression is discussed in more detail later.

Studies indicating the presence of inflammation before the onset of depression suggest that inflammation may be a trait as opposed to a state marker of depression. Evidence that inflammation is still present in remission and after antidepressant treatment (132; 147) further supports inflammation being a trait marker of depression, as it seems to be present independently of disease activity and severity. However, a large body of research indicates that acute psychosocial stress leads to increased levels of peripheral inflammation (148). The inflammation seen in depression could be stress-induced, implying that inflammation is a state as opposed to a trait marker of depression. This is supported by Rohleder & Miller who showed that IL-6 levels in women were related to short-term changes in mood but that average levels of depression measured over a year and a half were not associated with variability in IL-6 or CRP (149), suggesting that inflammation is associated with the state not the trait component of depression. The authors concluded that the mechanism behind their findings is comparable to those responsible for the raised peripheral inflammation seen in response to acute psychosocial stress. Determining whether inflammation is a trait or state marker of depression is important in terms of treatment implications and in unravelling its underlying aetiology. This is discussed in more detail in relation to stress and genetics later.

As described earlier, it is now known that peripheral inflammatory molecules are able to access the brain and induce a central inflammatory response. Some studies have shown evidence of increased inflammation in cerebro-spinal fluid (CSF) in depression (150). However, the majority of the research has looked at only peripheral inflammation and so the relationship between peripheral and central inflammation is not clear. Animal research has
consistently shown that activation of peripheral immune pathways leads to a pro-inflammatory response in the brain (27). In humans, the link between peripheral and central inflammation is not so clear. One study showed that the peripheral administration of IFNα, used to treat Hepatitis C patients, was associated with significant elevations in of CSF IL-6 and IFNα (151). Furthermore a recent PET study showed that a peripheral immune challenge is able to induce a central inflammatory response (152) suggesting that peripheral inflammation can induce a central inflammatory response. However, the concentrations of circulating inflammatory markers seen in depression are significantly lower than those used in immunotherapy. Therefore whether peripheral inflammation in depression is accompanied by a central inflammatory response remains to be seen. Microglia are the primary source of pro-inflammatory cytokines in the brain and, in their activated form, are the hallmark of a central inflammatory response. Using PET to image activated microglia would help determine whether depression is associated with central in addition to peripheral inflammation. If central inflammation is confirmed in depression, the question of direction of causality would still be left open: Is peripheral inflammation inducing a central inflammatory response or is the central inflammation primary and contributing to peripheral inflammation?

2.2.2 Sickness behaviour

We have all been unfortunate enough to experience a viral or bacterial infection and the resultant feelings of sickness. Feeling sick is a normal response to infection that is often overlooked. Rather than being a mere side effect of being ill, the behaviours associated with sickness are thought to be part of a highly organised strategy whereby the organism’s priorities are reorganised to fight infection and are therefore vital for survival (153). The dramatic changes in behaviour which occur in response to viral or bacterial infection are collectively termed 'sickness behaviour'. Symptoms of sickness behaviour include anhedonia, fever, cognitive dysfunction, loss of appetite, anxiety/irritability, fatigue, aching joints, sleep alterations, increased sensitivity to pain, social withdrawal and psychomotor slowing (154). These changes are thought to be part of a motivational state where the organism’s priorities are reorganised to promote resistance to pathogens and recovery from infection, for example through conserving energy and increasing body temperature. A rich evidence base has confirmed that such symptoms are triggered by pro-inflammatory cytokines, most notably IL-1α, IL-1β, IL-6 and TNF-α, that are induced by infectious agents in the periphery.

For example the peripheral or central administration of cytokines IL-1, TNF-α or the cytokine inducing lipopolysaccharide (LPS) to mice and rats induces the full spectrum of sickness behaviours (155). These cytokines mediate the local and systemic inflammatory response to invading pathogens but also infiltrate into the brain via the pathways described previously.
Once in the brain these cytokines induce a central inflammatory response, as evidenced by the peripheral administration of LPS inducing the synthesis and release of cytokines IL-1α, IL-1β and TNFα in the brain (156). These centrally produced cytokines interact with a network of cytokine receptors distributed throughout the brain and induce neurochemical changes that elicit the psychological, behavioural and cognitive changes associated with sickness, though the exact signalling pathways that mediate these changes have not been fully elucidated. It is clear that sickness behaviour is mediated by cytokines in the brain, as inhibition of its action by the central administration of the IL-1 receptor antagonist (IL-1ra) prevents the depressive effect of peripherally administered IL-1 on social exploration and food-motivated behaviour in rats (157).

The relevance of sickness behaviour to depression rests on their striking resemblance. In both sickness behaviour and depression there is a withdrawal from the physical and social environment, anhedonia, low mood, fatigue, impaired cognition, loss of appetite and sleep disturbance. If these symptoms are caused by cytokines in sickness behaviour, as the evidence suggests, then a reasonable assumption would be that cytokines are also involved in the development of symptoms in major depression. However, the resemblance between sickness and depression is only partial. Some aspects of sickness behaviour such as fever are not apparent in depression. Furthermore, once an infection has been dealt with, sickness behaviour subsides and the organism’s priorities return to normal. It is therefore a fully reversible phenomenon that is tightly regulated, for example by endogenous glucocorticoids and anti-inflammatory cytokines (154). In this sense, sickness behaviour can be viewed as a consequence of a reversible episode of brain inflammation that is triggered by peripheral immune activation. But this is not the case for depression. In depression, prevalent low mood and anhedonia can be present in the absence of infection. Instead there could be an underlying, non-specific inflammatory process contributing to depression. As outlined above, cytokines trigger adaptive behavioural and psychological changes which show a significant similarity to the symptoms of depression. Taken together with evidence of an overactive cytokine system in depression supports, this points to a causal role for cytokines in the development of depression. Accumulating evidence suggests that this may be associated with prolonged stress, overactivation of the HPA axis and subsequent glucocorticoid resistance. This is discussed shortly, in 2.2.7.

For more direct evidence for cytokine-induced depression, animal models have been used to demonstrate that cytokines can induce depressive symptoms specifically. This is challenging due to the overlap between sickness behaviour and depression-like behaviour. For example, the loss of appetite in sick animals mimics the decreased intake of food associated with anhedonia. Therefore, to demonstrate a role for cytokines in the aetiology of depression it is necessary to show that cytokine-induced depressive-like behaviour occurs independently of
sickness behaviour and that it is relieved by antidepressant treatment. One study demonstrated that administration of IL-1ra attenuated the avoidance deficit seen in rats faced with inescapable shock (158). Further experiments demonstrated that decreases in rewarding self-stimulation in rats administered LPS or IL-2, indicative of cytokine-induced anhedonia. It has also been shown that chronic antidepressant treatment attenuates depressive-like behaviours induced by a peripheral immune challenge (159). Thus there is evidence that cytokines can induce symptoms that are more in line with depression than sickness, though these studies must be interpreted with caution, as symptoms such as anhedonia can only be partly assessed, whilst others, like suicidal ideation, are impossible to measure in an animal. In humans though, symptoms of depression can be fully assessed and research into immunotherapy gives compelling evidence that cytokines induce symptoms of depression.

2.2.3 Immunotherapy & depression

Immunotherapy involves the administration of cytokines to activate the immune system and treat various medical illnesses. For example, IFN-α is used in the treatment of Hepatitis C and IL-2, IL-1 and TNF-α are used to treat certain cancers. Unfortunately though, alongside the therapeutic benefits of cytokine therapy, there are neuropsychiatric side effects including depressed mood, anhedonia, anxiety, fatigue and cognitive disturbances in a large proportion of patients (see table 2.1). Depression is seen as the hallmark of IFN-α induced neuropsychiatric side effects. Indeed studies indicate that 30-45% of patients being treated with IFN-α develop symptoms consistent with DSM criteria for depression (24; 160). Suicidal ideation has also been reported in immunotherapy and there have been incidences of attempted and completed suicide following IFN-α therapy (161). The neuropsychiatric effects of immunotherapy are time and dose dependent, usually appearing between the first and third month of treatment and disappearing once therapy ceases. The neuropsychiatric changes associated with immunotherapy strongly suggest a causative role for cytokines in the development of depression. These changes were observed almost twenty years ago when immunotherapy was first used to treat cancer and Hep C patients (162; 163) (see table ?). Since then, systematic investigations have confirmed that the neuropsychiatric side effects of immunotherapy are caused by cytokines accessing the brain (23; 160; 164–166). Specifically, it has been shown that immunotherapy induces activation of the cytokine network, which in turn results in symptoms of depression and other neuropsychiatric side effects. For example IFN-α is a potent inducer of pro-inflammatory cytokines. Accordingly Hep C patients treated with IFN-α exhibited higher serum IL-6 and IL-8 levels, which were significantly related to depression and anxiety scores (167). Immunotherapy can therefore provide a valuable model for studying the pathophysiology of cytokine-induced depression.
Research suggests that serious symptoms of depression develop in approximately half of
patients receiving immunotherapy. It seems that there are vulnerability factors which may
contribute to depression in some people and not others. For example studies have
demonstrated that a patient’s initial affective state, measured at baseline, is a risk factor for
the development of depression during IFN-α therapy (169; 170). Furthermore, baseline
immune activation (171) and heightened HPA axis activity (172) have been found to increase
the risk of developing depression during immunotherapy.

There appear to be two distinct categories of cytokine-induced side effects:
neurovegetative/somatic symptoms (fatigue, decreased appetite, pain, sleep disturbance)
and psychological symptoms (depressed mood, cognitive impairments, irritability, anxiety).
The two sets of symptoms appear at different timepoints during immunotherapy. For
example, in patients treated with IFN-α for malignant melanoma, neurovegetative and
somatic symptoms appeared early on, in week 2 of treatment and remained prominent
throughout the duration of treatment, whereas depressive and cognitive symptoms
developed later on, being most prominent in weeks 8-12 (23). The dissociation in timecourse
between these two symptom domains suggests distinct pathophysiological mechanisms
through which IFN-α induces vegetative/somatic and depressive/cognitive symptoms.
Furthermore, these symptom domains had differential responses to the SSRI paroxetine,

### Table 2.1 Neuropsychiatric side effects of immunotherapies (168)

<table>
<thead>
<tr>
<th>Immunotherapy</th>
<th>Neuropsychiatric side effects</th>
<th>Clinical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α</td>
<td>Fatigue, Psychomotor slowing, Depressed mood, Anxiety, Social withdrawal, Irritability, Anorexia, Cognitive disturbances (mental slowing, lack of concentration, memory impairment)</td>
<td>Cancer, Multiple sclerosis, Chronic hepatitis C, Other viral infections</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Fatigue, Depressed mood, Cognitive impairment</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>IL-1</td>
<td>Cognitive impairment</td>
<td>Cancer</td>
</tr>
<tr>
<td>IL-2</td>
<td>Fatigue, Anhedonia, Dysphoria, Cognitive impairment</td>
<td>Cancer</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Fatigue, Anorexia</td>
<td>Cancer</td>
</tr>
</tbody>
</table>
which improved depressive/cognitive symptoms but had minimal effect on vegetative/somatic symptoms. This finding has been replicated in numerous placebo-controlled trials, where SSRIs have been found to reduce the incidence and severity of the IFN-α depressive symptoms, but not the vegetative symptoms (24; 173; 174).

Consistent findings of SSRI efficacy in reducing the development and severity of depressive symptoms during immunotherapy suggest the involvement of serotonin in the mechanism underlying cytokine-induced depression. Indeed preclinical and clinical studies have shown that pro-inflammatory cytokines can interfere with the metabolism of serotonin. Serotonin (5HT) is synthesised within the brain from its precursor tryptophan (TRP) and is therefore entirely dependent on its availability. Tryptophan is metabolised through two main pathways: one is the serotonin pathway, initiated by the enzyme tryptophan hydroxylase, and the other is the kynurenine pathway, where the enzyme indoleamine 2,3-dioxygenase (IDO) catalyses the conversion of TRP into kynurenine (KYN) and then quinolinic acid (QA). Pro-inflammatory cytokines stimulate IDO and activate the KYN pathway, thus limiting the availability of TRP for the synthesis of serotonin (175). It might be this mechanism that underlies the depressive symptoms that develop in response to immunotherapy, which would be consistent with the monoamine hypothesis of depression. Indeed reduced concentrations of blood TRP is associated with reduced levels of 5HT availability in the brain (176) and TRP depletion is associated with low mood in healthy volunteers and relapse in depression (177).

In support of this, Capuron et al. showed that serum TRP concentrations decreased in cancer patients receiving either IL-2 or IFN-α and that the severity of depressive symptoms was correlated to the magnitude of the decreases in TRP concentrations. Furthermore, depressive symptoms developed on a matched timecourse with decreases in TRP concentration (178). In light of this, activation of the KYN pathway resulting in a reduction of TRP seems highly plausible. Another explanation is that cytokine therapy indirectly reduces TRP concentration as a result of reduced appetite and a subsequent decrease in food intake. However, studies have demonstrated significant increases in KYN in addition to decreases in TRP, which correlated with depressive symptoms, in patients receiving IFN-α (167; 179). This supports the hypothesis that the IDO-mediated activation of the KYN pathway leads to a reduction in TRP and subsequent depressive symptoms that develop during IFN-α therapy. It is possible that SSRIs counterbalance the effects of IFN-α on TRP metabolism by enhancing the central availability of 5HT through inhibiting synaptic reuptake. The effect of inflammation on neurotransmitter functioning is revisited when discussing the mechanisms underlying the association between inflammation and depression.

Although investigations into the neuropsychiatric effects of immunotherapy provide compelling evidence for the ability of pro-inflammatory cytokines to cause symptoms of
depression and cognitive impairment, they are limited in terms of generalisability. The patients in these studies have been severely ill and so cytokine administration may add to pre-existing, illness-associated medical and psychological factors. Also, cytokines are administered in very high doses during immunotherapy which also induce sickness and may affect emotional and cognitive processing. Therefore, the effects of a low dose of an endotoxin which potently induces a cytokine response, have been investigated in healthy volunteers (180). *Salmonella abortus equi*, administered intravenously to healthy volunteers, induced increased levels of cytokines which correlated with increases in anxiety and depressed mood in addition decreases in verbal and nonverbal memory. These changes were reversed once the cytokine levels normalised. This provides yet further evidence for a casual role for cytokines in the development of depression and suggests that the depression seen in immunotherapy studies cannot be solely explained by illness or high administration dosage.

That such a large proportion of patients develop depression when administered cytokines as part of immunotherapy, and that this is time and dose-dependent, and that mood changes also occur in healthy volunteers provides compelling evidence for the causal role of cytokines in depression. However, not all patients receiving immunotherapy develop symptoms severe enough to meet diagnostic criteria and it seems that there are both physiological and psychological risk factors that affect a person’s vulnerability to cytokine-induced depression. At a physiological level, both serotonergic system and the HPA axis seem to be involved, though the mechanisms underlying cytokine-induced depression may differ across individuals. There also seems to be a distinction between symptom domains, with neurovegetative/somatic symptoms developing at a different timescale to the psychological symptoms. It is clear that immunotherapy provides a valuable model for studying the pathophysiology of cytokine-induced depression. Continued work needs to fully elucidate the physiological mechanisms and neural substrates that underlie the neuropsychiatric side effects induced by cytokine administration. This will not only contribute to our understanding of depression but also enable the effective treatment of these distressing side effects that patients have to endure on top of the illness they are being treated for.

### 2.2.4 Medical comorbidity

The prevalence of depression is much higher in the medically ill compared to the general population (73) (see table 2.2). It is not surprising that the onset of a disabling medical disorder is associated with a greater risk for developing depression. However, mounting evidence suggests that depression is itself a risk factor for medical illness. Inflammation is a prominent feature of medical illness. Based on the involvement of inflammation in medical illness and depression, a logical explanation is that inflammation mediates the link.
The association between depression and cardiac disease has been studied intensively, perhaps owing to the 17-27% prevalence rates of depression in those suffering from coronary heart disease (CHD) (181). CHD is caused by the narrowing of the arteries due to the build-up of atherosclerotic plaque. It is a chronic inflammatory disease associated with an increase in local and systemic cytokines such as IL-6 and CRP. Depression is now established as a risk factor for CHD and so it has been hypothesised that inflammation may mediate the link between CHD and depression. Higher depression scores have been found to increase the risk for CHD 3-4 fold, despite other risk factors (182). Furthermore, depression significantly worsens clinical outcome. This is particularly apparent in myocardial infarction (MI) patients, where those with depression have a much poorer prognosis than those without depression. For example, in a prospective study of survival in MI patients, those with depression had a four-fold greater risk of cardiac death than those without depression (183). There is some evidence for a dose-response relationship between depression and CHD, suggesting a causal role. For example, the risk of cardiac events was twice as high in CHD patients with major depression than in patients with minor depression (184).

The mechanisms for this are not clear but the involvement of inflammation is biologically plausible. It has been hypothesised that an inflammatory response triggered by prolonged stress and a subsequent overactive HPA axis, along with maladaptive health practices associated with depression such as physical inactivity, poor diet and smoking may contribute to CHD progression (15). An alternative explanation for the increased incidence of depression in medical illness is that the release of proinflammatory cytokines as a function of disease-related inflammatory processes contributes to the pathophysiology of depression. This is in line with the ability of cytokines to signal to the brain and cause behavioural and

<table>
<thead>
<tr>
<th>Comorbid Medical Illness</th>
<th>Prevalence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac Disease</td>
<td>17–27</td>
</tr>
<tr>
<td>Cerebrovascular Disease</td>
<td>14–19</td>
</tr>
<tr>
<td>Alzheimer's Disease</td>
<td>30–50</td>
</tr>
<tr>
<td>Parkinson's Disease</td>
<td>4–75</td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
</tr>
<tr>
<td>Recurrent</td>
<td>20–55</td>
</tr>
<tr>
<td>Controlled</td>
<td>3–9</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Self-reported</td>
<td>26</td>
</tr>
<tr>
<td>Diagnostic interview</td>
<td>9</td>
</tr>
<tr>
<td>Cancer</td>
<td>22–29</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>5–20</td>
</tr>
<tr>
<td>Pain</td>
<td>30–54</td>
</tr>
<tr>
<td>Obesity</td>
<td>20–30</td>
</tr>
<tr>
<td>General Population</td>
<td>10.3</td>
</tr>
</tbody>
</table>
psychological changes consistent with symptoms of depression. The relationship between
depression, inflammation and medical illness is not simple. It could be that depression and
medical illness have a bidirectional relationship and that depression can be both a cause and
consequence of disease. Whether inflammation underlies this relationship is yet to be
confirmed but it seems biologically plausible.

It is not just in CHD that depression is so common. Depression has been estimated to affect
30-35% of patients recovering from stroke and is associated with increased mortality rates
and poor functional outcome (185). Depression is also very common in neurodegenerative
disease, diabetes, cancer, HIV and obesity (see table 2.2). It is likely that directionality differs
across medical disorders. For example, in neurological diseases such as Parkinson's Disease,
depression is likely to be a consequence of the neurodegenerative process. But in obesity, a
systematic review of longitudinal studies found a reciprocal link between depression and
obesity (186). Indeed obese people have a 55% increased risk of developing depression. This
seems to be related to depression having a causal effect on obesity, for example through an
unhealthy lifestyle involving physical inactivity and poor diet, but also to negative effects of
obesity on self-image and somatic consequences, leading to depression over time. Obesity is
associated with a chronic state of inflammation, induced by the accumulation of excess lipid
in adipose tissue, and is accompanied by elevated levels of inflammatory markers (187). As
inflammation plays a role in both obesity and depression, it is possible that inflammation
mediates their association.

Comorbid depression has been ignored in the past as the symptoms of depression are often
masked by the multitude of somatic and cognitive symptoms that can occur in medical illness.
Also, symptoms of depression are sometimes viewed as a predictable response to living with
a serious illness. But it is clear that depression is more than simply a consequence of medical
comorbidity. The symptoms can severely affect quality of life and studies have consistently
shown that depression is associated with poor prognosis and increased mortality. The
question of whether treating depression improves the overall clinical outcome has only been
partially addressed, but there is some evidence that treating depression does improve
functionality and mortality rates (188). The relationship between depression, medical illness
and inflammation seems complex and the direction of causality remains to be determined.
What is clear though is that there is a strong association between depression and medical
illness, and that mental and physical well-being are intimately related. Though this is yet to
be confirmed, it seems biologically plausible that inflammation mediates this link.

2.2.5 Mechanisms linking inflammation & depression

There are now several lines of evidence that support an association between immune
activation and depression; (1) depressed patients display increased concentrations of
inflammatory markers in comparison to healthy controls; (2) activation of the immune system in animals through administration of pro-inflammatory cytokines or cytokine-inducing LPS leads to dramatic behavioural changes known as ‘sickness behaviour’ that resemble symptoms of depression; these changes can be blocked by cytokine antagonists and potentially alleviated by anti-inflammatories/antidepressants in humans; (3) the use of cytokines to treat cancer and viral diseases such as hepatitis C is accompanied by neuropsychiatric side effects, particularly depressive symptoms and cognitive disturbances; (4) medical disorders, especially those with an inflammatory component, are associated with a high prevalence of depression. Taken together, there is compelling evidence that inflammation is involved in the pathophysiology of depression. However, the mechanisms underlying the association are yet to be fully established. Furthermore, the questions of direction of causality and whether activation of inflammatory pathways originates in the periphery or from within the CNS still need to be addressed. Over recent years though, a number of biologically plausible mechanisms able to explain the association between inflammation and depression have emerged.

2.2.6 Neurotransmitter functioning

A role for serotonin in the development of cytokine-induced depression is strongly supported by evidence that SSRIs administered during immunotherapy prevent the development of depression by up to four-fold, detailed earlier (24). Furthermore, pretreatment of rats with antidepressants has been found to block the behavioural changes associated with administration of bacterial endotoxin (159). These findings suggest that increasing the availability of serotonin in the synapse inhibits the depression-inducing effects of cytokines, implying that the depletion of serotonin as a function of cytokine exposure is a mechanism through which cytokines influence behaviour. As described earlier, serotonin is derived from the amino acid tryptophan and is entirely dependent on its availability. The enzyme indoleamine 2,3-dioxygenase (IDO) breaks down TRP into kynurenine (KYN) and then quinolinic acid (69). Immune activation, namely the presence of pro-inflammatory cytokines, stimulates the enzyme IDO, thus activating the kynurenine pathway leading to a lower availability of plasma TRP for serotonin synthesis (figure 2.3).
Supportive of this and mentioned earlier is evidence of decreased TRP and increased KYN in patients receiving IFN-α therapy (178). Blockade of IDO has been shown to inhibit LPS-induced depressive-like behaviour in mice (189). Furthermore, blockade of IDO following LPS administration resulted in attenuation of depressive-like behaviour as well as attenuated microglial activation (190), suggesting a direct relationship between IDO-induced activation of the KYN pathway and microglia. In addition to TRP depletion and reduced 5-HT, cytokine-induced activation of the kynurenine pathway via IDO produces TRP catabolites (TRYCATs) which can activate oxidative pathways, cause mitochondrial dysfunction and have neuroexcitatory and neurotoxic effects that can lead to neurodegeneration (191). The cytokine-induced production of TRYCATS also affects glutamatergic transmission.

In the TRYCAT pathway KYN is converted to kynurenic acid (KA) in astrocytes and quinolinic acid (QA) in microglia. Whilst kynurenic acid is an N-methyl-D-aspartate (NMDA) receptor antagonist, therefore inhibiting the release of glutamate, quinolinic acid is an NMDA receptor agonist and thus promotes the release of glutamate. Microglia are the only cells of the nervous system that express the complete enzymatic pathway required for synthesis of quinolinic acid. Therefore, any inflammatory mediators acting on microglia will increase the quinolinic acid to kynurenic acid ratio, leading to net NMDA agonism which could in turn lead to excess glutamate and neurotoxicity (192). The role of glutamate in depression has gained increasing support over recent years, evidenced by elevated glutamate levels in depressed patients, in addition to mounting evidence from the antidepressant effects of NMDA antagonists such as ketamine, which has almost immediate effects (193). Glutamate also causes TNF release from endotoxin-activated microglia. Therefore, NMDA agonism and a subsequent increase in glutamate could activate microglia and cause further release of inflammatory cytokines, leading to a vicious circle. In addition, there is evidence that
microglial activation and the release of inflammatory mediators within the CNS inhibit the removal of excess glutamate by excitatory amino acid transporters (EAAT) (194). The link between inflammation, glutamate and depression has been demonstrated in a study showing that ketamine abrogated the development of LPS-induced depressive-like behaviour in mice (195). This supports the theory that LPS-induced depressive-like behaviour is mediated by NMDAr activation following the production of QA from microglia.

Cytokines also affect the metabolism of dopamine (DA). IFN-α treated rats have been shown to exhibit decreases in CNS tetrahydrobiopterin (BH₄) and DA in association with stimulation of nitric oxide (NO). Tetrahydrobiopterin is the rate-limiting enzyme in DA synthesis; it converts tyrosine to dopamine's precursor L-3,4-dihydroxyphenylalanine (L-DOPA). Inhibiting NO synthesis has been found to reverse the inhibitory effects of IFN-α on BH₄. It is thought that cytokine influences on BH₄ through the activation of NO pathways might underlie reduced DA availability in certain brain regions (196).

It is possible therefore that the association between inflammation and depression is mediated by neurotransmitter function. Extensive research suggests a role for serotonin in the development of depression and more recently a role for glutamate has been proposed. Cytokine-induced activation of the IDO-mediated kynurenine pathway can explain both decreased serotonergic function and increased glutamatergic function. Dopamine availability also seems to be reduced by the KYN pathway through activation of NO. Therefore one possible mechanism linking immunity to depression is through the effects of pro-inflammatory cytokines on the kynurenine pathway, and the resultant disturbances in neurotransmitter functioning. However, given the complexities of depression and the intricate nature of the inflammatory response, it is likely that a disturbance of neurotransmitter metabolism only represents part of the picture.

2.2.7 Hypothalamic-pituitary-adrenal (HPA) axis

One of the most consistently observed findings in people with depression is hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, occurring in up to 80% of patients (197; 198). There is mounting evidence that this is associated with immune activation. Pro-inflammatory cytokines potently activate the HPA axis by stimulating the release of corticotropin releasing hormone (CRH), which has been consistently found to be elevated in depressed patients (197). CRH is the primary mediator of the hormonal response to stress and is considered to be the crucial link between psychosocial stress and depression (199). CRH is produced and released from the paraventricular (PVN) nucleus of the hypothalamus. CRH, in conjunction with vasopressin, then stimulates the pituitary gland to produce adrenocorticotropic hormone (ACTH), which then enters the bloodstream and causes the adrenal glands to release glucocorticoids such as cortisol (figure 2.4) (200).
HPA hyperactivity is normally regulated by means of an inhibitory feedback mechanism. In depression though, this feedback mechanism seems to be dysregulated. HPA interactions are regulated by glucocorticoids (GC) which are released to restrain neuroendocrine and immune responses to pathogens and stress. They are part of a feedback mechanism which dampens immune activity and downregulates HPA activation and are vital in maintaining homeostasis. One possible mechanism through which pro-inflammatory cytokines may activate the HPA axis is by inducing glucocorticoid resistance (decreased responsiveness to glucocorticoids) (197). It is thought that pro-inflammatory cytokines counteract the negative feedback action of glucocorticoids on the HPA axis, thus rendering it hyperactive. If this were the case, the normally inhibitory effects of glucocorticoids on cytokine production would no longer be as operative, which could ultimately lead to an unregulated feed-forward cascade of cytokine production.

Glucocorticoid resistance is demonstrated by the dexamethasone suppression test (DST). Dexamethasone binds to GRs and, normally, dampens the immune response by decreasing ACTH release, which in turn decreases cortisol levels. Non-suppression of cortisol secretion following dexamethasone administration is an indicator of glucocorticoid resistance and has been repeatedly found in patients with major depression (198). Not surprisingly, disorders characterised by excessive inflammation including inflammatory bowel disease, rheumatoid arthritis and asthma have also been associated with resistance to the inhibitory effects of
glucocorticoids (201). The diminished feedback regulation of the HPA axis due to glucocorticoid resistance is thought to be mediated, at least in part, by alterations in the glucocorticoid receptor (GR) (197). The mechanisms involved in glucocorticoid resistance are still unclear but the following have been proposed; 1) A downregulation of GRs in response to chronic raise levels of cortisol; 2) an alteration in the genetic structure of the GR; 3) interaction of ligand-independent pathways, e.g. cytokine signalling with GR signalling, causing impaired function (202). It is possible that impaired GR function and a subsequent hyperactive HPA axis may be secondary to chronic exposure to inflammatory cytokines during medical illness or stress. In further support of the involvement of GR dysfunction in depression comes from evidence that antidepressants may exert their clinical effects through GR upregulation (202).

A wealth of research indicates that the HPA system is overactive in depression. This includes evidence for increased levels of cortisol, ACTH and CRH in people with depression, in addition to non-suppression of cortisol following dexamethasone and some evidence of enlarged pituitary and adrenal gland in response to hypersecretion of CRH and ACTH, respectively (see (203) for a review). Considering that inflammatory pathways activate the HPA axis so potently and given all the evidence suggesting the involvement of inflammation and the HPA axis in depression, it seems obvious that inflammation, the HPA axis and depression are intricately involved. It is possible that impaired GR function and a subsequent hyperactive HPA axis may be secondary to chronic exposure to inflammatory cytokines during medical illness or stress. For example chronic stress can induce sustained elevations of cortisol which, over time, may cause immune cells to compensate for this by downregulating GR responsiveness to glucocorticoids, thus resulting in an overactive immune response. The stress response is driven by the HPA axis and it is highly plausible that the well-established relationship between stress and depression is mediated by the HPA axis.

2.2.8 Neurodegeneration

Neurodegeneration is defined as the progressive dysfunction and loss of neurons in the CNS. A common feature of neurodegenerative disease is chronic immune activation; in particular, activation of microglia. Depression is prevalent in neurodegenerative disease and neuroinflammation could be a mechanism shared by both. Depression itself may be associated with neurodegeneration. Indeed imaging studies in depression have demonstrated reduced volumes of hippocampus, amygdala, prefrontal cortex, anterior cingulate and basal ganglia (204). Structural changes in depression appear to be region-specific as opposed to global. Decreased hippocampal volume is one of the most consistent neurobiological findings in depression (205). Hippocampal volume reductions seem to be associated with duration of illness and memory impairment (206). Neuronal loss in depression could be related to a
neurodegenerative process and/or decreased neurogenesis. Neurogenesis is the process by which neurons are added to the brain. The hippocampus, specifically the dentate gyrus, is the primary area of neurogenesis in humans (207). Prolonged exposure to stress, as mentioned previously, results in activation of the HPA axis and the subsequent release of CRF, ACTH and glucocorticoids (see figure 2.4). Paradigms of prolonged exposure to stress in rodents result in glucocorticoid-dependent reductions in neurogenesis and volume in the hippocampus (208). Hippocampal neurons express receptors for glucocorticoids, suggesting glucocorticoids act directly on the brain. Taken together, these findings suggest that stress, depression and the hippocampus are linked through the action of glucocorticoids. Furthermore, hippocampal pathology might underlie the memory problems seen in depression.

Although many would object to the labelling of major depression as a truly neurodegenerative disorder, evidence does suggest that depression is associated with signs of neurodegeneration. The inflammatory and neurodegenerative (I&ND) hypothesis of depression has recently been proposed by Maes et al. (209). It posits that the enhanced neurodegeneration and defects in neurogenesis seen in depression are caused by inflammatory processes, including increased oxidative and nitrate stress (O&NS), increased circulation of proinflammatory cytokines and increased production of TRYCATs. These mechanisms are discussed briefly now.

The consumption of oxygen results in the generation of reactive oxygen (ROS) and reactive nitrogen species (RNS) which have neurotoxic effects. Oxidative stress is defined as the imbalance between oxidative and antioxidative systems. The brain is particularly vulnerable to oxidative stress as it uses 20% of the entire body's oxygen supply. Increased levels of reactive oxygen (ROS) and reactive nitrogen species (RNS) can have detrimental effects on membrane fatty acids, proteins and DNA which, unchecked, can lead to apoptosis (210). In normal conditions, antioxidants regulate both oxidative and nitrosative reactions to prevent damage. However, depression has been associated with limited antioxidant defences, as evidenced by lowered concentrations of tryptophan, tyrosine, albumin and vitamin E (211). A limited antioxidant defence system could lead to impaired protection against oxidative stress and could therefore be a contributing factor to neurodegeneration.

Neurodegeneration in depression might also stem from the production of neurotoxic tryptophan metabolites (TRYCATs) as a result of cytokine-induced activation of the IDO-mediated kynurenine pathway (figure 2.3). As mentioned previously the TRYCAT quinolinic acid is an NMDA receptor agonist and has neuroexcitatory and neurotoxic effects, perhaps contributing to neurodegeneration in depression. Inflammation has also been found to be associated with decreased neurogenesis, particularly in the hippocampus. For example, IL-1 exposure results in suppression of hippocampal neurogenesis in mice (212). Additionally,
TNF-α may contribute to neurodegeneration through inhibiting cell survival signals and potentiating glutamate neurotoxicity by stimulating microglial glutamate release (213). Likewise, glucocorticoids (raised in depression as described earlier) have been found to induce the release of glutamate and hence activate NMDA receptors, therefore potentially contributing to neurotoxicity, and ultimately neurodegeneration (214).

There is some evidence that depression, at least in some cases, is associated with neurodegeneration. One of the most-replicated findings is that stress and a subsequent excess in glucocorticoids inhibits neurogenesis in the hippocampus. This is in line with imaging studies indicating reductions in hippocampal volume in depression, with the established link between stress and depression and the memory impairments seen in depression. Whether the volume reductions reflect neurodegeneration, decreased neurogenesis or both are questions that are still open. Whichever mechanism underlies the volume reductions seen in some depressed patients, inflammatory processes are likely to be at play.

2.2.9 Neurotrophic factors

Neurotrophic factors are essential for neuronal development, function and survival. Brain-derived neurotrophic factor (BDNF) is an important neurotrophic factor abundant in the brain and periphery. Reduced expression of BDNF, indicative of impaired neuronal plasticity, has been associated with the pathophysiology of depression (215). In animal models, chronic stress paradigms have been found to reduce the expression of BDNF in the hippocampus, an effect which was reversed by treatment with different classes of antidepressants (216). This has been confirmed in humans; lower BDNF serum levels were found in depressed patients compared to controls and antidepressant treatment was able to restore BDNF levels to the normal value (217). Accordingly, the neurotrophic hypothesis of depression posits that loss of BDNF from the hippocampus contributes to an increased vulnerability to depression and that upregulation of hippocampal BDNF mediates antidepressant efficacy. One possible mechanism explaining a decreased expression of BDNF in depression is through the actions of pro-inflammatory cytokines. For example, a stress-induced increase in levels of IL-1β has been found to decrease glutamate release, which is thought to lead to a reduction in BDNF expression in the dentate gyrus (216). Also, hippocampal infusion of an IL-1β antagonist has been found to block the downregulation of BDNF resulting from social isolation (218).

The balance between cytokine levels and neurotrophic levels is thought to be associated with programmed cell death. For example, BDNF counteracts the actions of pro-inflammatory cytokines, protecting against neurotoxicity. Therefore, an increase in pro-inflammatory cytokines and a decrease in BDNF may synergistically function in favour of neurodegeneration. Consistent with this, a recent study found that patients with bipolar
disorder exhibited a pro-inflammatory state which worsened in the later stages of the illness and correlated with reduced BDNF levels, reflecting a decrease in neuroprotective mechanisms (219). As described previously, depression is associated with a hyperactive HPA axis and an increased release of glucocorticoids. Stress-induced glucocorticoid release has been found to inhibit the secretion of BDNF (220). Therefore the reduced BDNF expression found in depression could be at least partly due to excess glucocorticoid production.

### 2.2.10 Stress

Stress is associated with a dramatic increase in the risk of developing depression. The association between stressful life events and the onset of depression was first reported in the 1960s and is now one of the most replicated findings in the field (89). Strong evidence suggests that the link between stress and depression is mediated by inflammatory processes. It is now well established from animal and human research that psychological stress triggers an acute immune response. For example, a meta-analysis of 30 studies demonstrated robust increases in IL-6 and IL-1β in humans exposed to stress (148).

The link between stress and depression seems to involve altered HPA functioning. As described previously, perceived stress activates the release of CRH from the hypothalamus, which in turn stimulates the release of ACTH from the anterior pituitary and cortisol from the adrenal cortex (figure 2.4). Cortisol, in turn, normally inhibits the HPA system via negative feedback. As mentioned earlier, people with depression exhibit non-suppression of cortisol following dexamethasone administration. It is thus thought that glucocorticoid resistance disrupts the inhibiting effects of cortisol on the HPA axis, resulting in a heightened inflammatory state. This mechanism provides a clear biological link between stress and depression. People with depression exhibit an exaggerated stress-induced inflammatory response (221). This suggests that people with depression are more vulnerable to the physiological effects of stress. Indeed, life can be stressful and we have all experienced stressful life events, but not everyone goes on to develop depression. Therefore there are likely to be factors that increase an individual’s vulnerability to the destabilising effects of life. Both genetic and environmental influences are likely to underlie an individual’s reaction to stressful life events.

There is a strong association between early life stress and depression. Furthermore there appears to be a dose-response relationship between the severity of early life adversity and the subsequent development of depression (222). Evidence suggests that early life stress can have lasting effects on the immune system. For example, a recent prospective study showed that exposure to adverse events prior to age 8 was associated with increased inflammation at age 10 (223). Early life stress is also associated with increased levels of inflammatory markers in adulthood (146; 224; 225). There are also studies linking early life stress,
inflammation and depression. For example, in a large cohort study, depressed adults with a history of childhood maltreatment were more likely to have high levels of CRP compared to depressed people with no history of childhood maltreatment (146). Numerous studies have also linked severe life stress to the long-term disturbance of the HPA axis in depressed patients (226). It is thought that early life stress leads to sensitisation of the central stress response system, specifically to enhanced neuroendocrine, autonomic and behavioural responsiveness to stress via alterations in the HPA axis (227). In line with this, women who had experienced childhood abuse, with or without depression, had a significantly greater stress-induced ACTH response compared to controls and to women with depression (228). It is possible that a sensitised stress system caused by childhood adversity lowers an individual's threshold for experiencing subsequent stress, which might then lead to depression.

The neural mechanisms underlying stress-induced inflammatory response are beginning to be explored. In a recent study, healthy female volunteers were exposed to acute stress whilst undergoing an fMRI scan. They found that heightened neural activity in the amygdala in response to the stressor was associated with increases in peripheral inflammation (229). Another study used fMRI to investigate regions involved in a social stress paradigm following an inflammatory challenge (230). Increases in IL-6 were associated with greater activity in the anterior insula and dorsal ACC. The activity in these regions also mediated the association between IL-6 and depressed mood in the female participants.

Stress is not the only risk factor for depression. It is likely that other factors influence a person's vulnerability to depression. It is estimated that 30-40% of the risk for depression is genetically determined and it is now generally accepted that both genetic and environmental factors together underlie an individual's vulnerability to depression (222). Recent research suggests that genetic factors moderate the inflammatory response to stress (see (231) for a review). An individual's reaction to stress is likely to depend on the severity and frequency of the stressor but also the presence of psychological and biological variables. Recently, a 3-hit model has been proposed, where there is an interaction between a genetic predisposition early life stress and later stressful life events, resulting in a phenotype more vulnerable to stress and mental disorder (232). To summarise, a wealth of data from several lines of evidence indicates that stress triggers an inflammatory response. Stress and depression are strongly associated and inflammation might be one of the mechanisms that mediates this link. Indeed the mechanisms underlying the association between stress, inflammation and depression are yet to be confirmed but the research points to the involvement of the HPA axis. However, whether depressive symptoms are caused by external factors acting centrally on the brain, such as psychosocial stress, whether they stem from peripheral inflammatory processes from which cytokines subsequently enter the brain, or whether internal and
external stressors act synergistically to lead to the development of depression, remains unclear.

2.2.11 Antidepressants & inflammation

If pro-inflammatory cytokines play a causative role in the development of depressive symptoms then it would be reasonable to hypothesise that antidepressants may downregulate these cytokines. This seems to be the case to some extent, however there is a large degree of variation within the literature and findings are conflicting. One approach to studying the effects of antidepressants on cytokine levels is to look at differences in cytokine profiles before and after antidepressant treatment. A review of these studies indicated that whilst some antidepressants decreased levels of certain cytokines, others found no effect of antidepressants on cytokine levels and others even found an increase (233). It is likely that differences in methodology and heterogeneity in patient samples underlie these discrepancies. A more recent meta-analysis of twenty-two studies investigated the effects of numerous different classes of antidepressants on cytokines TNF-α, IL-6 and IL-1β (elevated in depression) (126). Overall, antidepressants reduced depressive symptoms, there was no effect on TNF-α, a reduction in IL-1β and a possible reduction in IL-6. Consistent with this, a large study found no change in TNF-α levels in responders or non-responders to SSRI treatment (234). These studies do not show overwhelming support for the hypothesis that antidepressants decrease cytokine levels. However, due to heterogeneity within the literature it is difficult to answer the question of the effects of antidepressants on cytokine levels sufficiently. Furthermore, different classes of antidepressants are likely to affect cytokine levels differently.

It may be the case that immune activation is only found in a subset of depressed patients and that it is only in this subset that antidepressants exhibit their anti-inflammatory properties by normalising cytokine production. Animal studies indicate that sickness behaviour and depressive-like behaviour can be attenuated by pretreatment with antidepressants (235) and, as mentioned previously, pre-treating cancer patients undergoing immune therapy with SSRI paroxetine significantly reduced symptoms of depression (23). Therefore there does seem to be some mechanism in which antidepressants are blocking the behavioural effects of cytokines. There are numerous pathways through which IFN-α may produce depressive symptoms, including an increased circulation of pro-inflammatory cytokines, depletion of TRP and the production of neurotoxic TRYCATS, and through stimulation of CRH and the subsequent activation of the HPA axis (23). Antidepressants may target any one, or perhaps a combination, of these pathways to alleviate depressive symptoms. Possible mechanisms of immune modulation by antidepressants are illustrated in figure 2.5 (168).
Due to all the evidence suggesting that inflammation plays a key role in the pathophysiology of depression, various anti-inflammatory agents have been trialled as antidepressants. One example is the cyclooxygenase-2 (COX-2) inhibitor celecoxib. COX-2 inhibitors inhibit the production of PGE₂, which normally stimulates the release of proinflammatory cytokines and has been found to be elevated in depressed patients (236). Patients treated with celecoxib as an addition to reboxetine showed significant improvements in depression scores compared to those treated with reboxetine alone (237). For example, Cytokine-induced sickness behaviour can be attenuated or even reversed by administering cytokine antagonists such as IL-1ra or anti-inflammatory cytokines such as IL-10 directly into the brain (238). The TNF-α receptor antagonist etanercept is used in the treatment of psoriasis. Patients treated with etanercept over placebo showed significant improvement in fatigue and symptoms of depression (239). In a RCT of another TNFα antagonist, infliximab, in treatment-resistant depression, there was no overall difference in symptom severity between treatment groups over a 12 week period (240). However, there was a significant improvement in those patients with high levels of baseline CRP, suggesting that anti-inflammatories may be effective in a subset of patients that have a heightened inflammatory state. That anti-inflammatory drugs have beneficial effects on depression symptomatology adds support to a role for inflammation in the aetiology of depression.
There is also mounting interest in the use of the second-generation tetracycline ‘minocycline’ as a new treatment for major depression, as emerging evidence from animal and human studies suggest that it holds both neuroprotective properties and anti-inflammatory properties. Specifically, administration of minocycline has been found to attenuate neuronal toxicity (241), restore hippocampal neurogenesis after LPS-induced inflammation (242) and protect against glutamate excitotoxicity (243). The neuroprotective properties of minocycline are thought to be due to suppression of microglial activation. Furthermore, it has been demonstrated that minocycline suppresses the hypoxic upregulation of pro-inflammatory agents NO, IL-1β and TNF-α (244), suggesting that minocycline is able to directly inhibit a heightened inflammatory state. In a recent study, minocycline demonstrated anti-depressant-like actions in the forced swimming test (FST) as evidenced by reduced immobility (245). An open-label study of minocycline as an adjunctive therapy to antidepressants showed significant improvements in symptoms (246). Taken together, these findings suggest that minocycline as a novel neuroprotective and anti-inflammatory antidepressant seems to be highly promising and a clinical trial in humans is called for.

2.3 Conclusion: Major Depressive Disorder

The reviewed research provides compelling evidence for the involvement of inflammatory processes in the pathophysiology of major depression. Despite inconsistencies within the literature it is difficult to deny some kind of association, based on convergent evidence from findings of; (1) increased concentrations of inflammatory markers in depressed patients compared to controls; (2) increased prevalence of depression in medical disorders, especially those with an inflammatory component; (3) cytokine-induced sickness behaviour in animal studies; (4) depressive symptoms as a side effect of cytokine therapy; (5) a possible role for anti-inflammatory drugs in alleviating depressive symptoms. However, whilst it might now be apparent that depression is associated with immune activation, the crucial question of whether inflammation is causally involved in the aetiology of depression, or whether inflammation is simply a consequence of depression, is yet to be answered. That the development of depression is frequent in inflammatory diseases, and that cytokine therapy induces symptoms of depression, suggest a causative role for cytokines in the pathophysiology of ‘depression due to a medical illness’. It remains to be seen whether inflammatory processes are causally involved in the development of major depression in the absence of comorbid medical illness or immunotherapy (110). However research into stress, its ability to induce an inflammatory response and its relationship with depression provides compelling evidence for the involvement of inflammation in depression in the absence of medical illness.
There are inconsistencies in the literature, with numerous studies citing a weak or even lack of an association between inflammation and depression. This may be explained by the inherently heterogeneous nature of major depression. Depression is an extremely complex mental disorder, with symptoms varying greatly between patients. Accordingly, there are numerous subtypes types of depressive episode based on symptomatology (catatonic, melancholic, atypical, postpartum, depression in bipolar affective disorder), longitudinal course (chronic, rapid cycling, seasonal) and response to treatment (247). Depression, as with other mental illness, is difficult to diagnose not only due to the variability in symptoms but also because, as of yet, there is no biological marker for its diagnosis. Despite decades of extensive research there is still no unifying pathophysiological basis for depression, illustrating a gap between diagnostic criteria and pathophysiology that does not fare well for the biological basis for depression. However, it could be that due to its heterogeneity and complexity, there is no existing final common pathway that is treatable with one class of drugs. Instead, it is possible that depressive symptoms develop as a result of a complex interaction between any number of the systems found to be involved in depression. Furthermore, this interaction may be different in each subtype or even individual case of depression, making depression a highly complex syndrome.

Mechanisms could include neurotransmitter disturbances, dysregulation of the HPA axis, and lower levels of antioxidants and neurotrophic factors, possibly leading to neurodegeneration. As described, an increase in pro-inflammatory cytokines is able to account for each of these mechanisms. The impact of stress in particular is thought to be associated with depression, as a long-standing clinical observation is that stressful life events often precede the first episode of depression. The biological changes accompanying this stressor, which may include a heightened immune response, are thought to result in an increased sensitivity to subsequent stressors. That depression may be caused by any number of these mechanisms could mean that the neurobiology underpinning each subtype or even each individual case of depression may be different. This would explain the fact that not all depressed patients are responsive to current antidepressant medication. It may also explain why inflammation only seems to be present in a subset of depressed patients. Each subtype of depression may have a distinct pathophysiological underpinning, rather than there being a distinct biological basis encompassing the whole of depression. Therefore, subtypes of depression may not only differ in terms of symptomology but also immunologically. For example, it has been found that melancholic and non-melancholic depressed patients show different immune patterns (134).

The ambiguity within the literature may be due to the heterogeneity of patient samples used in previous studies. Therefore, rather than dismissing the inflammatory hypothesis of depression based on these findings, it may be more beneficial to ask why evidence of inflammation is seen in some depressed patients and not others. The challenge remains to
elucidate the exact role of inflammatory processes in the aetiology of depression. Why
inflammation is only apparent in a subset of depressed patients and whether this is reflective
of a specific symptomatology, the exact role of anti-inflammatory agents or cytokine
antagonists in alleviating depressive symptoms, and the direction of causality all still remain
to be seen. Evidence of inflammation in the periphery is certainly suggestive of a role for
cytokines in depression. However, evidence of inflammation in the CNS would provide more
direct evidence for the involvement of inflammatory processes in the pathophysiology of
depression.

As far as I am aware, only two studies have investigated CNS inflammation in depressed
patients in-vivo using PET (248). The first used the TSPO tracer $[^{11}\text{C}]\text{PBR28}$ was to compare
the level of TSPO, indicative of activated microglia, between individuals in an episode of
major depression and controls. They found no significant difference in TSPO levels between
patients and controls and no correlation between TSPO levels and symptom severity. There
are a number of possible explanations for this. It is possible that this is a true finding and
depression is not associated with CNS inflammation. Alternatively, methodological issues
might account for the lack of difference. Firstly, the patients recruited to this study exhibited
mild-to-moderate symptom severity. It could be that any central inflammatory changes are
not severe enough to be detected by PET in mild cases of depression. In addition, a number of
the patients were taking antidepressants, which may have immunomodulatory properties.
Alternatively, it could be due to their choice of radiotracer. The quantification of $[^{11}\text{C}]\text{PBR28}$
is problematic. Because of its high affinity, it also binds extensively to the endothelium,
potentially masking the signal from microglia and the ability to detect group differences
(249). In contrast, a second study, using the radiotracer $[^{18}\text{F}]\text{FEPPA}$, found a significant global
increase in microglial activation in a sample of antidepressant-free MDD patients in a
moderate-to-severe MDE (250). Post-hoc they showed significant increases in PFC, ACC and
insula. Furthermore they showed a correlation between TSPO expression in the ACC and
symptom severity. The authors concluded that neuroinflammation is likely to be a causal
mechanism in the development of depression.

2.4 Research Aims: Major Depressive Disorder

This research aims to address some of the gaps in the field. The primary aim is to determine
the presence of CNS inflammation in MDD compared to matched controls. As stated
previously, much of the research has focussed on peripheral inflammation in MDD. Some
studies have shown raised CSF levels of inflammatory markers but investigations of CNS
inflammation in brain tissue are lacking. Indeed only two studies have looked at CNS
inflammation in brain tissue in-vivo (described above). This research addresses some of the
methodological issues apparent in the previous studies. Firstly, I have recruited a sample of
MDD patients who were free of antidepressant medication at for at least eight months prior to scanning. This is important due to the immunomodulatory effects of antidepressants. Whilst this research is at times conflicting, a repeated finding is that antidepressants reduce levels of cytokines. Their effects on the brain are largely unknown but it is highly possible that antidepressants have either a direct or indirect effect on microglial activation. The majority of studies in the field have included patients on a range of different classes of antidepressants. Measuring peripheral and central inflammation in a cohort of antidepressant free MDD patients sets this research apart from many studies in the field and removes the potential confound of antidepressant effects on inflammation.

The patients recruited to the study have also been in a major depressive episode (MDE) of moderate-to-severe severity (as measured by scores on Beck Depression Inventory (BDI), Hamilton Depression Rating Scale (HDRS) and Montberg-Asberg Depression Rating Scale (MADRS). This is important for two reasons. Firstly, studies have shown correlations between symptom severity and markers of peripheral inflammation. Patients with mild symptom severity exhibit lower concentrations of peripheral inflammatory markers than those with moderate-to-severe severity. Therefore inflammation in the CNS may not be detectable by PET in mild cases of depression. Secondly, whilst many previous studies have included heterogenous patient groups ranging from mild to severe depression we have selected a narrower range of severity thus making our sample more homogenous.

As mentioned previously, many factors can affect inflammation, including diet, smoking, alcohol/drug abuse, BMI, sleep, medical illness, mental illness, psychotropic medication, psychosocial stress and childhood adversity. We have tried to control for as many of these factors as possible. All of the recruited patients had a diagnosis of MDD and were in a MDE of moderate-to-severe symptom severity, were not taking antidepressants, were medically healthy, did not smoke, had no current or lifetime history drug or alcohol abuse, had no comorbid mental disorder or personality disorder and were not classified as obese. BMI was hard to control for, so although none of the patients were classified as obese, some were overweight. We have attempted to account for this through appropriate matching to controls (see methods chapter). Certain factors such as sleep and childhood adversity were out of our control but we took detailed information on these so that correlations with peripheral and central inflammation can be investigated.

As described earlier depression is associated with cognitive impairments including memory and executive function (109). The administration of pro-inflammatory cytokines to animals and to humans as part of immune therapy results in cognitive deficits including impairments in memory, attention and learning (251)(23). No studies have investigated correlations between cognitive function and measures of peripheral and central inflammation. We aim to
assess this by administering a small battery of cognitive tests assessing recall and recognition memory and emotional recognition.

This research aims to explore the involvement of both peripheral and central inflammation in MDD. It addresses some of the issues that might confound previous research by choosing a sample of antidepressant-free patients with moderate-to-severe symptom severity. It also explores the relationship between inflammation, cognitive function and MR measures of structural integrity. There will still be unanswered questions though. Whether inflammation is a cause or consequence of depression cannot be answered with this research. Also, whether the inflammatory process begins within the CNS or whether inflammatory signals reach the brain from the periphery thus triggering microglial activation, might still remain unclear. There is also the question of microglial phenotype; is the microglial activation measured with PET reflective of a pro- or an anti-inflammatory phenotype? This cannot be answered at present but one can speculate based on the literature that any microglial activation seen in depression is likely to reflect a pathological process. Clearly we are still a long way off fully elucidating the involvement of inflammation in depression. However, through combining PET, MRI, peripheral blood, neuropsychological and clinical data, this research should fill at least some gaps in the research and make a novel contribution to the literature. The hope is that the findings give us more insight into the pathophysiology of depression and that it contributes to the ultimate goal of depression research; to treat depression more effectively and end the suffering that it causes so widely.
3.1. Introduction to Schizophrenia

Schizophrenia is a mental disorder affecting approximately 0.5%-1% of the population worldwide (252). Despite the relatively low prevalence of the disorder, it is one of the major contributors to the global burden of disease (253), ranked among the top 20 causes of disability worldwide (254). It can severely affect mental and behavioural functioning, typically from late adolescence/early adulthood, continuing in episodes throughout the lives of those suffering from it. The hallmark of schizophrenia is psychosis, in which people lose touch from reality, for example through experiencing auditory hallucinations and paranoid delusions. Schizophrenia also consists of cognitive impairments and symptoms associated with affect and motivation. The culmination of these symptoms can significantly affect the ability to function in society (occupationally and socially) and can greatly diminish quality of life.

Schizophrenia remains a big challenge to psychiatry and to society as a whole. Its aetiology is still largely unknown and the current treatment approach has major limitations. These limitations present a problem not only for those suffering from schizophrenia but to society as a whole. Schizophrenia is associated with high rates of unemployment and reduced financial independence (255). Therefore the disorder can put a significant strain on resources. The predominant treatment approach is currently the use of antipsychotics which act as antagonists at dopamine D2 receptors. Antipsychotics however are not universally effective and have side effects which are often highly debilitating. There has been a distinct lack of progress in discovering more effective pharmacological treatments for schizophrenia since the advent of antipsychotics in the 1950s. This may reflect the difficulties in elucidating its underlying pathophysiology. Schizophrenia is a very complex brain disorder. It is highly heterogeneous in terms of clinical profile, severity of symptoms, course, treatment response and, in all likelihood, neurobiology. It is characterised by a number of psychopathological dimensions, described below, which vary in severity across patients and are likely to result from separate pathological processes. Furthermore, it is thought to result from a complex interaction between genetic and environmental risk factors which are only beginning to be unravelled.

Still, continued research into genetic and environmental interactions underlying schizophrenia, alongside advances in structural and functional neuroimaging and the search for novel biomarkers will widen our understanding of its causes. The ultimate goal of
Schizophrenia research is to identify and target pathological processes underlying all symptom domains and thus treat the disorder more effectively than at present. One novel research avenue that is receiving increasing attention as a potential treatment target is inflammation. Before reviewing the literature citing an association between schizophrenia and inflammation and providing rationale for the current study, an overview of the epidemiology, symptoms, course and treatment of schizophrenia is provided to give sufficient background to the disorder. Current dominant theoretical frameworks are also mentioned in order to place the current research into context.

3.1.1. Clinical symptoms

Schizophrenia is clinically characterised by the manifestation of symptoms that are a distortion of normal functions (positive symptoms), symptoms that describe a blunting or loss of range of affective and motivational functions (negative symptoms) and cognitive impairments (see figure 3.1). Positive symptoms include delusions, hallucinations and thought disorder. Delusions are false beliefs that are strongly held despite contrary evidence and fall into a number of categories. Persecutory delusions involve the belief that one is being followed, harassed or spied upon by others; delusions of reference describe the phenomenon of feeling that innocuous events have strong personal significance; delusions of control refer to the belief that one's thoughts and behaviour are controlled by another person, group of persons or external force. Common delusions of control include thought broadcasting (others can hear their thoughts), thought withdrawal (their thoughts are being taken), thought insertion (thoughts are being planted in their head). Grandiose and religious delusions also exist.
Hallucinations refer to the perception of a sensory experience in the absence of an external stimulus. Auditory verbal hallucinations (hearing voices) are the most frequent hallucinations experienced by people with schizophrenia; approximately three-quarters of patients hear voices and have a delusional interpretation of them (256). Visual hallucinations are less common, appearing in about half of patients, most frequently in the form of human figures showing movement (257). Somatic hallucinations can also occur, usually in the form of burning or tingling sensations, or in feeling that the body has changed in shape, size or form. Olfactory hallucinations also exist but these are less common. Delusions and hallucinations often fall under the umbrella term of ‘psychosis’: a mental state which reflects a loss of contact with reality.

Thought disorder also falls under the category of positive symptoms. This is where a person has difficulties in organising their thoughts and connecting them logically. A person with thought disorder’s speech may be characterised by loose associations or derailment, incoherence and tangentiality. Negative symptoms include a lack of motivation, blunted affect, anhedonia, apathy, poverty of speech and social withdrawal. Cognitive functioning is also severely affected in schizophrenia. Cognitive impairment in schizophrenia is substantially of a generalised nature, with additional specific deficits in memory, attention, processing speed and executive function. These cognitive impairments are present in the premorbid phase of schizophrenia and persist throughout its course (258) (259).

3.1.2. Epidemiology, course & diagnosis

The prevalence of schizophrenia varies across populations. A systematic review of prevalence estimates across 46 countries found lifetime prevalence rates to vary from around 1.6 to 12.1 per 1000 persons, with median lifetime prevalence of schizophrenia across all countries being 4 per 1000 (260). The incidence of schizophrenia is slightly higher in men than in women and men also tend to have an earlier onset (252). Childhood onset (below age 12) is rare but almost 20% of cases manifest in adolescence before age 18. The peak incidence for males and females is between the ages 18-25, but women have a second peak in the years 55-64 (261). The concept of what is now known as schizophrenia was introduced over a century ago by Emile Kraepelin. He labelled the illness ‘dementia praecox’ which emphasised the progressive deterioration that he believed to be central to the disorder. There was not always a progressive deteriorative course, however, and it was possible to show symptoms later in life. Accordingly Eugen Bleuler found the term ‘dementia praecox’ misleading and renamed the illness ‘schizophrenia’ (meaning ‘split mind’) to remove the emphasis from a deteriorating course and instead reflect the fragmented thought processes that he believed to be central to the illness.
There is a large degree of variation in the course of schizophrenia. In most cases the onset is gradual and there is a ‘premorbid phase’ where there are subtle changes in affect and cognition, often accompanied by unusual perceptions. This is often followed by a ‘prodromal phase’ with attenuated positive, negative and cognitive symptoms. The first episode of psychosis (FEP) symbolises the formal onset of schizophrenia. The subsequent course of illness varies substantially across individuals but the typical course of schizophrenia is characterised by exacerbations of psychotic symptoms with varying severity and inter-episode remissions of varying durations. It is thought that exacerbations can be triggered by stress, nonadherence to medication and substance abuse (262). Finally, patients enter a stable phase where psychotic symptoms subside but cognitive and negative symptoms are still prominent (263). Cognitive symptoms are generally stable throughout the course of the illness. Contrary to the Kraeplinian perspective of inevitable deterioration, some patients can recover and many have a good outcome. A recent meta-analysis of 50 studies revealed that approximately 1 in 7 patients met criteria for recovery (264) and a systematic review of longitudinal outcome studies in FEP found that 42% of patients had a good outcome (with 27% having a poor outcome) (265). Outcome seems to have improved over the past Century probably as a result of better treatment and, more recently, due to early intervention strategies (266). Outcome is determined by a multitude of factors including psychopathology, time of onset, social setting, treatment and environmental factors. Predictors of poor outcome include a family history of psychosis, male gender, greater cognitive impairment, premorbid symptoms, a long duration of untreated psychosis (DUP) and earlier age of onset (267).

Mortality rates in people with schizophrenia are approximately two to three times higher than those of the general population and life expectancy is cut short by approximately 15-20 years (268). A significant contributor to this increase in mortality is suicide. Individuals with schizophrenia are at a high risk for suicidal behaviour, with 10% of patients committing suicide and 20-40% making suicide attempts (269). These statistics highlight how distressing the symptoms of schizophrenia can be, but also the urgent need to treat the symptoms of schizophrenia more effectively and to prevent this devastating consequence of the illness. Whilst suicide is the biggest contributor to excess mortality in males, cardiovascular disease is the biggest contributor to excess mortality in females (270). Indeed medical comorbidity is common in schizophrenia and this substantially adds to the burden of the illness. Contributing factors are thought to include obesity, sedentary lifestyle, substance abuse and smoking. Indeed approximately 80% of individuals with schizophrenia smoke, often heavily (271) and there is a strong association between schizophrenia and substance abuse. Nearly 50% of patients have a co-occurring substance use disorder, with alcohol and cannabis being the most commonly abused substances (272). Substance use significantly worsens symptoms and substantially adds to the burden of the illness.
At present, the diagnosis of schizophrenia is based entirely on the presence and duration of symptoms. As with depression there are two main classification systems; the recent DSM-V and ICD-10. One of the main distinctions between DSM and ICD criteria for diagnosing schizophrenia relates to duration of symptoms. The ICD-10 stipulates that symptoms are present for just 1 month before a diagnosis of schizophrenia can be reached, whereas under DSM criteria symptoms must be present for 6 months and affect social and/or occupational functioning. Thus it is more difficult to reach a diagnosis of schizophrenia according to DSM compared to ICD criteria. The majority of research in schizophrenia to date has used DSM-IV criteria for assessing diagnosis. DSM-IV schizophrenia has good reliability and fair validity (273). Thus the essence of the DSM-IV criteria has been retained in DSM-V. However a number of key changes were made to address some of the shortcomings of DSM-IV. The most notable change is the abandonment of the clinical subtypes (paranoid, disorganised, catatonic, undifferentiated and residual) due to limited reliability and validity. As the current research was started before the release of the DSM-V, the DSM-IV criteria are used. However, as the symptom criteria necessary for the diagnosis of schizophrenia are largely the same between DSM-IV and V, this research remains in line with the current diagnostic system. In addition to the diagnostic systems, rating scales are used clinically and in research to determine severity of symptoms in each of the symptom domains, the Positive and Negative Syndrome Scale (PANSS) (274) being the most widely used.

3.1.3. Aetiology

The exact aetiology of schizophrenia is still unknown. But it is now apparent that schizophrenia is not caused by one single biological pathological process. Rather, a complex interaction between genetic and environmental risk factors seems to underlie an increased vulnerability for schizophrenia. The strongest known risk factor for developing schizophrenia is having a relative with schizophrenia. Indeed the lifetime risk increases from about 1% in the general population, to 10% in first degree relatives of those with schizophrenia and to around 50% in monozygotic twins and in children with two schizophrenic parents (275). Twin and adoption studies suggest that it is shared genes that determine this familial risk, with heritability estimates around 80% (276). Thus there is clearly a strong genetic underpinning to schizophrenia. However, it is also clear that schizophrenia is not caused by genes alone and that environmental factors must be involved too. Indeed it is now being increasingly realised that to gain a true understanding of schizophrenia, genetic susceptibility must be viewed in the context of the environment.

Over recent decades, a wealth of epidemiological studies has demonstrated that numerous environmental factors can increase the risk of developing schizophrenia. To outline all of the cited environmental risk factors for schizophrenia is beyond the scope of this overview and
so only some are listed. Exposure to early life environmental insults such as obstetric complications (277), prenatal exposure to infection (278) and childhood trauma (279) are associated with a greater risk for the development of schizophrenia. Other environmental risk factors include living in an urban environment (280), migration (281), psychosocial stress (282) and substance abuse (283). These environmental factors, along with psychological, sociocultural and even political factors are likely to interact with genes in a complex way to increase the risk for developing schizophrenia. Unravelling the complexity of these interactions remains a major challenge.

3.1.4. Neurobiological findings

Advances in neuroimaging techniques have enabled us to investigate the pathophysiology of psychiatric disorders in-vivo. As a result our understanding of the underlying neurobiology of disorders such as schizophrenia has widened considerably, but the precise mechanisms remain unclear and there are still many unanswered questions. In contrast to the incidental discoveries of pharmacological treatments of the past, neuroimaging should enable us to identify specific targets for drug development so that disorders can be treated in a more empirical manner. A range of neurobiological differences have been found between people with schizophrenia and controls. It is beyond the scope of this thesis to outline all of these in detail and so an overview of the dominant neurobiological findings is given, so as to place the current research into the context of the existing literature.

3.1.5. Dopamine Hypothesis

Schizophrenia research has focused on the dopamine system since the discovery of antipsychotics and their antidopaminergic properties in the 1950s. The dopamine hypothesis proposes that dopaminergic hyperactivity is responsible for the positive symptoms of schizophrenia, based initially on the correlation between the clinical efficacy of antipsychotic drugs and their ability to block dopamine D2 receptors (284) and on the psychotic symptoms produced by dopamine enhancing drugs (285). Since the advent of imaging technologies such as PET and SPECT, it has been possible to investigate the dopamine system in-vivo. Pioneering studies carried out by Farde and colleagues in the 1990s and more recent studies by Kapur et al. (286) have shown that striatal D2 receptor occupancy by antipsychotic drugs predicts clinical efficacy. Approximately 65% D2 receptor occupancy is necessary for antipsychotic efficacy and an excess of 80% occupancy results in a high risk of extrapyramidal side effects (287).

A recent meta-analysis of PET and SPECT studies investigating striatal dopamine function in schizophrenia found a significant increase in presynaptic dopaminergic function but no differences in dopamine transporter or receptor availability when compared to controls.
Specifically, increased presynaptic dopamine release seems to be specific to the associative functional (dorsal) division of the striatum and is strongly correlated with psychotic (positive) symptoms (289–291). Dopaminergic hyperactivity may be the final common pathway to psychosis, but it is not clear what is driving this. It could be secondary to prefrontal cortex dysfunction (292) or to abnormalities in glutamatergic function (293) (discussed below). All currently licensed antipsychotics block D2 receptors and so, whilst suppressing overall dopaminergic neurotransmission, they seem to be acting downstream from the source of abnormality.

It must be noted that not all patients exhibit elevated presynaptic dopaminergic function and the PET studies found substantial overlap between patients and controls. Furthermore patients that do not respond as well to antipsychotic treatment have shown lower levels of dopamine (294) and treatment-resistant patients have shown normal dopamine synthesis capacity (295). Therefore whilst an overactive dopamine system seems to underlie the positive symptoms in some schizophrenic patients, this is clearly not the case for everyone and it is likely that striatal dopaminergic hyperactivity explains only part of the picture. Indeed, this classical dopamine hypothesis cannot explain the negative and cognitive symptoms that are also present in schizophrenia. A revised dopamine hypothesis has since been proposed in attempt to account for the cognitive and negative symptoms (296). Based on findings from post-mortem, imaging and animal studies the revised DA hypothesis proposes that overactive dopamine function in the striatum is secondary to underactive dopamine function in the prefrontal cortex (PFC). However, there has been little direct evidence for frontal hypodopaminergia in schizophrenia. Furthermore it offers no explanation for the etiological basis of these regional dopaminergic abnormalities or for how these lead to the clinical manifestation of the disorder.

Kapur has since proposed an explanatory model linking dopamine to psychosis inspired by the incentive salience theory of addiction (297) The incentive salience theory of addiction is based on the idea that dopamine release in the ventral striatum signals that a given stimulus or event is important (or salient). All drugs activate dopamine release in the striatum and it is the salience attributed to drug taking rather than the pleasurable consequences that is thought to drive addiction (298). Kapur has proposed that psychosis is a disorder of aberrant salience. Specifically enhanced striatal dopamine release underlies delusions and hallucinations through the assignment of inappropriate salience to internal thoughts and external perceptions. Thus to a person experiencing psychosis, every thought or perception becomes loaded with meaning. Delusions can become crystallised as a top-down explanation is formulated in an attempt to make sense of these experiences of aberrant salience. Delusions are constructed by the individual and are strongly influenced by their environment, culture and psychological disposition. Once these delusions are formed,
patients seek confirmatory evidence from the environment that further feed their delusions. According to this theory, antipsychotics work through ‘dampening salience’, thus attenuating as opposed to curing psychosis. This would explain the high incidence of relapse once medication is stopped (299).

There is substantial evidence that presynaptic striatal dopamine abnormalities are associated with the positive symptoms of schizophrenia. However, the dopamine hypothesis has failed to account for the cognitive and negative symptom domains of schizophrenia. It is likely that multiple neurotransmitters and neural pathways underlie these symptoms, which often precede psychosis. Dopamine hyperactivity in the striatum might be the final pathway that accounts for the positive symptoms but this may not be the fundamental abnormality in schizophrenia. Indeed striatal DA hyperactivity likely reflects a psychotic ‘state’ as opposed to a trait. The challenge remains to identify the pathways that precede this, so that we can target upstream pathological processes and treat all symptoms domains effectively, or even prevent the onset of psychosis entirely. Stated in such a way, this sounds almost simple. But schizophrenia is an extremely complex disorder, in terms of heterogeneity, genetic susceptibility and interactions with a multitude of environmental risk factors. Unravelling its pathological mechanisms is a daunting task that requires continued epidemiological, biological, neuroimaging, psychological and preclinical research. Whilst, as stated by Laruelle and Abi-Dargham, dopamine may be the ‘wind of the psychotic fire’ (300), it is clear that to understand schizophrenia in its entirety we need to look beyond dopamine.

### 3.1.6. NMDA/Glutamate Hypothesis

Glutamate is the primary excitatory neurotransmitter in the brain. It is essential for most aspects of normal brain function but in excess can have toxic effects and reflect pathological states. There are a number of receptor subtypes that respond to glutamate. These are divided into ionotropic (N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainite receptors) and metabotropic receptors (G-protein coupled receptors (mGluR:1-8). The NMDA receptor/glutamate hypothesis has emerged as an alternative to the DA hypothesis as an explanation for the symptoms of schizophrenia. The NMDA/glutamate hypothesis is based on the observation that phencyclidine (PCP) and ketamine produce symptoms similar to those of schizophrenia (301). Both agents exert their effects by blocking neurotransmission at glutamate N-methyl-D-aspartic acid (NMDA) receptors. Therefore it was proposed that a disturbance in NMDA glutamate receptor function might model the pathophysiology of schizophrenia. The similarity between NMDA-antagonist induced psychosis and schizophrenia has been repeatedly demonstrated since the 1960s (302) (303). PCP and ketamine have been found to produce some of the positive symptoms, negative symptoms (emotional blunting, anhedonia,
social withdrawal) and executive cognitive dysfunction associated with schizophrenia (303). However, the primary psychiatric effect of ketamine is dissociation. Hallucinations and delusions are far less common. For example, ketamine administered to healthy volunteers produced perceptual distortions but not hallucinations (304) and so the use of ketamine as a model for schizophrenia must be questioned. Still, administration of NMDA receptor antagonists has been found to exacerbate positive, negative and cognitive symptoms in patients with schizophrenia (305).

Mounting evidence suggests that NMDAr dysfunction is associated with increased as opposed to decreased glutamatergic transmission (306). For example, NMDA antagonists have been shown to increase glutamatergic transmission at non-NMDA receptors (AMPA and kainate receptors) (307) and systemic administration of NMDAr antagonists increase cortical glutamate levels (307) (308). Glutamate and glutamine levels can now be directly detected in-vivo using Magnetic Resonance Spectroscopy (MRS). Elevated glutamine levels have been found in the anterior cingulate following ketamine administration (309). A recent review of MRS studies in schizophrenia indicates elevated glutamatergic levels in medial prefrontal cortex (mPFC) and basal ganglia of medication free patients and a possible relationship between increased glutamate and decreased volume in the hippocampus, though there was a large degree of variation across studies (310).

One mechanism through which NMDAr dysfunction might lead to excess glutamate is through cortical disinhibition. GABA is the primary inhibitory neurotransmitter in the brain whose principal role is to reduce neuronal excitability. Blocking NMDA receptors is thought to prevent glutamate from driving inhibitory GABAergic neurons, resulting in a loss of control over excitatory glutamatergic projections. Thus NMDA dysfunction could result in excitotoxic processes secondary to disinhibition of cortical glutamatergic projection neurons. The resultant effect of disinhibition of glutamatergic projections could be excessive stimulation of neurons within their projection field, which could lead to the psychomimetic effects of NMDA antagonists (311). This mechanism could provide a link between NMDAr dysfunction and dopamine, which is clearly involved in schizophrenia in some capacity. Altered glutamatergic neurotransmission could have a knock-on effect on the dopamine system. Specifically NMDA hypofunction and a subsequent disinhibition of glutamatergic projections could lead to hyperstimulation of DA neurons. Indeed administration of ketamine has been found to increase dopamine levels in humans and in animals (312). If hyperactive dopamine function is secondary to NMDAr dysfunction then targeting NMDA receptors might be a more effective treatment option than antipsychotics, which may be exerting their actions too far downstream to alleviate the full spectrum of symptoms experienced by those with schizophrenia. It must be noted that ketamine and PCP, in addition to their effects on the
NMDA receptor, have direct effects on the dopamine D2 and serotonin 5-HT2 receptors (313) and so findings using this model need to be interpreted with caution.

Excessive release of glutamate can lead to ‘excitotoxicity’, a pathological process caused by an influx of Ca+ and Na+ into postsynaptic neurons that can have toxic effects and lead to cell death (314). Glutamate excitotoxicity, secondary to NMDAr dysfunction and disinhibition of GABA-ergic neurons, could therefore account for the structural abnormalities that seem to be apparent in schizophrenia, described below (section 3.1.7). As mentioned above, there is some evidence of increased glutamate levels in patients with schizophrenia (310). If excessive glutamatergic transmission is associated with schizophrenia, then reducing glutamate transmission would be a potential target for drug development. In support of this, pretreating healthy volunteers with lamotrigine, a compound that attenuates cortical glutamate release via inhibition of Na+ channels, decreased the ketamine-induced symptoms that resemble the positive, negative and cognitive symptoms of schizophrenia (315). Lamotrigine in conjunction with clozapine has been found to improve negative, positive and cognitive symptoms in treatment-resistant schizophrenic patients (316). Further studies are needed to determine whether attenuating glutamate release is a viable treatment option for schizophrenia.

Whilst antipsychotics are largely effective in treating the positive symptoms of schizophrenia, their inefficacy in treating the cognitive and negative symptoms indicate that the dopamine hypothesis of schizophrenia is only relevant to the positive symptoms. Targeting NMDA dysfunction offers the potential to treat the negative and cognitive symptoms also. Further work needs to determine whether NMDAr dysfunction, excess glutamate and excitotoxicity precede the onset of psychosis and whether targeting these mechanisms would alleviate all symptom domains or even prevent the transition from an at-risk state to schizophrenia.

3.1.7. Structural abnormalities

Kraepelin considered progressive deterioration to be central to dementia praecox and insisted it was a disease of the brain that would one day be proven by pathological research. However, efforts to identify post-mortem brain abnormalities in the late nineteenth and early twentieth centuries did not produce any consistent findings, leading to the labelling of schizophrenia research as the ‘graveyard of neuropathologists’ (317). A lack of any concrete evidence of an underlying pathology to schizophrenia led to a psychodynamic approach to schizophrenia research which focussed on functional rather than biological causes and took precedent over the Kraepelinian biological approach for some time. Although the discovery of antipsychotics and the emergence of the dopamine hypothesis rekindled interest in the biological approach to schizophrenia, it was not until the first computed tomography (CT) study of schizophrenia in the 1970s that there was a return to the study of the
neuropathology of schizophrenia. Johnstone et al.’s landmark study (318) found enlarged ventricles in a small number of chronic schizophrenic patients. This led to a proliferation of CT and, later, MRI studies searching for structural abnormalities in schizophrenia. Numerous meta-analyses of volumetric MRI studies have found ventricular enlargement and decreased grey matter volume in schizophrenia (319–322). Meta-analyses of volumes of specific regions indicate reduced volumes of anterior cingulate (323), thalamus (324), amygdala (325) and hippocampus (326) in patients with schizophrenia.

However, the degree of ventricular enlargement and cerebral reduction is subtle and there is a significant overlap between patients and controls. The average volume reduction is around 3% and it is not clear whether such a subtle difference is related to schizophrenia itself or related to confounding factors such as antipsychotic medication, substance abuse, malnutrition or stress (327). A recent large meta-analysis of 317 volumetric studies distinguished between medicated and antipsychotic-naïve patients. In medicated patients there was an average total volume reduction of 2%. In antipsychotic-naïve patients there was evidence of grey matter loss although this was less extensive. Indeed there is evidence that antipsychotic medication is associated with brain volume reductions. For example, patients on typical but not atypical antipsychotics showed greater grey matter frontal, temporal-insular and precuneus reductions compared to drug-free patients (328). Another study in first-episode psychosis patients found that patients treated with high doses of haloperidol had smaller cortical grey matter volumes than those on lower doses (329), suggesting that the effect of haloperidol on brain volume may be dose-dependent.

Similarly, a longitudinal study in patients with first-episode psychosis found that haloperidol, but not olanzapine, was associated with frontal cortical loss over 2 years (330). Another longitudinal study found a 3% volume decrease over 1 year which was positively correlated with antipsychotic dose but that this was not affected by antipsychotic type (331). A large longitudinal study found that greater intensity of antipsychotic treatment was associated with smaller grey matter volumes and also progressive decrement in white matter volume (332), again suggesting a dose-dependent effect. A systematic review of MRI studies investigating effects of antipsychotic drugs suggests that antipsychotics do have regional effects on brain structure and that the type of antipsychotic has differential effects. Thus the initial studies citing structural abnormalities and progressive loss of grey matter must be interpreted cautiously as the type, duration and dose of antipsychotics are highly likely to confound their findings. The effect of antipsychotics on the brain is revisited later.

Whether enlarged ventricles and a reduction of grey matter reflect the presence of a progressive disease process as advocated by Kraepelin is still unclear. Longitudinal studies have produced inconsistent results in regards to both ventricular enlargement and grey matter loss (133), though a recent meta-analysis of 27 longitudinal MRI studies suggests that
schizophrenia is associated with progressive structural abnormalities, with greater reductions in grey and white matter over time compared to controls (333).

There is a great deal of variation across volumetric studies in schizophrenia. This is likely to be due to a culmination of the inherent heterogeneity of schizophrenia, confounds in samples such as medication status and duration of illness, small sample sizes and differences in imaging parameters and analysis. This highlights the need to investigate brain structure in clearly defined patient groups, such as first episode antipsychotic-naïve patients and medicated patients in a later stage of the illness. This would help determine whether structural abnormalities are likely to reflect a neurodevelopmental or a neurodegenerative aetiology. Despite significant contradictions within the literature, there is evidence of structural abnormality in schizophrenia which, however slight, should not be ignored. Whether these structural abnormalities progress over time is unclear. However, if this were the case, then structural abnormalities could reflect an ongoing underlying process in the brain to which psychotic symptoms are secondary. Thus, whilst antipsychotics target positive symptoms by blocking D2 receptors, this may be too far downstream from an underlying pathology which could be causing a progressive loss of brain tissue and which may be involved in the development of the cognitive and negative symptoms of schizophrenia.

3.1.8. Antipsychotics

Schizophrenia is primarily treated pharmacologically with antipsychotics. All currently licensed antipsychotics act as antagonists at dopamine D2 receptors and thus decrease the transmission of dopamine. The dopamine hypothesis of schizophrenia, described earlier, provides an explanation for their therapeutic efficacy. However, as discussed there are limitations to the use of antipsychotics in the treatment of schizophrenia, some of which undermine the dopamine model. Prior to the 1950s, people with schizophrenia were institutionalised in asylums where treatments were ineffective and often cruel. However, in 1952, following the serendipitous discovery of the first antipsychotic chlorpromazine, the treatment of schizophrenia and indeed psychiatric practice was revolutionised. For the first time, the positive symptoms of schizophrenia (delusions, hallucinations, thought disorder) could be relieved with a drug. Control of the florid symptoms of psychosis led to the discharge of large numbers of patients from asylums and hospitals and, along with other contributing factors such as the human rights movement, ultimately led to psychiatric deinstitutionalisation.

The discovery that chlorpromazine seemed to work through blockade of the dopamine D2 receptor led to the development of dozens of drugs sharing this common mechanism of action, known as ‘first-generation’ or ‘typical’ antipsychotics. These first-generation antipsychotics (FGAs), however, resulted in ‘extrapyramidal side effects’ such as
Parkinsonian symptoms and tardive dyskinesia due to the blockade of D2 receptors in the basal ganglia. This led to high rates of discontinuation and relapse. Owing to such side effects, the search for a new class of better tolerated antipsychotic drug began. Clozapine, a D2 antagonist which did not produce EPS was soon discovered. However, due to a potentially fatal side effect – the lowering of white blood cell counts, it was withdrawn from the market and the search for new antipsychotics continued. This led to the development of more 'second generation antipsychotics' (SGAs) or 'atypical' antipsychotics which promised enhanced efficacy and safety. These differ pharmacologically, with lower affinity for D2 receptors and greater affinities for serotonin and norepinephrine receptors (334). SGAs including clozapine, risperidone and olanzapine have largely replaced FGAs due to their more tolerable neurological profiles. However, claims of superior efficacy over FGAs appear to have been largely unfounded. With the exception of clozapine, trials have failed to consistently or robustly replicate findings of superior efficacy of SGAs (335) (336).

To properly address the efficacy, safety and tolerability of antipsychotic drugs, the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) trial was initiated by the National Institute of Mental Health (NIMH) (337). A large cohort of patients with chronic schizophrenia was randomly assigned to receive first-generation antipsychotic perphenazine or second-generation antipsychotics olanzapine, quetiapine, risperidone or ziprasidone. Overall, 74% of patients discontinued medication within 18 months owing to inefficacy or intolerable side effects (338). Results from perphenazine and the SGAs were similar in most respects. The CATIE trial highlights that SGAs are not necessarily more effective or better tolerated than FGAs and that antipsychotics are severely limited in their ability to schizophrenia effectively.

Whilst the advent of antipsychotic drugs has contributed to a more humane treatment of those with schizophrenia and significantly reduced the burden to society, antipsychotics are limited in terms of efficacy and tolerability. In terms of efficacy, a meta-analysis of 38 studies (339) found that SGAs were more effective than placebo in improving overall symptoms. However, the pooled effect size was moderate (0.49) and the absolute difference in responder rates was just 18% (41% responded to drug and 24% to placebo). The number needed to treat was 6. Furthermore, they found that the drug-placebo difference diminished over time. The findings of this study also pointed to evidence of publication bias. Such figures cast serious doubts over the claimed efficacy of antipsychotics. The newer generation of antipsychotics were also marketed as being safer than the conventional antipsychotics. However, mounting evidence suggests that this too is misleading.

Although extrapyramidal side effects are less severe following SGA treatment, they are still common and often warrant treatment with antimuscarinic drugs (340). Another serious side effect of antipsychotic treatment is excessive weight gain. The first systematic meta-analysis
of weight gain during antipsychotic treatment showed beyond doubt that many SGAs had a
greater propensity to induce excessive weight gain than the preceding FGAs (341). Numerous
studies have replicated this finding (342). Generally, clozapine and olanzapine are associated
with the greatest degree of weight gain, but risperidone, quetiapine, amisulpride and
zotepine are also associated with a significant risk for inducing weight gain (343).

Treatment with SGAs also increases the risk of developing type 2 diabetes to a much greater
extent than FGAs (344). SGAs can act directly to impair glycaemic control, leading to insulin
resistance, impaired glucose tolerance and type 2 diabetes (345). It is also likely that weight
gain contributes to the increased risk for developing type 2 diabetes and also cardiovascular
disease, a common cause of mortality in schizophrenia. The concept of ‘metabolic syndrome’
has been proposed to explain the relationship between metabolic abnormalities and CV
disease. This ‘metabolic syndrome’ encompasses obesity, hyperglycemia and hypertension. It
was initially claimed that the greater risk for developing these metabolic effects was related
to schizophrenia itself, however a recent systematic review finding no difference in
cardiovascular risk between untreated patients and controls seems to confirm that the
increased risk is related to antipsychotic medication (346).

In addition to these metabolic side effects, some first and second generation antipsychotics
can induce an increase in prolactin, which in turn can lead to sexual dysfunction (347).
Sedation is another side effect that can severely impact on social and occupational
functioning. Both first and second-generation antipsychotics are also associated with
ventricular arrhythmia and a dose-related increased risk of sudden cardiac death (348). For a
recent review of efficacy and tolerability of 15 of the most common first and second
generation antipsychotics see (349). It is not surprising, then, that discontinuation rates are
so high in clinical trials and that medication compliance is such an issue in the treatment of
schizophrenia. Patients that choose not to take antipsychotic medication are more likely to
relapse (350). Nonadherence to medication is largely driven by lack of efficacy and
intolerable side effects. Therefore it is clear that antipsychotics, at present, are inadequate in
the treatment of schizophrenia. Indeed there have been calls to re-evaluate the risk-benefit
ratio of antipsychotics based on the overestimation of their evidence base and an
underestimation of the seriousness of their adverse effects (351).

Another important limitation is that whilst antipsychotics are relatively efficacious for the
positive symptoms of schizophrenia, they are largely ineffective in treating the negative and
cognitive symptom domains. Previously these symptom domains have been largely ignored.
The hallmark of schizophrenia is psychosis and up until fairly recently the central focus has
been on treating delusions and hallucinations. However, it is the negative and cognitive
symptoms not the positive symptoms, that seem to predict poor social and occupational
functioning (352). All in all it seems that the predominant treatment for schizophrenia – the
use of antipsychotics – whilst being effective in alleviating the positive symptoms of schizophrenia in some patients, are unable to treat the negative and cognitive symptoms, and often lead to unwanted side effects which lead to medication nonadherence and perhaps increased mortality. Clearly there is room for a great deal of improvement in the pharmacological treatment of schizophrenia and there is a long way to go in improving quality of life. It has been over 60 years since the discovery of antipsychotics and since then little progress has been made in the ability to treat schizophrenia effectively or safely. It seems that it is time to look beyond the dopamine system in an attempt to unravel the underlying pathology that is not being targeted by current antipsychotics. Alternatively, it could be that a ‘magic bullet’ does not exist for such a heterogenous disorder and that more precise treatments for specific clusters of symptoms will be the best approach. Indeed alternatives to antipsychotic treatment have shown promising findings. A wealth of research indicates that CBT can be effective in treating psychosis/schizophrenia (115). Furthermore, exercise (353) and lifestyle interventions (354) are showing promising findings.

Therefore it is clear that we need to investigate novel avenues of research to inform the development of more effective treatments in schizophrenia. Novel treatments should have minimal side effects and target each symptom domain. As discussed in the next section, inflammation is a highly promising as a potential biomarker and treatment target.

3.2. Schizophrenia & inflammation

A link between immunology and schizophrenia was first described over a Century ago based on observations that individuals sometimes present with psychosis during the course of a microbial disease. Menninger added further support to the infectious theory of schizophrenia through his investigations around the 1918 influenza epidemic. He observed that psychiatric admissions for psychosis surged around the time of the epidemic, with many of these patients being labelled as having ‘dementia praecox’ (355). Interest in infectious theories of psychiatric disorders faded though, following the emergence of Freudian theories and the psychodynamic approach. However, an immunological basis to schizophrenia has been revisited over recent decades following the publication of key studies that suggest a role for inflammation in schizophrenia, and it is now one of the leading research areas in the field. I now summarise and evaluate the various lines of research which suggest an association between schizophrenia and inflammation. I then provide a rationale for the current investigation into schizophrenia, followed by the aims and hypotheses of the research as a whole.
3.2.1. Infection to the developing brain

A wealth of epidemiological research indicates that prenatal exposure to infection is a risk factor for developing schizophrenia (278). Early evidence for this came from the observation that individuals born in late winter or early spring are at a higher risk of developing schizophrenia later in life. Despite the questionable methodologies of the earliest studies, this finding has been reproduced in multiple epidemiological studies (356). Due to the peak incidences of infectious diseases in the winter and spring, it has been hypothesised that infants born in these months experience a greater exposure to infectious agents, which may in turn contribute to the development of schizophrenia. Further epidemiological studies of data from influenza epidemics found an association between infection during pregnancy and the later development of schizophrenia. However, findings from these studies are limited due to an inability to accurately determine the presence of prenatal infection. Since then, more rigorously designed studies have investigated the presence of prenatal infection based on serological markers. Using this approach Brown et al. demonstrated a seven-fold increased risk of developing schizophrenia following influenza exposure during the first trimester (357). Epidemiological studies using serological data have also confirmed a higher risk for developing schizophrenia following prenatal exposure to Toxoplasma gondii (T. gondii) (358), herpes simplex virus (HSV-2) (359) and rubella (360). Furthermore, increased maternal levels of cytokines IL-8 (361) and TNF-α (362) have been found in mothers of offspring with schizophrenia and psychosis, respectively. It could be that each individual infection adds to an increased risk for schizophrenia via unique effects. However, mounting evidence suggests that infections act via one common mechanism to increase the vulnerability to schizophrenia, through disruption of neurodevelopment.

Indeed a proliferation of animal studies has provided robust evidence for the emergence of long-term functional and structural brain abnormalities following prenatal exposure to infection (363). Maternal Immune Activation (MIA) models have given us significant insights into the effects of prenatal exposure to infection on the brain and behaviour. For example a mouse prenatal influenza model has been investigated extensively and demonstrated neurodevelopmental impairment and behavioural abnormalities in adulthood that are relevant to schizophrenia (364). Another class of animal model uses agents that invoke a cytokine-associated immune response without using viral or bacterial pathogens. One of the most common agents is polyriboinosonic-polyribocytidilic (poly[I:C]). A multitude of studies provide robust evidence for behavioural, cognitive, pharmacological and structural abnormalities in adult rodents following maternal exposure to poly[I:C] (365). Interestingly, the abnormalities only emerge after the offspring have reached late adolescence, in line with the manifestation of clinical symptoms in schizophrenia. Another approach is to investigate the effects of administration of specific cytokines to pregnant mice. Indeed maternal
administration of IL-6 is sufficient to induce structural and functional abnormalities in adulthood (366). Furthermore, blocking the effects of IL-6, exposure to poly[I:C] is no longer able to induce behavioural dysfunction in the resultant offspring, suggesting that IL-6 may be crucial in linking prenatal infection to abnormal brain development.

It is likely that there are numerous etiologically relevant risk factors that interact and culminate in the development of schizophrenia. Prenatal infection is a relatively frequent occurrence and it is clear that this alone is not sufficient for the development of schizophrenia. It has therefore been proposed that the synergistic effects of a genetic predisposition to psychosis and adverse environmental exposure (prenatal infection) lead to the development of schizophrenia (367). This is supported by a recent study indicating that the brain and behavioural responses to prenatal immune activation (using poly[I:C]) are exacerbated in mice with a genetic predisposition to neurodevelopmental abnormalities (368). Another possibility is that prenatal exposure to infection creates an ‘at-risk’ state and that the individual is more vulnerable to a subsequent postnatal stimulus, such as chronic stress or drug abuse, known as the ‘two-hit’ hypothesis (369). This has recently been modelled also: prenatal immune activation and subsequent stress in puberty produced synergistic effects on behavioural abnormalities in adulthood, with neither immune activation nor stress alone producing these effects (370). Thus prenatal infection is thought to act as a ‘disease primer’, increasing the vulnerability to the neuropathological effects of subsequent environmental insults. It must be noted that MIA models are not necessarily disease-specific, but rather represent a general vulnerability to neurodevelopmental abnormalities. But given the association between immune activation and schizophrenia, it is likely that these models are highly relevant to schizophrenia.

### 3.2.2. Autoimmune Disease

An association between autoimmune disease and schizophrenia was first observed in the 1960s when a high proportion of celiac disease was noticed in those with schizophrenia (371). Since then, a number of autoimmune diseases have been associated with an increased risk for schizophrenia, including further evidence for celiac disease (372), autoimmune hepatitis, type 1 diabetes, multiple sclerosis, psoriasis (373). A Danish population-based study found that the relative risk for schizophrenia for an individual with a history of autoimmune disease is increased by 45% (374). That autoimmune disease infers such a large increase in risk suggests a role for immune activation in the aetiology of at least some incidences of schizophrenia. The immune system can produce autoantibodies that react against its own antigens, leading to autoimmune disease. Various autoantibodies with cross-reactivity against brain antigens have been identified in schizophrenia, however findings have not been always been consistent. Perhaps the strongest evidence for autoimmunity
causing psychosis comes from investigations into NMDAR antibody encephalitis – a recently
discovered disorder where antibodies produced by the body’s own immune system attack
NMDA receptors in the brain. The antibodies are highly specific and are not present in
healthy or disease controls (375). Over two thirds of patients with NMDAR antibody
encephalitis present with symptoms that resemble those of schizophrenia, namely delusions,
hallucinations and thought disorder (376) (377). Indeed symptoms of psychosis are the most
common initial presentation (68-80%) and patients are often misdiagnosed initially. Left
untreated, however, the illness progresses and memory deficits, seizures and language
deficits emerge, followed by a state of unresponsiveness with catatonic features (378).
Patients with schizophrenia also show an increased prevalence of NMDAR antibodies (378)
and it has been suggested that 6.5% of those with first-episode psychosis may have specific
antibodies amenable to immunotherapy (379). As described previously (section 3.1.6) there
is mounting evidence that schizophrenia is associated with NMDA receptor hypofunction.
This lends support to the relevance of NMDAR antibodies in schizophrenia and provides a
plausible mechanism for the manifestation of schizophrenia-like symptoms. That patients
with NMDAR antibody encephalitis present with symptoms of schizophrenia provides
compelling evidence for a link between immune activation, altered NMDA receptor function
and schizophrenia.

3.2.3. Autopsy studies

Whilst there is some evidence of structural abnormality in patients with schizophrenia, these
findings do not seem to translate to neuropathological findings. The neuropathology of
schizophrenia remains largely unknown. Still, numerous autopsy studies have attempted to
confirm the existence of an underlying neuropathology to schizophrenia by searching for
evidence of microglial or astrocytic activation. The earliest finding of microglial alterations in
schizophrenia was in 1975 (380). Since then, findings of reactive glia have been contradictory
and largely inconclusive (381). Furthermore, there are limitations to the methodologies of
the earlier studies, some of which employed questionable counting procedures. More recent
post-mortem studies have found evidence for microglial activation in schizophrenia (383;
384) however, equally, others have found an absence of microglial activation in
schizophrenia (386). Despite such discrepancies, the studies that have found evidence of
microglial activation should not be ignored. More recent autopsy studies have shown
evidence for inflammation in schizophrenia. Fillman et al. reported increased inflammatory
markers (including IL-1β, IL-6, IL-8 and inflammatory mRNA expression) in post-mortem
brains of schizophrenic patients compared to matched controls (382). Furthermore,
increased microglial density has been demonstrated in the ACC of patients with
schizophrenia compared to controls, post-mortem (384). Another recent study, using the
TSPO ligand PBR28 in post-mortem brains reported increased TSPO density in the DLPFC of schizophrenia patients which was independent of antipsychotic medication (385).

It could be that microglial activation and an inflammatory process is only present in a subset of schizophrenic patients. An interesting finding from a recent study is increased microgliosis in the brains of schizophrenic patients who had committed suicide (530), suggestive of a role for immunological factors in suicide. Whether microglial activation is consequential of pre-suicidal stress or a causal factor in suicidal behaviour is unclear. It could be that the cytokines and neuroendocrine factors released by microglia induce neurotransmitter changes which could exacerbate psychotic symptoms and promote suicidal behaviour. In line with this, it may be that microglial activation is only present during episodes of psychosis, and as microglial activation is relatively short-lived, evidence of microgliosis might not be present at autopsy. This could explain the discrepancies in the post-mortem literature. It also suggests that studying the schizophrenic brain at autopsy is inadequate in addressing the question of microglial activation. Investigating the presence of microglial activation in-vivo in patients in a psychotic episode could better address the question.

### 3.2.4. Peripheral inflammation

It is clear that schizophrenia is a heterogeneous disorder in its clinical symptomatology, course, treatment response and, in all likelihood, its aetiology. Such heterogeneity might explain the wide-ranging results found in most investigations into the neurobiology of schizophrenia. Findings from immunological studies of schizophrenia are no exception to this. Multiple studies have investigated the presence of markers of inflammation in the periphery and in the CSF of schizophrenic patients. Whilst there is a considerable amount of inconsistency in the literature, there are some inflammatory markers that have been consistently shown to be elevated in schizophrenic patients.

It has been repeatedly observed that cancer patients treated with immune-activating cytokine IL-2 develop severe psychiatric side effects, specifically perceptual and cognitive disturbances that are characteristic of schizophrenia and that are dose-dependent (162). This led to the macrophage theory of schizophrenia which proposed that a failure of macrophages to properly suppress T-lymphocyte secretion of IL-2 plays a causative role in the development of schizophrenia (387). Accordingly, IL-2 has been found to be increased in the CSF of patients with schizophrenia (388). Furthermore, an increase in CSF IL-2 levels has been found to predict the recurrence of psychotic episodes in relapse-prone schizophrenics (389). This is suggestive of a heightened inflammatory response in schizophrenic patients. Additionally, IL-2 has been found to have a potent effect on dopaminergic neurons (390), therefore heightened levels of IL-2 could contribute to the increased striatal dopaminergic activity observed in schizophrenia. However, several studies have demonstrated no
difference between IL-2 levels in schizophrenic patients and controls (391) (392) highlighting the possibility that a heightened immune response is only present in a subset of schizophrenic patients.

Increased levels of pro-inflammatory cytokine IL-6 in patients with schizophrenia has also been observed in multiple studies (393; 397; 398). Moreover several studies have found a relationship between increased IL-6 concentration and clinical features of schizophrenia such as duration of illness (393) (394) (395) and treatment response (396) (396) (171). Another study found that IL-6 levels were elevated in acutely psychotic patients, but that in patients in remission, levels were no different from controls (398), suggesting that a heightened inflammatory state may be specific to psychotic episodes. This is analogous to evidence for increased DA release in periods of exacerbation compared to periods of stabilisation (300). Indeed a meta-analysis found differences in cytokine abnormalities in acute and stable phases of schizophrenia (399), with cytokines IL-6 and IL-1β appearing to be state markers and IL-12, IFN-γ and TNF-α appearing to be trait markers.

A recent meta-analysis of 62 studies produced effect size estimates for the following cytokines/receptors; IL-1β, IL-1RA, IL-2, sIL-2R, TNFα, IL-6, sIL-6R and IL-10 (9) (179). Of the studies assessing in-vivo peripheral cytokine levels, a moderate and highly significant effect size was obtained for IL-6, IL-1RA and sIL-2R, suggesting that these cytokines are raised in schizophrenia, thus providing evidence that schizophrenia is associated with some degree of immune activation.

There is also evidence that schizophrenia is accompanied by an acute-phase response, as indicated by an increase in acute-phase proteins (APPs) such as haptoglobin, α1-antitrypsin and fibrinogen, and a decrease in negative APPs such as albumin and transferrin (405). Consistent with this, one study found an association between elevated CRP levels and symptom severity in patients with schizophrenia and schizoaffective disorder (407). A follow-up study, however, found no association between elevated CRP and symptom severity as measured by PANSS, although there was an association between elevated CRP levels and severity of cognitive impairment (409). Meta-analyses have confirmed the presence of other abnormalities associated with inflammation in schizophrenia too, for example increased autoantibodies (400), markers of oxidative stress (401) and circulating lymphocytes (402).

In accordance with the hypothesis that schizophrenia may be mediated by an inflammatory response to prenatal infection or obstetric complications (414), it has been found that cytokine levels, including IL-1β, IL-6 and TNFα are elevated in amniotic fluid where pregnancies have been complicated (416). Such cytokines are able to cross the placenta and disturb neurodevelopmental processes which might ultimately present as schizophrenia later in life.
That multiple studies have found an association between immune activation and schizophrenia makes the association difficult to deny. However, discrepant findings highlight the need to further research the role of inflammatory processes in schizophrenia. Conflicting findings across studies could be explained by the heterogenous nature of schizophrenia, differences in illness state (first episode vs chronic), antipsychotic medication, smoking and obesity. Numerous meta-analyses, however, have found raised inflammatory markers in first-episode, antipsychotic-naïve patients (399; 401; 402), suggesting that the association is independent of the effects of antipsychotic medication. A recent meta-analysis of cytokine function in medication-naïve first episode psychosis reported highly significant elevations in IL-1β, sIL-2r, IL-6 and TNFα (403). Furthermore, it has recently been shown that plasma analytes including cytokines, cortisol and markers of oxidative stress in individuals with clinical high risk (CHR) can predict the transition to psychosis with reasonable sensitivity and specificity (~0.8) (404). This provides further evidence that inflammation might be causal in the development of psychosis and highlights the clinical utility of using a blood biomarker assay to improve the identification of individuals that are at high risk of transitioning to psychosis. The challenge remains to determine what is specific about the subset of patients who show an increased inflammatory state. Most studies have investigated the presence of inflammatory markers in the periphery. Determining whether there is evidence of inflammation in the CNS would provide more direct evidence for the involvement of inflammation in the pathophysiology of schizophrenia.

3.2.5. Genetics & inflammation

As mentioned previously, schizophrenia has a heritability of around 80% (276). Until recently, the search for genes that contribute to its susceptibility has been fairly unsuccessful. Over 1000 candidate genes have been tested, making schizophrenia one of the most intensively studied disorders. However, these studies have been plagued by a failure to replicate their findings. For example a comprehensive study of 14 of the most cited candidate genes found no association with schizophrenia (406). Since the advent of genome-wide association studies (GWAS) though, the search for the genetic basis of schizophrenia has progressed significantly. GWAS studies systematically test the entire genome rather than hypothesised candidate genes. This is important for a disorder such as schizophrenia because a) our understanding of its pathophysiology is so limited and b) it is a highly complex genetic disorder, with many genes each conferring only a small effect on the phenotype.

Three large GWAS studies have implicated the Major Histocompatibility Complex (MHC) in schizophrenia (408; 410; 411). The MHC region, located on chromosome 6, encodes hundreds of genes that control immune function. Variation within the MHC has been found to be associated with almost every autoimmune disease, in addition to some infectious and
inflammatory diseases (412). The MHC finding remains the most significant and consistent across GWAS studies in schizophrenia (413). Indeed a meta-analysis of 17 GWAS studies identified 129 genome-wide significant single nucleotide polymorphisms (SNPs) which mapped onto the extended MHC region (415). Further studies with additional subjects replicated the genome-wide significance of 5 of these SNPs with the most significant being rs2021722. For a review of GWAS findings at the MHC locus in schizophrenia, see (413). In their review, Corvin and Morris conclude that ‘the MHC may be a risk factor more specific to schizophrenia than to other psychiatric disorders’. Given the importance of the MHC region in immune function, the genome-wide significance of MHC regions provides compelling evidence for a link between schizophrenia and immune function.

3.2.6. Stress

It has long been established that stress is closely associated with symptoms of schizophrenia. The diathesis-stress model was first proposed over 40 years ago and has been a dominant framework for understanding the aetiology and course of schizophrenia (417). According to the model, symptoms of schizophrenia are triggered or worsened when environmental stressors (stress) act upon a genetic vulnerability (diathesis). Early support for the theory came from research into psychosocial stress. There is some evidence that patients with schizophrenia experience more psychosocial stress then healthy controls, however these findings have not always been replicated (418). Stronger support comes from studies indicating that relapse and symptom exacerbation are preceded by an increase in stressful life events (419). Therefore, whilst people with schizophrenia do not necessarily experience more stressful life events, stress appears to affect the course of the disorder. Stress is a highly subjective experience: what is deemed stressful to one person may be entirely innocuous to someone else. Therefore cross-sectional studies based on self-report measures of life events may be underestimating the association between stress and psychosis.

More recently, research has focussed on the biological response to stress. The HPA axis, described previously, facilitates the physiological and behavioural response to threat and is activated by exposure to stress. Walker et al. give a detailed review of the HPA axis in the context of schizophrenia (420). To summarise, patients with schizophrenia have higher levels of cortisol and ACTH at baseline, indicative of a hyperactive HPA axis. Interestingly, patients treated with antipsychotics have lower levels of cortisol and ACTH, raising the possibility that one of the mechanisms of action of antipsychotic could be suppression of the HPA axis. Both medicated and unmedicated patients with schizophrenia also exhibit non-suppression of cortisol in response to dexamethasone challenge (421). Some studies have shown an association between negative symptom severity and cortisol/ACTH levels (422), whilst others have shown an inverse correlation between performance on cognitive tasks and
cortisol levels (423). This is consistent with research showing that disorders involving excessive cortisol production and steroid treatment is associated with cognitive deficits (424). The stress-diathesis model incorporated these findings by proposing that the HPA axis mediates the link between stress and psychosis (425). Interestingly, conditions associated with excessive cortisol production, such as Cushing’s Disease. As mentioned previously, an overactive HPA axis is closely related to inflammatory processes. It is thought that chronically high levels of cortisol results in glucocorticoid resistance, so that the dampening effects of cortisol and the resultant negative feedback system is no longer effective. The HPA axis and associated inflammatory response subsequently becomes overactive.

Glucocorticoid receptors are ubiquitously distributed in the hippocampus. Research suggests that persistently elevated levels of glucocorticoids can be neurotoxic and the hippocampus may be especially sensitive to this. For example animal studies have shown that GCs can decrease neurogenesis in the dentate gyrus, decrease neuronal survival following insults and contribute to neuronal death (426). Stress-induced decreases in neurogenesis in the hippocampus appears to be common across species, life stages and most types of stressors (427). This would explain the reductions in hippocampal volume and memory impairments seen in patients with schizophrenia. The HPA axis is thought to be involved in the link between prenatal infection or maternal stress and the increased risk for developing schizophrenia (428). It is thought that such prenatal insults results in a sensitised HPA axis so that it is more reactive to subsequent insults.

Interestingly, an association between stress, HPA activity and dopamine function is now becoming apparent. Glucocorticoid secretion increases DA activity in certain brain regions, most notably the mesolimbic system (429). Furthermore a PET study found that exposure to a psychosocial stressor was associated with an increase in dopamine release in the ventral striatum and that this was correlated with cortisol release (430). This suggests that stress and its biological substrates may precede excessive dopamine transmission. Thus targeting the biological substrates associated with stress may be targeting a more upstream pathophysiology than dopamine.

Another interesting line of evidence linking stress and schizophrenia comes from research into the effects of living in urban environments. More than half of the World’s population lives in cities and this is projected to rise to 67% by 2050 (United Nations, 2012). Despite a general health advantage in physical health in city dwellers, living in an urban environment seems to increase the risk for developing mental disorders. The most striking increase in risk attributable to living in an urban environment is for schizophrenia. A meta-analysis of 10 studies indicates that the incidence of schizophrenia is around double the rate in urban compared to rural areas (431). Meta-analyses also show a dose-response relationship between an urban upbringing and schizophrenia risk (432)(431)(433), suggesting that the
relationship may be causal. Longitudinal studies suggest that it is an urban environment as opposed to other epidemiological factors that increase the risk for schizophrenia (434). Indeed many of the studies control for possible confounders such as substance abuse and genetic risk (433). Furthermore, longitudinal studies indicate that moving from an urban to a rural environment in childhood brings about a corresponding decrease in risk for psychosis (435). In their review, March et al. conclude that the findings in the literature do not seem attributable to reverse causation or to better access to services, but that the timing, consistency and dose-response relationship point to an aetiological role for urbanicity in the development of psychosis (432). One explanation is that certain risk factors for schizophrenia are particularly prevalent in urban environments. The relationship between urbanicity and psychosis could in part be explained through exposure to infection, which spreads more readily in urban environments. Urbanisation and migration can lead to overcrowding which can increase the spread of infection. Furthermore, political, economic and sociological processes can lead to deprived neighbourhoods where people live in unhealthy conditions and are exposed to pollutants and toxins. Perhaps the most accepted explanation for the association is that social stress as a result of living in an urban environment contributes to the development of psychosis. A supportive social environment is one of the most important factors necessary for physical and mental health. It is thought that loss of group support may be perceived as a threat to survival and thus elicit a stress response. Indeed threats to social connection are thought to tap into the same neural ‘alarm system’ triggered by threats to physical danger (436). Living in an urban environment is associated with increased social stress, potentially triggered by factors such as social isolation (being ‘alone in a crowd’), social inequality, social defeat and lack of perceives safety. Other factors associated with urban living that can contribute to stress include overcrowding, lack of green space and environmental pollutants (282).

A highly cited paper demonstrates that urban upbringing and city living have measurable impacts on social evaluative stress processing (434). Using fMRI they showed that urban upbringing was associated with increased activity in the ACC, which followed a dose-response relationship and was specific to the social stress condition. The ACC is important for social processing and in regulating the stress-response system. It is also though to play a pathophysiological role in both schizophrenia and depression, whose incidences are both increased in cities. Thus social stress seems highly plausible in its ability to explain the link between the urban environment and increased psychiatric risk. It is clear that urban upbringing alone is not sufficient for the development of schizophrenia but that it culminates from a complex interaction between genetic and environmental factors. In support of this is evidence of the synergistic interaction between familial risk for psychosis and urbanicity increasing the risk for schizophrenia (437).
Another consistent finding is that migration and belonging to an ethnic minority increases the risk for schizophrenia (438). It has been shown that this does not reflect an increased risk in a person's country of origin but rather depends on the ethnic diversity of the area in which they are living (439). This has been attributed to social stress stemming from the feeling of being different from others. Such research lends further support to the notion that social stress is a contributing factor to the development of schizophrenia.

A wealth of epidemiological research shows that childhood adversity (exposure to harmful experiences such as social neglect or abuse) is a major risk factor for the development of psychosis and schizophrenia (440). However, the specificity for schizophrenia relative to other psychiatric disorders has been challenged (441). It is possible that early life stress in addition to a genetic predisposition creates an at-risk state where an individual is more vulnerable to subsequent stressors, culminating in the development of symptoms of schizophrenia.

All of the above research suggests that stress, whether in the context of urbanicity, migration, childhood abuse or heightened cortisol levels, is associated with schizophrenia. Stress activates the HPA axis and can, under chronic conditions, induce inflammation. Therefore inflammation may be the biological substrate linking stress and the development of or exacerbation of symptoms of schizophrenia.

### 3.2.7. Antipsychotics & inflammation

As mentioned previously, there is evidence of increased peripheral inflammation in schizophrenia. However, increases in specific cytokines have not always been replicated and the findings are at times conflicting. Antipsychotic medication may be a contributing factor to such inconsistent findings, with mounting evidence that they have immunomodulatory properties. Investigations into the effects of antipsychotics on cytokine levels have yielded inconsistent findings however. Indeed the picture is far from clear: different antipsychotics seem to have differing effects on cytokine profiles, and whilst some antipsychotics have inhibitory effects on certain cytokines, they can stimulate others. Furthermore, there are discrepancies between the in-vivo studies investigating cytokine levels in peripheral blood following antipsychotic treatment, and in-vitro studies investigating the effects of antipsychotics on LPS or mitogen-stimulated cytokines. It is beyond the scope of the thesis to review the in-vitro and in-vivo findings from investigations into all antipsychotics, but these are given in the following reviews (442)(399) (443).

One of the most consistent findings is an inhibitory effect on pro-inflammatory cytokine IL-2 that seems to be common to all investigated antipsychotics (chlorpromazine, haloperidol, clozapine and risperidone). However, the effects of individual antipsychotics are more
variable across other cytokines are more variable. For example, clozapine has been found to stimulate IL-6 and TNF-α (444), whereas risperidone has been found to decrease IL-6 and TNF-α (445). A meta-analysis carried out by Miller et al. found that in a total of 488 patients whose blood cytokine levels were measured pre- and post-antipsychotic treatment, there were significant increases in IL-12 and sIL-2R and significant decreases in IL-6, IL-1β and TGF-β (399). A recent meta-analysis of 23 studies found antipsychotic treatment to be associated with decreases in IL-1β and IFN-γ, and increases in IL-12. Cytokines IL-6, IL-2, IL-10, IL-1RA, sIL-6R, TGF-β and TNF-α were unaffected (443).

The various reviews of studies on antipsychotics and cytokines conclude that antipsychotics have anti-inflammatory properties. It has been hypothesised that the therapeutic effects of antipsychotics reflect their ability to suppress or normalise inflammation and that antipsychotics act as a ‘fire extinguisher’ in the brain (446). Whilst anti-inflammatory properties have been shown with certain antipsychotics for certain cytokines, a failure to robustly replicate these findings means this conclusion must be taken tentatively. For example, the significant increase in IL-12 following risperidone treatment challenges the general conception that antipsychotics are anti-inflammatory in nature (443). Indeed in a recent study, patients treated with risperidone exhibited initial decreases in IL-1β and IL-6, however these then increased to baseline levels, and pro-inflammatory cytokine TNF-α was significantly increased at both 3 and 6 months (447). The patients also experienced steady and significant weight gain over the 6 month treatment period. As mentioned previously, antipsychotics are associated with severe weight gain and, subsequently, obesity (342). Obesity is closely associated with an increased production of pro-inflammatory cytokines and chronic inflammation (448). Therefore it is possible that atypical antipsychotics such as risperidone have the ability to induce inflammation indirectly through their propensity to induce weight gain. Findings from studies investigating the effects of antipsychotics on microglia have been more consistent. In-vitro studies have shown that a number of antipsychotics, including olanzapine, risperidone, aripiprazole and spiperone can inhibit microglia (449)(450)(451)(452).

The general consensus is that antipsychotics have some anti-inflammatory properties. However the findings in the literature are so contradictory that it is difficult to conclude that antipsychotics are purely anti-inflammatory in nature. The issue of heterogeneity likely contributes to the inconsistent results, but another likely contributing factor is that alterations in the cytokine system are often subtle and difficult to reproduce. Furthermore, cytokine concentrations in the blood fluctuate greatly and are affected by a wide variety of factors that can be difficult to control for, including age, gender, time of day, amount of sleep, diet, smoking and exercise. Investigating the effects of antipsychotics on microglia is likely to give a more accurate measure of the immune mediating properties of antipsychotics. As far as
is known at the time of writing, the effects of antipsychotics on microglia have not been investigated in a clinical population in-vivo. Labelling antipsychotics as anti-inflammatories seems to be an oversimplification. At present it is perhaps more accurate to acknowledge them as having immunomodulatory properties. Further research is needed to determine the exact nature of the impact of antipsychotics on peripheral and central inflammatory processes.

3.2.8. Anti-inflammatory treatments

In response to mounting evidence for a role for inflammation in the pathophysiology and possibly aetiology of schizophrenia, researchers have begun to explore whether anti-inflammatory agents can be used to treat schizophrenia. A number of randomised placebo-controlled trials (RCTs) have investigated the efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) used in conjunction with antipsychotics in the treatment of schizophrenia. NSAIDs exert their efficacy through inhibition of cyclooxygenase (COX), an enzyme responsible for the synthesis of pro-inflammatory molecules such as prostaglandins. A meta-analysis of 5 RCTs indicated moderate but significant positive results: NSAIDs, used in conjunction with antipsychotics, had a moderate effect on total and positive symptoms, with a small effect on negative symptoms (453). However, another meta-analysis of 8 RCTs found no significant effects of NSAIDs in PANSS total or negative scores, though there was a small effect on positive symptoms (454). A larger meta-analysis of 26 RCTs found promising improvements in symptoms when aspirin, N-acetylcysteine (NAC) and estrogen were added to an antipsychotic (455). Arguably the best evidence is for aspirin. Further, Laan et al. indicated that those with the highest levels of inflammation at baseline responded the most favourably to adjunctive treatment with aspirin (456), suggesting that stratification based on immune status may be a successful predictor of response to anti-inflammatories.

Another anti-inflammatory agent that has received much attention in the treatment of schizophrenia is minocycline - an antibiotic that readily passes through the BBB. It is used safely and effectively as an antibiotic and as an anti-inflammatory drug for treating acne and arthritis. The main mechanisms of action are thought to be inhibition of microglial activation, attenuation of apoptosis and suppression of reactive oxygen species (457). It has also been found to block the toxic effects of NMDAr antagonists and is thought to affect NMDA signalling (458). In neurodegenerative disease models, minocycline has inhibited microglial activation and the subsequent release of cytokines TNF-α, IL-1β and IL-6 (459). Miyaoka et al. were the first to publish a case report and open-label study of minocycline as an adjunctive therapy and indicated global improvements in patients with schizophrenia (460). (461).

Since then, 5 RCTs have investigated the effects of minocycline as an add-on treatment in schizophrenia. Chaudrey et al., in a study of 94 patients in Brazil and Pakistan, found that the
addition of minocycline to TAU early in the course of schizophrenia significantly improved negative symptoms (462). Another trial found a greater decline in PANSS negative scores in patients treated with minocycline compared to placebo (in addition to risperidone) however this did not reach statistical significance (463). Levkovitz et al. found a beneficial effect of minocycline on the negative symptoms of schizophrenia and on general outcome. Furthermore, improvements were seen in cognitive (executive) functioning (464). A recent trial in 40 patients with chronic schizophrenia demonstrated a significant reduction in PANSS total and PANSS negative scores in those treated with minocycline as opposed to placebo in addition to risperidone (465). Another recent RCT, this time in early-phase schizophrenia, also showed significant improvements in negative symptoms in those patients who received minocycline in addition to risperidone for 16 weeks (466). There is a clear need for a RCT investigating minocycline as a stand-alone treatment, to determine its efficacy independently of antipsychotics. However, these studies are highly promising in terms of the potential of minocycline for the treatment of the negative, and possibly cognitive symptoms of schizophrenia. The observed efficacy of minocycline provides further evidence for the role of inflammation in schizophrenia. Minocycline potently inhibits microglia and so this could be one mechanism through which it exerts its therapeutic effects. Indeed microglial activation, as described earlier, can have neurotoxic effects. Thus microglia could be a potential target for reducing grey matter reduction and clinical deterioration in schizophrenia.

3.2.9. PET studies of CNS inflammation

Before the advent of sophisticated imaging modalities such as PET and SPECT, researchers had to rely on autopsy studies to investigate the presence of microglial activation in schizophrenia. This was far from ideal as microglial activation can be relatively short-lived, therefore evidence of microglial activation may not be present at autopsy. However, as already described, it is now possible to measure microglial activation, albeit indirectly, in-vivo and non-invasively using PET and radiotracers specific to TSPO. Several groups have utilised PET to investigate the presence of neuroinflammation in schizophrenia.

Van Berckel et al. were the first to quantify microglial activation in patients with schizophrenia in-vivo using TSPO tracer $[^{11}\text{C}](R)$-PK11195 (519). In a sample of 10 patients with recent-onset schizophrenia, they found a moderate yet significant increase in binding of $[^{11}\text{C}](R)$-PK11195 for total grey matter compared to controls, suggesting the presence of microglial activation in the early years of schizophrenia. However, no significant correlation was found between tracer binding and clinical symptoms. This was the first study to demonstrate the presence of microglial activation in schizophrenia in-vivo. It suggests that neuroinflammation is present in the early years of schizophrenia. The authors theorised that this microglial activation reflects neuronal damage. However, whether psychosis precedes or
is a result of neuronal damage, is not clear. An important limitation of this study is that all 10 patients were on antipsychotics at the time of scanning. As mentioned previously, antipsychotics have immunomodulatory properties: they affect cytokine levels in-vivo (442) and, importantly, have been found to suppress microglial activation in-vitro (446). Therefore antipsychotics could be masking the true extent of microglial activation in schizophrenia. Also, patients only displayed moderate levels of psychotic symptoms which may have affected degree of microglial activation. If microglial activation is associated with psychosis specifically, then scanning patients in a psychotic episode would be crucial.

The second study investigating neuroinflammation in schizophrenia in-vivo scanned seven patients who were in a psychotic phase at the time of study (467). They also used \([^{11}C](R)-PK11195\) PET and found whole-brain binding, indicative of microglial activation, to be 30% higher in patients compared to controls but this failed to reach statistical significance. There was, however, a highly significant increase in binding in the hippocampus of patients. Hippocampal pathology has been repeatedly reported in the literature however the focal neuroinflammation was not associated with structural abnormalities. As with Van Berckel et al.’s study, all patients were taking antipsychotic medication at the time of scanning and medication effects are a potential confound.

A third study used a second-generation TSPO tracer \([^{11}C]DAA1106\) to investigate the presence of neuroinflammation in schizophrenia (468). There was no significant difference in tracer binding between fourteen patients with schizophrenia and controls. However, there were significant positive correlations between \([^{11}C]DAA1106\) binding and positive symptom scores and also with duration of illness and age. This suggests that neuroinflammation may be reflective of psychosis symptom specifically and/or the more chronic stages of schizophrenia. Again, all patients were taking various types of antipsychotics which could have confounded the findings. Also, as is described in more detail in the next chapter, the second-generation TSPO tracers, including \([^{11}C]DAA1106\) are affected by a genetic polymorphism which renders some individuals as high-binders, some as mixed-affinity binders and some as non-binders. This information has not surfaced at the time of this study and so there were no correction for genotype. It is likely that this seriously confounds their findings.

The most recent PET investigation into neuroinflammation in schizophrenia used another second-generation TSPO tracer, \([^{18}F]FEPPA\), in a sample of sixteen patients with schizophrenia with ongoing psychotic symptoms (469). They found no significant differences between patients and healthy volunteers in grey or white matter after correcting for genotype. Furthermore there were no correlations between total volume of distribution (VT) and any measures of psychopathology or cognition, or regional MRI volumes. These findings are in contrast to the previous PET findings which showed global (470) or focal
neuroinflammation (467) and correlations with symptom severity and illness duration (468). Differences may be attributable to unique properties of the radioligand used and other methodological factors or from differences in the patient populations. As with all the previous studies, all patients were undergoing antipsychotic treatment at the time of scanning (14 on atypical antipsychotics; 2 on typical), and the possibility that medication is suppressing the true extent of microglial activation cannot be ruled out. The authors note that given the high variability in their results, they would need around 21 subjects per group to detect a 20% difference between groups, meaning that their study could be underpowered. The PET studies point to the presence of neuroinflammation in some patients with schizophrenia. The major confound of medication, however, makes the findings difficult to interpret. It is clear that there is a gap in the research which, as outlined below, this study aims to fill.

3.3. Conclusion: Schizophrenia

There are now multiple lines of evidence which suggest a role for inflammation in the pathophysiology of schizophrenia; (1) infection/trauma to the developing brain increases the risk for schizophrenia; (2) autopsy studies show some evidence for gliosis in schizophrenia; (3) peripheral inflammatory markers are raised in a subset of patients with schizophrenia; (4) autoimmune disease increases the risk for schizophrenia; (5) antipsychotics exhibit some anti-inflammatory properties which could contribute to their therapeutic efficacy; (6) PET studies show some indication of CNS inflammation in patients with schizophrenia; (7) the anti-inflammatory drug minocycline demonstrates high therapeutic efficacy, particularly in treating the negative symptoms. However, there are inconsistencies in the literature and some of the findings are obscured by the confound of antipsychotic medication.

It is likely that the inherent heterogeneity of schizophrenia contributes to the inconsistencies in the literature. It is now generally accepted that such a complex and heterogenous disorder is unlikely to arise from one single cause. It seems more likely that multiple genetic and environmental factors create an at-risk state which, under certain pathological conditions such as chronic stress or substance abuse, can lead to immune activation, dysfunctional neurotransmission and structural abnormalities. The pathophysiology and underlying aetiology is therefore likely to differ across patients. This would explain the variations in the clinical presentation of schizophrenia. Indeed each clinical dimension could reflect a distinct neurobiological pathway. Accordingly it has been proposed that the DSM entity of schizophrenia be dissected into component symptom complexes (positive, negative, cognitive) (220). Treating these narrower targets as opposed to attempting to treat all symptoms with one class of drug may be a more effective way of treating schizophrenia.
The origin of immune activation in schizophrenia remains to be established. The recent GWAS studies suggest that immune genes are involved in the aetiology of schizophrenia. A genetic susceptibility to a heightened inflammatory response to environmental stimuli is a promising hypothesis. Indeed all of the environmental risk factors for schizophrenia can have an impact on the inflammatory response: prenatal infection, obstetric complications, substance abuse and stress. Related to stress are the risk factors of childhood adversity, living in an urban environment and migration. That environmental insults that are known to increase the risk for schizophrenia are associated with inflammation provides strong support for the involvement of inflammation in schizophrenia. But whether such environmental insults, on top of a genetic susceptibility, lead to an inflammatory process in the brain, is yet to be established. An increase in circulating inflammatory cytokines may enter the CNS and activate microglia. As described previously, activated microglia can release excessive glutamate and free radicals which can have neurotoxic effects and lead to cell death. Such a process may be contributing to the structural abnormalities associated with schizophrenia. Excessive glutamate transmission may also lead to overstimulation of dopamine neurons in the striatum. Antipsychotics may therefore be acting too far downstream from the core of the pathophysiology. Targeting inflammatory processes which may be more upstream may result in more effective treatments (see figure 3.2).

Figure 3.2 A Possible immune pathway to schizophrenia
A possible genetically-mediated inflammatory response to environmental insults may lead to microglial activation which, if chronic, can release free radicals and glutamate which can have neurotoxic and possibly neurodegenerative effects. Activated microglia also release glutamate which, alongside NMDA receptor dysfunction, may lead to excessive Glu transmission and excitotoxicity. Hyperactive striatal DA may be secondary to excitatory glutamatergic projections. Thus the action of antipsychotics may be too far downstream. Anti-inflammatories such as minocycline may target a more upstream pathophysiology. Glycine transport 1 (GlyT-1) inhibitors and metabotropic glutamate receptor (mGluR) agonists tackling Glu dysfunction are currently in phase III trials.

It might be that inflammatory processes are related to specific symptom domains. Indeed, the trials of minocycline to date indicate that it is selectively effective in treating the negative, and
possibly cognitive symptoms. Future treatment strategies may well include several classes of medication that target specific symptom domains. This might yield better results than attempting to develop a magic bullet for what is a highly heterogenous disorder, seemingly caused by a complex interaction between genetic and environmental factors.

To conclude, there is convergent evidence that inflammation is involved in the pathophysiology, and possibly aetiology of schizophrenia, at least in a subset of patients. However, there are still many unanswered questions. Further research is needed to determine the source of the inflammation, why it only seems apparent in a subset of patients, its relationship with symptomatology, the relationship between peripheral and central inflammation, the effects of antipsychotics on peripheral and central inflammation, and its relationship to other neurobiological processes such as neurotransmitter function. Nonetheless, inflammation provides a plausible mechanistic explanation for the main risk factors associated with schizophrenia (prenatal infection, childhood adversity, urbanicity, migration). It also fits in with the neurobiological findings. For example, neuroinflammation has been associated with white matter disruption, which might underlie the functional dysconnectivity seen in schizophrenia. Furthermore, microglial activation and the subsequent release of neurotoxic free radicals could explain the regional reductions in grey matter. Microglial activation may also be able to explain the increased glutamate levels seen in some patients with schizophrenia. Perhaps the strongest evidence for inflammation having an aetiological role in schizophrenia comes from the GWAS studies implicating the MHC region in schizophrenia. Taken together, the literature strongly suggests a role for inflammation in schizophrenia.

3.4. **Research aims: Schizophrenia**

A review of the literature has revealed that the role of inflammation in schizophrenia is far from clear. This research cannot address all of the unanswered questions but it aims to fill some of the gaps. As with the depression arm of the study, we aim to use PET and the TSPO tracer \(^{[11]C}\)(R)-PK11195 to determine the presence of neuroinflammation in schizophrenia. The majority of research surrounding inflammation and schizophrenia has focussed on peripheral inflammation, whereas establishing the presence of neuroinflammation \textit{in-vivo} may be crucial in underlying its pathophysiology. The four preceding PET studies show mixed findings – two indicating neuroinflammation is present in schizophrenia and two indicating no association. All participants in these studies were taking antipsychotics however. As antipsychotics have been shown to have immunomodulatory properties this might have confounded their results.
To address this issue we recruited two groups of patients with schizophrenia; patients who are not taking antipsychotics (for at least 3 months prior to scanning) and patients who were on atypical antipsychotic risperidone (Long Acting Injection; LAI). We measured peripheral and neuroinflammation in both patient groups and in healthy controls in a first attempt to address the questions of whether neuroinflammation is present in medication-free schizophrenia and whether this is affected by a common antipsychotic. In this initial investigation we explored the effects of just one antipsychotic on peripheral and central inflammation. Different antipsychotics have been found to have varied effects on inflammatory processes, which would have complicated the analysis. We chose patients who were on a bi-weekly LAI or ‘depot’ of risperidone to overcome the issue of medication compliance. We chose risperidone because it is one of the most common of the atypical antipsychotics and a large pool of patients in the Manchester area is on risperidone. To complement the clinical work we carried out a small preclinical study examining the effects of risperidone on microglial activation in-vivo. Hooded rats treated intravenously with risperidone or placebo for 21 days were scanned on the Siemens Inveon small animal PET scanner using the TSPO tracer $[^{18}\text{F}]$-DPA714. Findings will be confirmed ex-vivo using immunohistochemistry and autoradiography. As far as is known at the time of writing, no study has investigated the effects of an antipsychotic on microglial activation in-vivo.

To investigate the role of inflammation in schizophrenia comprehensively, we collected data on a number of factors that could affect inflammation. These included symptom severity as measured with the PANSS, BMI, smoking status, amount of exercise, sleep quality, alcohol use and childhood adversity. The PANSS is split into positive, negative and general subscales, but does not offer a comprehensive assessment of cognitive function. Cognitive impairment, as described earlier, is a key feature of schizophrenia and is thought to have a significant impact on quality of life and functional outcome (471). Previous research has shown an association between inflammation and cognitive impairment. For example, administering inflammatory-inducing molecules can induce cognitive impairments in both animals (27) and humans (472). To assess the relationship between inflammation and cognitive function we carried out a mini battery of cognitive tests assessing recall and recognition memory and emotional recognition. We also investigated the relationship between peripheral and central inflammation, which the PET studies have failed to show detect so far.

Whilst the current research cannot address all the questions surrounding the involvement of inflammation in schizophrenia, it does fill some of the gaps in the literature. Perhaps most importantly, we investigate the presence of neuroinflammation in a sample of antipsychotic-free patients for the first time. A comparison to a sample of patients on just one antipsychotic is also entirely novel. This, along with the preclinical data, should be able to give us more insight into the role of antipsychotics on neuroinflammation in-vivo. This may also have
implications for the interpretation of the previous PET studies. As with the depression arm of the study there will still be unanswered questions regarding causality and the phenotype of the microglia we are imaging. But through combining PET, MRI, peripheral blood, neuropsychological, clinical and preclinical data, this research should make a novel and meaningful contribution to our understanding of the role of inflammation in schizophrenia. The ultimate goal is that the findings help to inform the development of more effective treatment for those enduring both the debilitating symptoms of schizophrenia and the side effects of current antipsychotics, so that their quality of life and indeed life expectancy, meets that of the general population.

The rationale for this study and how it fits in to the existing research should now be clear. The previous chapters have tackled depression and schizophrenia separately, for ease and clarity. The value of studying them together must not be overlooked, however. Using the same methodology to investigate two distinct, but at times overlapping, disorders enables us to make important comparisons between them. Symptoms of depression are common in schizophrenia (11). Whilst we have screened out those with a comorbid diagnosis of MDD, patients often report sub-clinical levels of depression. Indeed the negative symptoms of schizophrenia overlap with the symptoms of depression, for example the difficulties in motivation and social withdrawal. Furthermore, minocycline seems to be effective in treating the negative symptoms specifically. It could be that there are shared neurobiological mechanisms common to both depression and schizophrenia that possibly reflect an overlap in symptomatology. Studying them together allows us to determine whether there is any symptomatic overlap and any regional overlaps in the neuroimaging findings, for the first time.
3.5. Hypotheses

The aim of this study was to use PET with the radiotracer $^{11}$C(R)-PK11195 to investigate whether depression and schizophrenia are associated with evidence of microglial activation. Secondary aims were to measure correlations between $^{11}$C(R)-PK11195 binding and peripheral inflammatory markers, symptom severity and cognitive functioning. Specific to the schizophrenia cohort, we compared $^{11}$C(R)-PK11195 binding between medicated and unmedicated patients to give us insight into the effects of an atypical antipsychotic on inflammation. The preclinical PET study complemented the clinical study by comparing $^{18}$F-DPA714 in rats treated with risperidone compared to placebo. Based on the literature, we hypothesised that:

i. $^{11}$C(R)-PK11195 binding, indicative of microglial activation, will be increased in both patient groups (depression and schizophrenia) in comparison to controls.

ii. The degree of $^{11}$C(R)-PK11195 increase will be positively correlated with illness severity.

iii. $^{11}$C(R)-PK11195 binding will be decreased in medicated compared to unmedicated schizophrenic patients

iv. $^{18}$F-DPA714 uptake will be decreased in rats treated with risperidone compared to placebo
This chapter is divided into two sections. Section 4.1 gives an overview PET, the scanner used in this study - the High Resolution Research Tomograph (HRRT) and modelling of PET data. I also explain how we can image neuroinflammation using PET and radiotracers that bind to the Translocator Protein (TSPO) is then discussed. The TSPO tracer used in this study, \([^{11}\text{C}]\text{(R)-PK11195}\) is introduced and compared to the newer generation TSPO tracers. Section 4.2 outlines the experimental procedures adopted for this study.

### 4.1 Positron Emission Tomography & neuroinflammation

Positron Emission Tomography (PET) is a molecular functional imaging technique that allows the quantitative measurement of a biochemical or physiological process *in-vivo*. A physiologically active compound is tagged with a positron-emitting radioisotope, typically \(^{11}\text{C}\) or \(^{18}\text{F}\). The radiolabelled compound is then administered, usually intravenously, in tracer amounts so as not to disturb the biological process of interest. The radioisotope undergoes decay and the emitted positrons collide with nearby electrons, leading to the annihilation of both particles and the resultant release of two photons in opposite directions (close to a 180° angle) (see figure 4.1). The emitted gamma rays are detected by the PET scanner and enable the quantification and localisation of the annihilation events.

A PET scanner consists of a ring of detectors that detect the co-incident pairs of annihilation photons. Because the photons are detected in coincidence at an angle close to 180°, the original event can be located along the line of response (LOR). Image reconstruction
algorithms are then used to produce an image from the projection data (the total number of counts detected along each LOR over the duration of the scan). Tissue and bone can attenuate some photons, preventing them from reaching the detectors and being counted. Attenuation can be corrected for by conducting a short 'transmission' scan using an external photon source before the 'emission' scan (the dynamic scan following injection of the radiotracer). The difference in signal detected between the transmission scan with the subject in the scanner and a 'blank' scan without the subject gives an estimate of the attenuation caused by the subject’s bone and tissue. This attenuation map is then used to correct for attenuation during the emission scan.

4.1.1 The High Resolution Research Tomograph (HRRT)

The HRRT is a dedicated brain PET scanner with a spatial resolution of approximately 2.5mm throughout the field of view (FOV; axial 252 mm, transaxial 312mm), providing a higher resolution than any other clinical PET scanner currently available. This high resolution is achieved through its unique design: it consists of eight planar detector heads arranged in an octagon equipped with a dual layer of scintillator crystals. Scintillator crystals absorb the gamma photons produced by positron annihilation and amplify the signal. The dual layer of scintillators in the HRRT allows for Depth of Interaction (DOI) detection (the depth at which the gamma ray strikes the detector). The difference in light decay between the two layers allows for distinction of events between the layers. The DOI information helps in reducing parallax errors that can occur near the edge of the FOV and allows for more accurate location of PET events, accounting for the high resolution of the HRRT. Such high resolution and corresponding reduction in partial volume effects allows for imaging of small structures in the brain with accuracy superior to any clinical PET scanner, making it an invaluable tool for investigating the pathophysiological processes underpinning psychiatric and neurological disorders of the brain.

4.1.2 Modelling of PET data

Kinetic modelling of PET data is necessary to define the relationship between the measured data and the physiological parameters that affect the uptake and metabolism of the tracer. The concentration of radioactivity in a given tissue is dependent on two factors; the physiology of the tissue (bloodflow, metabolism) and the input function (the amount of radioactivity in blood or plasma, reflecting the amount of tracer being delivered to the tissue). A kinetic model is a mathematical description of tissue concentration and these factors. Factors affecting measurement of a receptor-binding radiotracer include regional bloodflow, capillary permeability, non-specific tissue binding, receptor association and dissociation rates, tracer clearance from blood and tracer metabolism (473).
Various types of model exist for extracting meaningful physiological data from PET measurements. Compartmental modelling is the most common method for describing the uptake and clearance of radiotracer in tissue. In compartmental modelling, each compartment defines a possible state of the tracer; either its physical location (for example in intra/extracellular space) or its chemical state (for example it’s binding state). It also describes the ability of the tracer to move between compartments.

The compartmental model below (Two tissue compartment model: figure 4.2) describes the distribution of radiotracer in the blood and in the brain. After injection, the tracer is initially distributed throughout the blood volume. It must then pass the blood-brain-barrier (BBB) before it can bind to the receptor of interest (specific binding). However it will also bind to other molecules and other tissues such as blood, which contaminates the signal (non-specific binding). Additionally, metabolism of the radioligand may also need to be accounted for, as radiolabelled metabolites might cross the BBB and further contaminate the signal.

The concentration of radiotracer in each compartment changes over time, reflecting the initial delivery and uptake of the radiotracer, followed by a wash-out phase. In some studies, arterial blood samples are taken throughout the scan to determine the concentration of radiotracer and radiolabelled metabolites over time. Using an arterial input function is the gold-standard in kinetic modelling of PET data, as it allows for accurate quantification of delivery of radiotracer to the brain and therefore more accurate quantification of receptor binding. However, arterial sampling is highly invasive and technically demanding. Less invasive input functions are therefore often used as an alternative to the arterial input function.

**Simplified Reference Tissue Model**

It is possible to simplify the two-tissue compartment model and use a ‘reference’ region as an indirect input function as an alternative to the arterial input function. A reference region
must be a region devoid of specific binding. The measured radioactivity in the reference region must then reflect free and non-specific binding of the radiotracer (figure 4.3). The Simplified Reference Tissue Model (SRTM) is based on the following assumptions (474):

i) The reference region is devoid of specific binding

ii) The volumes of free and non-specific binding are the same in the target and reference regions

The cerebellum is often used as a reference region. There are, however, limitations of using the cerebellum as a reference input function in TSPO radiotracer studies, discussed later.

Figure 4.3 Simplified Reference Tissue Model

Cp refers to concentration of radioligand in plasma. The ROI consists of free, non-specifically bound and specifically bound radioligand. The reference region (cerebellum) ideally consists of free and non-specifically bound radioligand (i.e. is devoid of specific binding). The SRTM consists of four rate constants: k1 and k2 describe the exchange of tracer between plasma and the region of interest; k'1 and k'2 describe the exchange of tracer between plasma and the reference region (usually the cerebellum).

**Binding potential**

All receptor binding studies aim to measure a target receptor in terms of specific radioligand binding. The ‘binding potential’ (BP) is the primary outcome measure used to define specific binding of radioligand to the receptor of interest. It describes the linear role of two parameters: receptor density and radioligand affinity (475). Specifically, BP is the ratio of Bmax (receptor density) to K_D (equilibrium dissociation constant of radioligand). Radioligand affinity is the inverse of K_D and so BP can be viewed as the product of B_max and affinity (equation 1) (476). In in-vivo studies only a subset of receptors are available for radioligand binding, for example because some of the receptors are bound to endogenous ligand. Therefore the term Bavail is used to denote the subset of receptors available for binding in in-vivo molecular imaging.

\[
BP = \frac{B_{\text{max}}}{K_D} = B_{\text{max}} \times \frac{1}{K_D} = B_{\text{max}} \times \text{affinity} 
\]

(1)
There are several ways of defining binding potential. BP quantifies the concentration of specific binding as a ratio to some other reference concentration. There are three different reference concentrations, which give rise to BP<sub>F</sub>, BP<sub>P</sub> and BP<sub>ND</sub>. BP<sub>F</sub> refers to the ratio at equilibrium of radioligand that is specifically bound in tissue to the concentration of free radioligand in tissue. This is assumed to be equal to the free concentration in plasma. BP<sub>P</sub> refers to the ratio of specifically bound radioligand to that of the total parent ligand in plasma (equal to the free plus protein bound radioligand). BP<sub>ND</sub> refers to the ratio at equilibrium of the specifically bound radioligand to that of non-displaceable radioligand in tissue. Non-displaceable radioligand refers to the concentration of ‘free’ and non-specifically bound radioligand. BP<sub>ND</sub> is obtained from reference-tissue methods, where the radioligand concentration in receptor-rich regions is compared to the concentration in a receptor-free region.

Both BP<sub>F</sub> and BP<sub>P</sub> require measurement of the arterial input function, whereas BP<sub>ND</sub> can be quantified without arterial sampling. In reference tissue methods where arterial sampling is avoided, binding potential is calculated as a ratio of specifically bound radioligand to non-displaceable (free and non-specific) radioligand in tissue:

\[
BP_{ND} = \frac{f_{ND} \text{avail}}{K_D} \quad (2)
\]

4.1.3. The Translocator Protein (TSPO)

As described earlier, microglia are the brain's resident macrophages. They become activated in response to damaging stimuli and over-express the translocator protein (TSPO). As a result, the Translocator Protein (TSPO), formerly known as the Peripheral Benzodiazepine Receptor (PBR), is currently under investigation as a biomarker of neuroinflammation (see figure 4.4). It is an 18kDa protein found on the outer mitochondrial membrane of glial cells in the brain and has been consistently shown to be a sensitive biomarker of reactive gliosis and inflammation in response to a variety of brain insults including physical trauma (477), degenerative disease (37)(478), CNS inflammatory disease (479) and stroke (480).

The TSPO has a number of biological functions. It is primarily involved in the translocation of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in steroid synthesis. It is also thought to be involved in cell growth and proliferation, calcium flow, bile acid synthesis, chemotaxis and cellular immunity, mitochondrial respiration and apoptosis (see (480) for review). That TSPO knockout mice die at an early embryonic stage (481) suggests that it is a protein that is vital to survival from an early stage. A number of endogenous ligands for TSPO have been identified, including endozepines and cholesterol, which are thought to be associated with steroid synthesis (480).
Due to its involvement in biological processes such as steroid synthesis it is highly expressed in peripheral tissues. In contrast, the expression of TSPO in the brain is low. In the normal brain, TSPO expression is minimal and limited to endothelial cells of blood vessels, meninges, ependymal cells and choroid plexus. Under pathological conditions, however, TSPO expression greatly increases in microglia, astrocytes and monocytes infiltrating into the CNS (480; 482). Most animal studies show that the greatest increase in TSPO expression comes from activated microglial cells (483), with some studies reporting a stronger correlation of [3H]-PK11195 binding with microglia versus astrocytes (484; 485). Furthermore, in-vitro studies using [3H]-PK11195 suggest higher $B_{max}$ values in microglia versus astrocytes but whether this translates to clinical PET studies is not clear. Based on the evidence to date, it seems as though there is a greater contribution to the increased TSPO expression in pathological conditions from microglia than astrocytes.

![Figure 4.4 Transition of microglia from resting to activated form & corresponding overexpression of TSPO (PBR).](image)

**4.1.4. TSPO radiotracers**

Using high-affinity, selective TSPO ligands in conjunction with PET, we can investigate microglial activation, indicative of neuroinflammation, in the living brain. The most established TSPO radioligand is $[^{11}C]$-PK11195, which has been used to investigate neuroinflammation in PET studies for almost 30 years, with the first study using $[^{11}C]$-PK11195 in humans in 1986 (486). In 1994 it was discovered that the $R$-enantiomer displayed a greater affinity for PK binding sites than the $S$-enantiomer (487). Subsequently, $[^{11}C]$-(R)-PK11195 has since been used in most studies due to its increased sensitivity. $[^{11}C]$-(R)-PK11195 has been used to show evidence of neuroinflammation in numerous neuropathological states, including Alzheimer's Disease (478; 488), Parkinson's Disease (37), stroke (47), Multiple Sclerosis (479), human immunodeficiency virus (HIV) disease (489), encephalitis (490) and schizophrenia (467; 470). Despite its ability to detect microglial
activation in pathological brain states with high selectivity and high sensitivity, $[^{11}C](R)$-PK11195 as a TSPO tracer is limited by high levels of non-specific binding and poor signal-to-noise ratio (48), which complicates its quantification. Further, PK11195 is labelled with Carbon-11 (half life: 20.38 min) which limits its dissemination and widespread clinical use (studies using Carbon-11 radiotracers require an on-site cyclotron).

Consequently there is an ongoing effort to develop novel TSPO tracers with improved specific to nonspecific binding and signal-to-noise ratios. During the last several years over 40 new TSPO radioligands have been reported in the literature (491). Of these second generation TSPO ligands, several have been investigated in humans and have demonstrated improved signal-to-noise ratio, for example $[^{11}C]$PBR28, $[^{18}F]$PBR06, $[^{11}C]$DAA1106 and $[^{11}C]$DPA713. At first glance such second generation TSPO tracers seem to be promising alternatives to $[^{11}C]$-PK11195. However, a recent study using $[^{14}C]$PBR28 found that 14% of healthy volunteers did not display a specific binding signal in either the brain or the periphery (492).

Further investigation into this apparent nonbinding of PBR28 subsequently demonstrated substantial variability in PBR28 binding across subjects. Using brain tissue from donors with MS, Owen et al. showed that subjects fell into one of three groups depending on their PBR28 affinity; high-affinity binding with a single site (HAB), low-affinity binding with a single site (LAB) or mixed-affinity binding (MAB) with one high- and one low-affinity site (493). It was then found that all other second-generation TSPO ligands in clinical application at the time (PBR06, PBR111, DPA713 and DAA1106) also demonstrated intersubject variability in binding affinity (494). Such findings represent a challenge for quantification of TSPO binding using these second-generation tracers, as differences in PET signal across subjects cannot be accurately inferred as differences in receptor density, seriously confounding the interpretation of between-group differences.

It has recently been shown that binding affinity of PBR28 is determined by a common polymorphism (rs6971) in the TSPO gene (50), which is thought to be the case for all other TSPO tracers displaying a trimodal distribution. For example, the rs6971 polymorphism has since been shown to predict $[^{18}F]$FEPPA total distribution volume in human brains, with distinct differences in time activity curves between genetic groups (495). This is problematic for studies using second-generation TSPO tracers, due to an increase in variance and the differential inclusion of the three genotypes in clinical and control groups. Genotyping subjects in advance and sub-selecting a genetically homogenous sample of high-affinity binders can overcome this limitation. However this selection process has obvious implications for patient recruitment. For example, up to 50% of the population may need to be excluded to ensure a homogenous sample of high-binders (50). In a patient population that may already be difficult to recruit, having to exclude half of the patients is not practically feasible.
In contrast, PK11195 appears to bind in only one way, showing similar affinity across all subjects. In-vitro studies suggest that $^{[1]}$-[PK11195 binds to a different site on the TSPO, with no apparent difference in affinity between HABs and LABs (493). Therefore, until the development of tracers with consistent binding affinities across subjects, $^{[1]}$-[PK11195 is still favourable in difficult to recruit patient populations. Another potential limitation of the second generation TSPO tracers is that due to their higher affinity for TSPO, there is a greater propensity to bind to TSPO in endothelial cells of blood vessels, which then masks the TSPO that is being expressed from microglia. Therefore the higher affinity could result in further complications in quantification. Despite the limitations of $^{[1]}$-[PK11195, its use is not complicated by the TSPO polymorphism and it remains the best characterised radiotracer for imaging activated microglia in humans.
4.2 Study methods

I now outline the methodology of the study, beginning with details of the participants; the inclusion/exclusion criteria, recruitment methods and demographic/clinical characteristics. Next I outline the procedure, followed by a description of the materials used; the questionnaires, rating scales and cognitive tests. The scanning acquisition and image analysis protocols are then defined. Finally, the statistical tests used to analyse the data are outlined.

4.2.1 Participants

A total of 30 patients were recruited to the study between July 2011 and July 2015. Sixteen patients had schizophrenia (diagnosed according to DSM-IV criteria). Eight of these were antipsychotic-free for at least 3 months prior to scanning and the remaining eight were receiving either Risperidone or Paliperidone Long Acting Injections (LAI). Fourteen patients had Major Depressive Disorder (DSM-IV), were in a major depressive episode (MDE) and were antidepressant-free at the time of scanning. Eight age and gender matched healthy volunteers were screened and scanned under this study protocol, and eleven were taken from an existing dataset. All participants were medically healthy and had no drug or alcohol abuse. Participants were screened and scanned at the University of Manchester’s Wolfson Molecular Imaging Centre (WMIC). Ethical permission was obtained from Greater Manchester East Research Ethics Committee (Ref no. 09/H1013/78). The study was also approved by the Administration of Radioactive Substances Advisory Committee (ARSAC, ref no. 595/3586/25501).

Inclusion criteria

I. Male and Female
II. Age 18 – 60 years
III. Diagnosis of Schizophrenia or Major Depressive Disorder according to DSM-IV criteria
IV. Absence of Axis II disorder (personality disorders) according to DSM IV criteria
V. At least moderate clinical severity:
   Schizophrenia:
   a) Positive and Negative Syndrome Scale (PANSS) ≥ 70
   Depression:
   a) Hamilton Depression Rating Scale (HADS) ≥ 17
   b) Beck Depression Inventory (BDI) ≥ 19
VI. Capacity and willingness to give informed consent.

Exclusion criteria

I. Research involving the administration of ionising radiation (e.g. PET scans, X-rays) within the previous 12 months
II. Drug or alcohol dependence or misuse within the previous year
III. Suicidal ideation, unless the patient is an inpatient and accompanied by ward staff during their study participation

IV. Patients detained under the Mental Health Act 1983

V. Female patients will be excluded if they have:
   a. Confirmed pregnancy or lactation (all females will have a urine pregnancy test on the day of PET scanning to exclude unknown or undisclosed pregnancy)
   b. Lack of effective contraception (this can include abstinence) in the 15 days prior to PET scanning, to exclude a pregnancy too early to be detected by urine pregnancy test.

VI. For major depressive disorder:
   a. Presence of psychotic symptoms
   b. Use of antidepressant medication in the last 3 months

VII. Use of medications that might interfere with PK11195 binding (e.g., benzodiazepines or hypnotics, including zaleplon, zolpidem, zopiclone and eszopiclone), or that are known to suppress microglial activation (e.g. minocycline) or possibly suppress microglial activation (e.g anti-inflammatory drugs)

VIII. Presence of another comorbid Axis 1 disorder

IX. Significant co-morbid medical disorders:
   a. History of head injury with unconsciousness > 3 minutes
   b. Current, or history of, epilepsy
   c. CVA or TIA
   d. Hypertension or coronary artery disease

X. Contraindications to MRI scan, including ferromagnetic implants, claustrophobia, cardiac implants etc. (according to MRI safety guidance)

4.2.2 Patient recruitment

Schizophrenia

Patients with schizophrenia were recruited from NHS mental health services in the Greater Manchester area, specifically from Greater Manchester West (GMW) and Manchester Mental Health and Social Care (MMHSC) Foundation Trusts. Within the trusts, patients were recruited from Community Mental Health Teams (CMHTs) and Early Intervention Services (EIS). Most of the patients were referred to the study through Care Coordinators (CCs), Community Psychiatric Nurses (CPNs) or Support Workers. Patients were also able to self-refer to the study, which was advertised via posters and leaflets placed on Trust noticeboards/in waiting areas (see figure A-1 in appendix for a study poster). Permission to recruit was obtained from each patient’s lead Consultant.

In total, 76 patients were identified as being potentially eligible (either off antipsychotic medication or on risperidone/paliperidone depot). Of these, 21 were brought in for screening. The majority of excluded patients did not meet inclusion criteria, based on either telephone screening or information from patients’ key workers. A total of 22 patients consented to taking part in the study. Of these, 3 were excluded due to the presence of a significant medical disorder (e.g. diabetes) or drug abuse. Three patients had their MRI scan
but failed to have their PET scan. 16 patients had both MRI and PET scans (see consort diagram below, figure 4.5)

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**Figure 4.5 Consort diagram: Schizophrenia patients**

**Depression**

Patients with MDD were mostly recruited through self-referral. Posters were placed in CMHTs but also in Primary Care sites such as local GP surgeries and Psychological Services. Patients were also recruited through the Voluntary Sector, for example from Manchester's Self Help Services, which offers support, counselling and CBT to people suffering from symptoms of depression. Adverts were also posted online; on the ‘Citizen Scientist’ website, where members of the public are given the opportunity to take part in health research in Manchester, and on the University’s volunteering webpages. The posters and online advertisements included a link to the study webpage (embedded on the University’s website) which outlined details of the study and enabled people to fill out a confidential screening questionnaire, the 9-item Patient Health Questionnaire (PHQ-9).

In total, 238 people filled in the online screening questionnaire. Of these, 97 had symptoms that were of at least moderate severity. Of those that were telephone screened, 19 met all inclusion/exclusion criteria and verbally agreed to take part in the study. Of these, 17 had MRI scans but no PET, and 14 had both MRI and PET scans (see figure 4.6).
Participant information sheets were given to each potential participant at least 24 hours before informed consent was taken. The information sheet was gone through in detail before consent to ensure that participants understood why the research was being carried out and what was involved.

### 4.2.3 Healthy volunteers

Healthy volunteers (HVs) were aged 18-60, medically healthy, had no past or present drug or alcohol abuse and no axis I or axis II DSM-IV disorder. The exclusion criteria were the same as for the patients (listed above). Most of the healthy volunteers (n=13) were taken from an existing dataset of [11C]-PK11195 scans performed at WMIC between 2009 and 2013. All of the healthy volunteers were medically screened. Four of the controls taken from the existing dataset did not undergo psychological screening. However they reported no current or previous mental disorder. These controls are starred in the demographic data provided in table 4.3. To ensure accurate matching, 8 healthy volunteers were also recruited as part of this study (all in 2015) through advertising on the Citizen Scientist website and the University volunteering webpages. Of these 8 volunteers, 3 have been excluded due to abnormally high signal from outside the brain (scalp, meninges, blood vessels) resulting in contamination of PK11195 binding within the brain (more details given in image analysis section). Of note, the synthesis of [11C](R)-PK11195 was less efficient in the older scans (2009-2013), resulting in a lower injected dose. However, as long as injected dose is within the minimum and maximum range (between 340 and 740MBq), differences in injected dose are not problematic as the outcome measure ($BP_{ND}$) is independent of injected dose.

This is described further in section 4.2.8.
Demographic & clinical data

The age, sex, Body Mass Index (BMI) and smoking status are listed for the fourteen depression patients (table 4.1), then the sixteen schizophrenia patients (table 4.2) and for all of the healthy volunteers (table 4.3). Note that the healthy volunteers used for matching to each patient cohort are slightly different. Symptom severity as indicated by scores on various rating scales is also listed for the patients. More detailed clinical data is given in the results chapters.

Table 4.1 Clinical & demographic data: MDD patients

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<th>BMI</th>
<th>Smoker</th>
<th>BDI(^a)</th>
<th>MADRS(^b)</th>
<th>HAM-D(^c)</th>
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<td>31 ± 6</td>
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\(^a\)BDI: Beck Depression Inventory (mild severity = 14-19; moderate = 20-28; severe = >29)
\(^b\)MADRS: Montgomery-Asberg Depression Rating Scale (mild severity = 7-19; moderate = 20-34, severe = >29).
\(^c\)HAM-D: Hamilton Depression Rating Scale [17-item] (mild severity = 8-13, moderate = 14-18, severe = >18)

Table 4.2 Clinical & demographic data: schizophrenia patients

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<tr>
<td>16</td>
<td>40</td>
<td>M</td>
<td>25.5</td>
<td>Y</td>
<td>105</td>
<td>Risperidone LAI</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>33 ± 9</td>
<td>-</td>
<td>28 ± 6</td>
<td>-</td>
<td>85 ± 9</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)PANSS: Positive and Negative Syndrome Scale (Total score)
### Table 4.3 Demographic data: healthy Volunteers

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Age</th>
<th>Sex</th>
<th>BMI</th>
<th>Smoker</th>
<th>Matched to</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>M</td>
<td>20.0</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>M</td>
<td>31.7</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>F</td>
<td>25.6</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>M</td>
<td>24.5</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>F</td>
<td>22.8</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>6*</td>
<td>50</td>
<td>M</td>
<td>23.0</td>
<td>-</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>7*</td>
<td>43</td>
<td>F</td>
<td>24.5</td>
<td>-</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>8*</td>
<td>44</td>
<td>F</td>
<td>22.3</td>
<td>-</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>9</td>
<td>41</td>
<td>M</td>
<td>21.9</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>M</td>
<td>21.6</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>F</td>
<td>24.6</td>
<td>N</td>
<td>Depression/Schizophrenia</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>F</td>
<td>18.1</td>
<td>N</td>
<td>Depression</td>
</tr>
<tr>
<td>13</td>
<td>45</td>
<td>M</td>
<td>23.0</td>
<td>N</td>
<td>Depression</td>
</tr>
<tr>
<td>14*</td>
<td>46</td>
<td>M</td>
<td>27.7</td>
<td>N</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>M</td>
<td>30.8</td>
<td>N</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>16</td>
<td>38</td>
<td>M</td>
<td>23.7</td>
<td>N</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>17</td>
<td>34</td>
<td>M</td>
<td>25.8</td>
<td>N</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>18</td>
<td>24</td>
<td>M</td>
<td>24.0</td>
<td>N</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>33 ± 10</td>
<td>24 ± 3</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Controls taken from an existing dataset who were not administered the SCID/NP

Both depression and schizophrenia patients were age- and gender-matched to their respective cohorts of healthy volunteers, though it was not possible to match the schizophrenia patients on BMI or smoking status (tables 4.3 and 4.4).

### Table 4.4 Comparison of age, gender, BMI and smoking status in depression patients & HVs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depression patients (n=14)</th>
<th>Healthy Volunteers (n=13)</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>30 ± 12</td>
<td>33 ± 11</td>
<td>0.673</td>
</tr>
<tr>
<td><strong>Gender (male:female)</strong></td>
<td>7 : 7</td>
<td>7 : 6</td>
<td>-</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>23 ± 6</td>
<td>23 ± 3</td>
<td>0.615</td>
</tr>
<tr>
<td><strong>No. of smokers</strong></td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Values given as mean ± SD

<sup>a</sup>p-value obtained from independent-samples t-tests

### Table 4.5 Comparison of age, gender, BMI and smoking status in schizophrenia patients & HVs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Schizophrenia patients (n=16)</th>
<th>Healthy Volunteers (n=16)</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>33 ± 9</td>
<td>33 ± 10</td>
<td>0.871</td>
</tr>
<tr>
<td><strong>Gender (male:female)</strong></td>
<td>11 : 5</td>
<td>11 : 5</td>
<td>-</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>28 ± 6</td>
<td>24 ± 4</td>
<td>0.020*</td>
</tr>
<tr>
<td><strong>No. of smokers</strong></td>
<td>11</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Values given as mean ± SD

<sup>a</sup>p-value obtained from independent-samples t-tests

<sup>*</sup>Significant at p<0.05 level
4.2.4 Materials

Various structured interviews, rating scales and questionnaires were used to confirm diagnosis, assess symptom severity and obtain information on other study relevant factors (alcohol use, smoking, exercise, sleep, childhood adversity). These are listed below:

Rating scales

- Structured Clinical Interview for DSM-IV Axis I disorder (SCID-I/P) (247)

All patients were administered the SCID-I/P to confirm diagnosis and to exclude other comorbid Axis I disorders. Healthy volunteers were administered the non-patient version (SCID-I/NP)

For MDD:
- Beck Depression Inventory (BDI) (496)
- Hamilton Depression Rating Scale (HAM-D), 17-point version (497)
- Montgomery-Asberg Depression Rating Scale (MADRS) (498)
- Patient Health Questionnaire (PHQ-9) (499)

For Schizophrenia:
- Positive and Negative Syndrome Scale (PANSS) (274)

Questionnaires

- Alcohol Use Disorders Identification Test (AUDIT) (500)
- Godin Leisure-time Exercise Questionnaire (501)
- The Sleep Condition Indicator (SCI) (502)
- Childhood Adversity Questionnaire (503)

Cognitive tests

All patients and some of the controls were administered the following computer-based tasks to assess the relationship between cognitive functioning and inflammation. Cognitive tests were designed and administered using E-Prime software (v 2.0) and displayed on a Microsoft Surface Pro tablet. The main outcome measures used for the emotional recognition and recognition memory tasks were 'hit rate' and 'false alarm rate'. In both tasks there are four possible categories of response (see table 4.6). These are calculated through counting the number of hits, misses, false alarms (FA) and correct rejections (CR) (see table 4.6 and equations below).

<table>
<thead>
<tr>
<th>Word type</th>
<th>Subject response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old</td>
<td>Hit</td>
</tr>
<tr>
<td>Old</td>
<td>Miss</td>
</tr>
<tr>
<td>New</td>
<td>False alarm</td>
</tr>
<tr>
<td>New</td>
<td>Correct rejection</td>
</tr>
</tbody>
</table>

Table 4.6 Possible responses in memory recognition task
Calculating the proportion of correct responses is known as the ‘hit rate’, calculated as:

\[
\text{Hit rate} = \frac{\# \text{ hits}}{\# \text{ hits} + \# \text{ misses}}
\]

This however is an incomplete measure of an individual's performance on a task as it is influenced by response bias. For example, if a participant responds with ‘old’ to every word, they would get a hit rate of 100% which would not be an accurate reflection of performance. Therefore a more complete description of performance is to calculate the false alarm rate, which takes the proportion of false alarms and correct rejections into account:

\[
\text{false alarm rate} = \frac{\# \text{ false alarms}}{\# \text{ false alarms} + \# \text{ correct rejections}}
\]

Recall memory

Participants were first administered the encoding stage of the memory task. This involved the presentation of 30 words that they were asked to memorise. Of these 30 words, 10 were positively valenced, 10 were negative and 10 were neutral. Examples of positively valenced words are ‘intelligent’ and ‘likable’, negative words included ‘thoughtless’ and ‘gloomy’, and neutral words included ‘standard’ and ‘level’. After a delay of approximately 15 minutes participants were given a free recall task in which they had to write down as many words from the original list in 3 minutes. Each word was presented on the computer screen for 1 second.

Recognition memory

After the recall task, participants were given a recognition task where they had to identify the original 30 words (from the encoding stage) as distinct from 30 distractor words (see appendix 1 for word list). Participants were required to make a yes/no judgement in relation to whether they recognised the words from the original encoding task or not. Participants had 3 seconds to respond to each presented word.

Emotional recognition

The facial expression recognition task used Ekman and Friesen's 'Pictures of Facial Affect' battery of stimuli (504) to assess participants' ability to recognise facial expressions of four emotions (anger, fear, happiness and sadness) (figure 4.7). The emotional expressions consisted of two different intensities (50% and 70%) in addition to neutral expressions. Four actors out of the ten from the original Ekman study (505) were chosen. Sticky labels were placed on the keys that corresponded to anger, fear, happy, sad and neutral responses and participants were asked to respond as quickly as possible. Faces were shown for 1 second, followed by a fixation cross shown for 3 seconds. Participants had a short practise before beginning the task.
4.2.5 Procedure

Taking part in the study involved two visits to the WMIC. After establishing that patients met the main eligibility criteria, either through telephone screening or through contact with the patient’s key worker, a screening session was arranged. If participants met criteria following psychological and medical screening, they returned for the scanning session (figure 4.8).

Screening

Informed consent was first obtained. Participants then underwent psychological screening. Diagnosis was confirmed using the SCID/P (and a lack of diagnosis was confirmed in healthy volunteers using the SCID/NP). The appropriate rating scales were then administered followed by the questionnaires listed in the Materials section. The structured clinical interviews were audio recorded for each patient. Next, participants carried out the cognitive tasks.

Participants then underwent medical screening, administered by a WMIC Clinician. A medical history and physical examination ensured that no obvious medical condition could be causing
inflammation. All participants had screening blood tests (total 15ml) to ensure normal full blood counts and normal liver, renal and thyroid functioning. A urine sample was also taken for urinalysis and to test for recent drug use.

**Scanning**

The scanning session was arranged as soon after the screening session as possible (usually within one week). Participants first had a 30-minute MRI scan on a 1.5 Tesla scanner. This was to exclude abnormality, for co-registration with the PET data and to assess white matter structural integrity. All MRI scans were checked and reported on by a Radiologist. The scanning acquisition parameters are detailed below. After their MRI scan participants had a cannula inserted into a suitable vein (usually the antecubital fossa of the right arm). A 10ml blood sample was taken through this cannula for measurement of peripheral inflammatory markers (detailed in section 4.2.9). The participant was then given a motion-tracking cap to wear for assessment of head motion during the scan, and positioned on the PET scanner bed. After a seven minute transmission scan, the radiotracer \([^{11}\mathrm{C}]\)(R)-PK11195 was injected through the venous cannula and emission data was collected for 60 minutes. In total participants were lying in the PET scanner for approximately 70 minutes. Following the PET scan, the participant was brought out of the camera, the venous cannula was removed, and the participant was discharged following a standard post-scanning assessment.

### 4.2.6 MRI acquisition

All participants were scanned using the 1.5 Tesla Philips Achieva whole body scanner at the WMIC. The MR imaging consisted of the following sequences, which came to a total of 28 minutes:

- **High resolution, three-dimensional T1-weighted gradient-echo sequence (MP RAGE)**
  Standard anatomical sequence used for excluding structural abnormality and for co-registration with PET data
- **T2-weighted Fluid Attenuation Inversion Recovery (T2-FLAIR)**
  Standard anatomical sequence used for detecting lesions
- **Diffusion Weighted Imaging (DWI)**
  Used as a means of measuring tissue micro-structure, often in white matter tracts
- **Magnetisation Transfer Ratio (MTR)**
  Another sequence enabling the investigation of the structural integrity of white matter [DWI and MTR data not shown in this thesis].
PET acquisition

Radiotracer production

The radiotracer \([11C]\)-(R)PK11195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide) was produced onsite at the WMIC. It was synthesised by reaction of \([11C]\)methyliodide with the precursor N-Desmethyl-(R)PK11195, as described previously (487) (figure 4.9). Intravenous administration of a radiotracer in humans must comply with radiation and pharmaceutical standards, to ensure both safety and efficacy. Quality control (QC) checks were carried out before the radiotracer was released for administration. These included checks for pH levels, radiochemical identity, radiochemical purity, specific activity, radionuclide purity, residual solvents, endotoxins and sterility testing.

![Figure 4.9 Chemical structure of [11C](R)-PK11195](image)

Image acquisition

Once the participant was positioned in the PET scanner, a 7 minute transmission scan with a \(^{137}\)Caesium point source was acquired for subsequent attenuation and scatter correction. The emission scan was started immediately after the transmission scan. Shortly after this, following release of the radiotracer from the QC team, the radiotracer was drawn up into a 10ml syringe. The \([11C](R)-PK11195\) was then injected intravenously by hand as a slow bolus (10ml) over approximately 15 seconds and flushed with 0.9% saline (10ml) at the same rate. See table 4.8 for comparisons of injected dose, specific activity, injected mass and radiochemical purity between both patient groups and healthy volunteers.
Emission data were then acquired for 60 minutes post-injection in list mode. Dynamic PET data were binned into 18 frames of varying lengths (durations: 1 x variable background frame prior to injection, 1 x 15s, 1 x 5s, 1 x 10s, 1 x 30s, 4 x 60s, 7 x 300s, 2 x 600s). The multi-frame PET images were reconstructed with the HRRT user’s software using iterative ordered subsets maximum likelihood expectation algorithm (OP-OSEM) (506). We employed an ultrafast implementation of this algorithm (507) using 12 iterations and 16 subsets. Corrections for attenuation and scatter were included in the algorithm. The PET images were reconstructed with a voxel size of 1.22 x 1.22 x 1.22 mm. 3D Gaussian smoothing filters of 2 mm and 4 mm full width at half maximum (FWHM) were applied to the reconstructed images to reduce noise at the voxel level.

### Table 4.8 Comparisons of radiotracer characteristics across groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy volunteers (n=18)</th>
<th>Depression patients (n=14)</th>
<th>Schizophrenia patients (n=16)</th>
<th>p-value</th>
<th>HVs vs depression</th>
<th>HVs vs schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected dose (MBq)</td>
<td>594 ± 123</td>
<td>662 ± 96</td>
<td>676 ± 54</td>
<td>0.12</td>
<td>0.02*</td>
<td></td>
</tr>
<tr>
<td>Injected Mass (μg)</td>
<td>1.9 ± 1.7</td>
<td>1.8 ± 0.9</td>
<td>2.0 ± 0.8</td>
<td>0.47</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Specific Activity (Gbq/μmol)</td>
<td>328 ± 145</td>
<td>417 ± 169</td>
<td>411 ± 208</td>
<td>0.01*</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Radiochemical purity (%)</td>
<td>98 ± 0.8</td>
<td>99 ± 1.0</td>
<td>98 ± 0.6</td>
<td>0.15</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

Numbers presented as mean ± SD
*Significant at threshold p<0.05

Injected dose of radiotracer was significantly higher in the schizophrenia patients compared to healthy volunteers. However, as long as the dose is not too low or too high, this will not affect binding potential. This is because BP_{ND} is calculated from the ratio of radioligand uptake in the target tissue to a reference tissue. See equation below where V_{ROI} refers to the total distribution volume in the target region (ROI) and V_{ROR} to the total distribution volume in the region of reference (ROR).

\[
BP_{ND} = \frac{V_{ROI} - V_{ROR}}{V_{ROR}}
\]

Therefore if injected dose is higher in a particular subject, uptake will be higher in both the target and reference tissue, and the ratio will stay the same. Equally, if injected dose is lower, uptake will be lower in both the target and reference regions. It is the ratio between them which gives us BP_{ND}, which is therefore independent of injected dose. It is important, though, that the injected dose is within minimum and maximum limits (340-740 MBq). If radioactivity is too low, the image will have a lower signal-to-noise ratio and BP will be less reliable. Equally if radioactivity is too high, there will be an increase in noise as the camera will become overloaded with counts. The injected doses of all scans were within these limits. The
most important radiotracer characteristic is injected mass. This must be within tracer dose limits (<5% occupancy of the target receptor) and must not differ between groups.

4.2.8 Inflammatory markers

A 10ml blood sample was taken before the PET scan to measure peripheral inflammatory markers. Samples were immediately spun down to plasma (centrifuged for 15 minutes at 1000g), separated into aliquots and stored in a -80°C freezer. Once all samples had been collected, plasma proteins (CRP, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFNγ, BDNF, Eotaxin, RAGE, ICAM-1 and IP-10) were simultaneously quantified using a multiplex immunoassay magnetic bead panel (R&D Systems; Minneapolis, MN). The assay was performed in a 96-well plate according to the manufacturer’s instructions. Aliquots of frozen plasma and assay reagents were allowed to thaw to room temperature prior to processing. The plate consisted of six standards in duplicate, a blank (background) and the subject sample wells in duplicate. A magnetic plate washer (Bio-Tek ELx405; Bio-Tek, Bedfordshire, UK) was utilised during the plate washing stages. The plate was processed on a Luminex 200 instrument (Luminex). Protein concentrations were calculated and analysed with the xPONENT software (Luminex, v. 3.1.871) based on the median fluorescent intensity (MFI) data.

4.2.9 PET image analysis

The following image analysis steps were carried out for all participants. See figure A-2 in appendix for a flowchart of the pre-processing and kinetic modelling steps needed to obtain BP_{ND} values for each ROI.

Image co-registration

First, a dynamic PET image was created by summing frames 2 to 18, resulting in a 60 minute summation image. This image contains bloodflow dependent signal and provides good anatomical detail for accurate co-registration. The T1-weighted MRI image was then co-registered to this dynamic PET image using a rigid body transformation algorithm in VINCI v2.55 (Max-Planck Institute for Neurological Research, Cologne, Germany). The MRI was co-registered to the PET image as opposed to the other way round in order to preserve the quality of the PET data. Although the image values and dimensions differed between the MRI and PET scans, the brain was structurally identical allowing for accurate within-subject alignment across modalities. Each co-registration was visually inspected to ensure accuracy (see figure 4.10).
Segmentation & normalisation

Segmentation of the MRI is necessary to account for differences in radiotracer uptake in different tissue classes. Using Statistical Parametric Mapping (SPM8; Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, University College London, UK) the co-registered T1-weighted MRI (in PET space) was segmented into grey matter (GM) and white matter (WM) probabilistic maps (see figure 4.11: B&C). To define all the voxels belonging to grey matter, a threshold was applied whereby all voxels greater than 0.5 were assumed to be GM, resulting in a binary map.

The Hammers atlas, a three-dimensional maximum probability brain atlas (508) consisting of 83 ROIs in template (Montreal Neurological Institute; MNI) space, was used for automatic delineation of brain regions. This atlas was normalised from template space into individual space via a non-rigid warping algorithm using the inverse transformation parameters from segmentation. This resulted in an individualised maximum probability brain atlas of 83 regions. By combining the binarised GM map with the normalised brain atlas, an individualised atlas specific to the grey matter was created. The fit of these maps were checked against the corresponding T1-weighted MRI (see figure 4.11: D&E).
Generation of parametric images

As described previously, the gold standard in kinetic modelling of PET data is to use a metabolite-corrected arterial input function. Indeed it has been demonstrated that a two-tissue reversible model using a plasma input function is optimal for $^{[11]}$C(R)-PK11195 studies.
Due to its highly invasive nature, arterial sampling was not deemed appropriate for these patient cohorts. However, a comparison of BP values obtained from plasma input function and $B_{\text{ND}}$ values obtained using a reference region indicated a strong correlation (510). The SRTM is the recommended reference region model in PK11195 studies when a plasma input function is not available. The cerebellum has been used consistently as a reference region in previous studies (36; 511; 512). Therefore a grey matter cerebellum input function (see figure 4.12) was used with the SRTM to obtain parametric $[^{11}\text{C}](R)$-PK11195 $B_{\text{ND}}$ maps. TSPO is expressed ubiquitously throughout the brain: in endothelial cells of blood vessels, in perivascular macrophages, lymphocytes and neutrophils, in the choroid plexus and in ependymal tissue (513). Therefore, specific binding in the cerebellum is likely to contaminate the reference region even in healthy controls.

![Figure 4.12 Example of a grey matter cerebellum Time Activity Curve (not decay corrected).](image)

An alternative approach to using the cerebellum as a reference region is to use cluster analysis to segment voxels into classes and select as a reference those voxels that exhibit kinetic behaviour closest to that of grey matter in healthy controls. Turkheimer et al. developed a supervised clustering (SVC) technique which segments voxels in the raw dynamic data into six pre-defined tissue classes (normal GM and WM, blood pool, muscle, skull and pathological tissue with high TSPO density) based on their Time Activity Curves (TACS) (513). The cluster algorithm then extracts as a reference region a cluster of GM voxels which exhibit kinetic behaviour closest to that of GM in a population of healthy controls. Previous research has shown high correlation between BP values obtained from a plasma input function and values obtained from a reference region extracted from supervised cluster analysis (27). Indeed SRTM combined with supervised cluster extraction has been used in $[^{11}\text{C}](R)$-PK11195 studies to detect neuroinflammation in numerous disorders including...
Alzheimer’s Disease (515) and traumatic brain injury (516). The six-tissue SVC method has been validated for use on the HRRT (517).

In practice, neither $\text{BP}_{\text{ND}}$ values obtained from a cerebellum or a supervised cluster reference region are fully reliable. Due to the ubiquity of TSPO expression in the brain, both are likely to be subject to contamination from specific binding, resulting in an underestimation of specific binding in target regions. In chapter eight I compare consistency between $\text{BP}_{\text{ND}}$ values obtained using a cerebellum reference region and values obtained from a cluster extracted reference region. The clustering code, adapted for data acquired from the HRRT, was implemented in the software package SUPERPK (Imperial Innovations, Imperial College London, UK) (513). Two recent papers have concluded that the cerebellum is the preferred reference region over a supervised cluster region (518)(514). Therefore the main findings of this study shown in the subsequent results chapter are presented as BP values obtained from a cerebellum reference input function.

**Obtaining regional readouts**

The GM and unsegmented individualised object maps, created previously, were then projected onto the corresponding parametric PET images. Due to high levels of $[^{11}\text{C}](\text{R})$-PK11195 binding outside the brain, for example from TSPO expressed in blood vessels in the scalp and in the meninges, there is often spill-over of signal into the brain. The object maps were therefore manually edited using Analyze software (Analyze AVW, Mayo Clinic, Rochester, USA) to exclude any contamination of signal from outside the brain (see figure 4.13). Object maps were edited in all orientations (transverse, coronal and sagittal). The edited object maps were then projected onto the corresponding parametric maps, resulting in readouts of mean $\text{BP}_{\text{ND}}$ values from each ROI.

Three of the controls had abnormally high levels of $[^{11}\text{C}](\text{R})$-PK11195 outside the brain, seemingly coming from the meninges, that resulted in excessive spill-over into the brain. These participants were excluded based on visual inspection and on data points being $>2$ standard deviations away from the mean (i.e. they were outliers).
4.2.10 Statistics & power analysis

Group differences were assessed using a repeated-measures ANOVA with region as a within-subjects factor and group as a between-subjects factor. Secondary, exploratory independent-samples t-tests were used to assess differences in each ROI. Correlations were reported with Pearson's correlation coefficient. A threshold of \( p < 0.05 \) was used to determine statistically significant findings, with \( p < 0.1 \) used to indicate trend significance.

Group sizes of 16 were determined based on the literature and standard power analysis for PET studies. Power analysis was based on the largest \( [^{11}C](R)\)-PK11195 PET study in schizophrenia at the time of the design of the study (519). Mean and standard deviation

![Figure 4.13 Object map editing to correct for spill-over of signal from outside brain](image)

A: Unsegmented object map overlaid onto parametric PET image (left) and corresponding MRI (right) before editing. Arrows indicate areas showing spill-over of signal from behind the left eye, from the scalp/meninges and from blood vessels. B: Object map edited for spill-over of signal from outside the brain. Arrows indicate areas where the object map was edited.
values obtained from this study showed that a group of size of \( n=16 \) would be sufficiently powered (79\%) to detect an increase in \([^{11}C](R)-PK11195\) binding of 16.7\% or greater in the schizophrenia group (1-tailed, \( p=0.05 \)). At the time that the study began there was no equivalent data on depression, however assuming similar variation in \([^{11}C](R)-PK11195\) binding amongst patients with depression, we hypothesised that a sample size of 16 would be able to detect the same increase. It was not possible to recruit all 16 depression patients. A sample of 14, however, still had 75\% power to detect the same difference.

### 4.3 Preclinical PET study

To complement the clinical PET study, we also conducted a pilot preclinical PET study to examine the effects of atypical antipsychotic Risperidone on TSPO expression in the rodent brain. Placebo-treated and risperidone-treated animals were scanned on the Inveon small animal PET/CT camera using the TSPO radioligand \([^{18}F]DPA-714\). Details of this pilot study are given below.

#### 4.3.1 Animal model

Ten adult male Lister-hooded rats, initially weighing 300-320g were maintained under standard laboratory conditions at a temperature of 21 °C (±2 °C) and humidity of 40–50\%. Rats were housed in groups of 5, and maintained on a 12-h light/dark cycle (lights on at 0700 hours), with experimental procedures performed during the light phase. Rats had free access to food (standard laboratory chow, Special Diet Services, Essex, UK) and water. The rats were randomly assigned to receive either risperidone (0.5mg/kg) or placebo (saline) once per day for 21 days. Risperidone or placebo was administered in a volume of 1ml/kg via the intraperitoneal route. Experiments were conducted in accordance with the Animals (Scientific Procedures) Act UK (1986), and approved by the University of Manchester ethical review process.

![Figure 4.14 Outline of preclinical PET project](image.png)
4.3.2 PET data acquisition

DPA-714 was labelled with $^{18}$F (half-life, 109.8 min) as described previously (520). Animals were scanned on the Siemens Inveon® small animal PET/CT scanner. Four animals were scanned at baseline (i.e. prior to treatment and then allocated to the risperidone and saline groups, $n=2$ per group). These and the remaining 6 animals ($n=3$ risperidone, $n=3$ saline) were then scanned post-treatment, 3 weeks later. Prior to each scan, anaesthesia was induced by 5% isoflurane and maintained by 2.0-2.5% isoflurane in 70%;30% NO$_2$:O$_2$. A 24-gauge catheter was then inserted in the tail vein for intravenous administration of radiotracer. Animals were placed in the scanner on a heating blanket to maintain temperature. A CT scan was performed prior to PET acquisition to obtain attenuation correction factors.

$[^{18}F]$DPA-714 was then injected through the catheter, coinciding with the start of PET acquisition. PET data were acquired for 60 minutes in list-mode and were binned into 16 dynamic frames. Finally, the emission data were normalised, corrected for attenuation and radioactive decay, and reconstructed using OSEM-3D (16 subsets, 4 iterations) into images with voxel sizes 0.776 x 0.776 x 0.796mm. After their last scan, rats were killed by cervical dislocation. Brains were then frozen in -40°C isopentane and later cut into 20μm-thick sections using a cryostat and mounted on slides. The frozen sections were then post-fixed in paraformaldehyde (4% in 100mM Phosphate Buffered Saline; PBS), allowing immunohistochemistry and autoradiography to be carried out in the future.

4.3.3 Image analysis

Summed PET images between 20 and 60 minutes were co-registered to a stereotaxic MRI rat brain template (521) using rigid registration with mutual information in BrainVisa software (http://brainvisa.info/). The ROIs were automatically delineated using this atlas. $[^{18}F]$DPA-714 uptake was quantified for each ROI using Standardised Uptake Values (SUVs) (injected dose/mass). Weights of the animals were Kleiber corrected (522). Group differences between risperidone and saline-treated animals were assessed using a two-way ANOVA with treatment and ROI as independent factors. Regional differences were assessed post-hoc using independent-samples t-tests.
In this chapter, I first present demographic, questionnaire and clinical data for the depression patients (table 5.1). Next I display the main PET findings: the BP<sub>ND</sub> values for patients followed by correlations of PET data and various other measures, including rating scale scores, demographic information, questionnaire results, cognitive test results and peripheral markers of inflammation.

5.1. Demographic & clinical data

All patients were medically healthy and non-smoking. Most of the patients were within healthy BMI range (see table 5.1). Although four of the patients were classed as being overweight, they were deemed medically healthy in all other areas and patients were matched to healthy volunteers on BMI such that there was no significant difference (\(p=0.67\)). None of the patients abused drugs or alcohol (assessed using DSM-IV criteria). Alcohol use was further assessed through the self-report of average units consumed per week and the Alcohol Use Disorders Identification Test (AUDIT) (see table 5.1). Alcohol use was below harmful levels in all patients. Amount of exercise varied substantially across patients, as shown by scores from the Godin Leisure-time Exercise Questionnaire (scores less than 24 indicate an inactive lifestyle, 24-50 moderately active and scores over 50 highly active). All but one patient experienced some form of childhood adversity, with half of patients exhibiting scores of 5 or greater (each point represents an adverse childhood experience such as physical, emotional or sexual abuse, neglect or family dysfunction) (see table 5.1). All patients were matched to healthy volunteers for age (within 6 years), gender, smoking status and BMI.

Table 5.1 Demographic and questionnaire data for patients with depression

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>BMI</th>
<th>Smoke</th>
<th>Alcohol: units/wk</th>
<th>AUDIT</th>
<th>Exercise</th>
<th>Childhood adversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>M</td>
<td>29.1</td>
<td>N</td>
<td>0</td>
<td>7</td>
<td>68</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>M</td>
<td>23.8</td>
<td>N</td>
<td>5</td>
<td>4</td>
<td>55</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>F</td>
<td>22.7</td>
<td>N</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>M</td>
<td>22.7</td>
<td>N</td>
<td>0</td>
<td>6</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>F</td>
<td>19.4</td>
<td>N</td>
<td>10</td>
<td>6</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>F</td>
<td>19.3</td>
<td>N</td>
<td>6</td>
<td>4</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>M</td>
<td>24.7</td>
<td>N</td>
<td>4</td>
<td>3</td>
<td>56</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>F</td>
<td>29.0</td>
<td>N</td>
<td>5</td>
<td>3</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>49</td>
<td>M</td>
<td>30.4</td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>F</td>
<td>28.7</td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>M</td>
<td>24.0</td>
<td>N</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>19</td>
<td>M</td>
<td>18.5</td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>32</td>
<td>F</td>
<td>21.7</td>
<td>N</td>
<td>11</td>
<td>9</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>24</td>
<td>F</td>
<td>22.8</td>
<td>N</td>
<td>0</td>
<td>4</td>
<td>101</td>
<td>4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>31 ± 12</td>
<td>-</td>
<td>24 ± 4</td>
<td>-</td>
<td>3 ± 4</td>
<td>4 ± 3</td>
<td>40 ± 29</td>
<td>5 ± 3</td>
</tr>
</tbody>
</table>
All patients were in a moderate to severe MDE (see table 5.2). None of the patients took antidepressants for at least 3 months prior to taking part in the study. Half had previously taken antidepressants and half had never taken antidepressants. Most of the patients were relatively young at onset of first symptoms (ten were teenagers), though duration of illness was variable (ranging from 2 to 39 years) (see table 5.2).

**Table 5.2 Clinical data for depression patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>BDI</th>
<th>MADRS</th>
<th>HAM-D</th>
<th>Age at onset</th>
<th>Duration of illness</th>
<th>Previous antidepressants</th>
<th>Months without antidepressant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>26</td>
<td>21</td>
<td>13</td>
<td>11</td>
<td>Citalopram</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>29</td>
<td>18</td>
<td>15</td>
<td>5</td>
<td>Citalopram</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>34</td>
<td>19</td>
<td>15</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>35</td>
<td>25</td>
<td>18</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>24</td>
<td>17</td>
<td>17</td>
<td>12</td>
<td>Citalopram, fluoxetine, venlafaxine</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>29</td>
<td>17</td>
<td>17</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>35</td>
<td>21</td>
<td>16</td>
<td>39</td>
<td>N/A</td>
<td>&gt;60b</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>30</td>
<td>18</td>
<td>24</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>30</td>
<td>18</td>
<td>44</td>
<td>5</td>
<td>Fluoxetine</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>41</td>
<td>34</td>
<td>26</td>
<td>30</td>
<td>15</td>
<td>Amitriptaline, mirtazapine</td>
<td>25</td>
</tr>
<tr>
<td>11</td>
<td>23</td>
<td>32</td>
<td>22</td>
<td>24</td>
<td>16</td>
<td>Reboxetine, sertraline, paroxetine</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>33</td>
<td>37</td>
<td>23</td>
<td>16</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>33</td>
<td>32</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>22</td>
<td>24</td>
<td>21</td>
<td>18</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>30±6</td>
<td>31±4</td>
<td>20±3</td>
<td>20±8</td>
<td>11±10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*aDuration of illness calculated by age at scanning minus age at onset

bPatient number 7 had not taken antidepressants for at least 5 years (medical records before this time were not available).
5.2. PET data

A repeated-measures ANOVA revealed no significant main effect of group ($F_{1,25}=1.66, p=0.21$) and no significant interaction between group and region ($F_{1,25}=1.43, p=0.24$). However, mean $B_{\text{ND}}$ values were 26% higher in MDD patients ($\text{mean±SD}=0.10±0.07$) compared to healthy volunteers ($\text{mean±SD}=0.08±0.03$). A one-tailed independent-samples $t$-test indicated a trend toward significance ($p=0.10$). See table 5.3 for output values from the repeated-measures ANOVA.

### Table 5.3 Repeated-measures ANOVA table for MDD patients vs controls

<table>
<thead>
<tr>
<th>Within-subjects effects</th>
<th>F</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region$^a$</td>
<td>33.487</td>
<td>3.053</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Region*Group$^a$</td>
<td>1.433</td>
<td>3.053</td>
<td>0.239</td>
</tr>
<tr>
<td>Error (region)$^a$</td>
<td>76.313</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-subjects effects</td>
<td>F</td>
<td>df</td>
<td>p</td>
</tr>
<tr>
<td>Group</td>
<td>1.658</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>Error</td>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Greenhouse-Geisser correction used due to violation of the assumption of sphericity.

**Figure 5.1** $B_{\text{ND}}$ values for all MDD patients vs controls across cortical ROIs

Each dot represents an individual participant. Red lines represent mean values. DLPFC: Dorsolateral prefrontal cortex; VLPFC: Ventrolateral prefrontal cortex; OFC: Orbitofrontal cortex; ACC: Anterior cingulate cortex; PCC: Posterior cingulate cortex. Temporal, parietal, occipital and insula are grey matter (cortical) regions.
A secondary, exploratory analysis using independent-samples t-tests revealed a significant increase in the anterior cingulate cortex (ACC) \( (p=0.02) \) and posterior cingulate cortex (PCC) \( (p=0.04) \) in the patients compared to controls, with mean values being 37\% and 34\% higher, respectively. There was an increase in TSPO BP\(_{\text{ND}}\) in all regions (see table 5.4) but no other ROI reached statistical significance at the \( p<0.05 \) level.

Table 5.4 Regional BP\(_{\text{ND}}\) values for MDD patients and HVs with % differences and \( p\)-values

<table>
<thead>
<tr>
<th>Region</th>
<th>MDD patients ((n=14))</th>
<th>Healthy Volunteers ((n=13))</th>
<th>% difference</th>
<th>( p)-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC</td>
<td>0.11 ± 0.09</td>
<td>0.08 ± 0.05</td>
<td>26%</td>
<td>0.31</td>
</tr>
<tr>
<td>VLPFC</td>
<td>0.15 ± 0.09</td>
<td>0.13 ± 0.04</td>
<td>10%</td>
<td>0.60</td>
</tr>
<tr>
<td>OFC</td>
<td>0.11 ± 0.08</td>
<td>0.08 ± 0.06</td>
<td>22%</td>
<td>0.37</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.01 ± 0.05</td>
<td>-0.01 ± 0.04</td>
<td>-</td>
<td>0.24</td>
</tr>
<tr>
<td>Parietal</td>
<td>0.08 ± 0.07</td>
<td>0.07 ± 0.02</td>
<td>17%</td>
<td>0.76</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.06 ± 0.04</td>
<td>0.05 ± 0.03</td>
<td>16%</td>
<td>0.51</td>
</tr>
<tr>
<td>ACC</td>
<td>0.15 ± 0.08</td>
<td>0.10 ± 0.06</td>
<td>37%</td>
<td>0.02*</td>
</tr>
<tr>
<td>PCC</td>
<td>0.10 ± 0.06</td>
<td>0.07 ± 0.02</td>
<td>34%</td>
<td>0.04*</td>
</tr>
<tr>
<td>Insula</td>
<td>0.13 ± 0.10</td>
<td>0.11 ± 0.07</td>
<td>17%</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Figure 5.2 Representative BP\(_{\text{ND}}\) maps for MDD patient and age- and gender matched control

BP\(_{\text{ND}}\) maps overlaid onto T1-weighted MRIs in transverse, coronal and sagittal planes.
A: Patient 11 (40 year old male with MDD). B: Control 9 (41 year old male).
### 5.3. Correlations

There were no correlations between $[^{11}\text{C}](\text{R})$-PK11195 binding and scores on the BDI, MADRS or PHQ-9. There were two negative correlations with scores on the HAM-D and $[^{11}\text{C}](\text{R})$-PK11195 binding in the parietal and dorsolateral prefrontal cortex (figure 5.3). See figure A-3 in appendix for correlation matrix of symptom severity scores and regional $[^{11}\text{C}](\text{R})$-PK11195 binding.

There were no correlations between $[^{11}\text{C}](\text{R})$-PK11195 binding and age, BMI, childhood adversity, alcohol consumption (units/wk), AUDIT scores (alcohol use), SCI scores (sleep quality) or childhood adversity. There was however a significant positive correlation between duration of illness and $[^{11}\text{C}](\text{R})$-PK11195 binding in the PCC and a significant negative correlation between $[^{11}\text{C}](\text{R})$-PK11195 binding in the ACC and amount of exercise, as measured by the Godin Leisure-Time Exercise Questionnaire (figure 5.4). Note the correlation between $[^{11}\text{C}](\text{R})$-PK11195 and duration of illness was driven by an outlier. See figure A-4 in appendix for correlation matrix of demographic/clinical data and PET data.

![Figure 5.3](image)  
**Figure 5.3 Negative correlations with HAM-D scores and BP_{ND} in parietal cortex and DLPFC**

![Figure 5.4](image)  
**Figure 5.4 Correlations between $[^{11}\text{C}](\text{R})$-PK11195 binding, duration of illness and exercise**
5.4. A closer look at symptoms

MDD patients were divided into those that were currently experiencing suicidal thoughts (n=9) and those that were not (n=5), based on a score of 3 or higher on the suicidal thoughts item of the MADRS. A repeated-measures ANOVA indicated significantly higher binding in suicidal patients compared to non-suicidal patients ($F_{1,12}=7.43$, $p=0.01$), but no interaction between group and region ($F_{3,36}=1.89$, $p=0.15$). Mean BP$_{ND}$ values across all ROIs were 64% higher in the patients with suicidal thoughts (see table 5.5 & figure 5.5). The main effect remained significant after including age, duration of illness and severity scores (BDI, MADRS and HAM-D) as covariates. Exploratory t-tests revealed significantly higher [11C](R)-PK11195 binding in ACC, PCC, insula, OFC and VLPFC.

Table 5.5 Regional BP$_{ND}$ values for MDD patients with and without suicidal thoughts

<table>
<thead>
<tr>
<th>Region</th>
<th>Suicidal thoughts (n=9)</th>
<th>No suicidal thoughts (n=5)</th>
<th>% difference</th>
<th>$p$-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC</td>
<td>0.14 ± 0.08</td>
<td>0.06 ± 0.08</td>
<td>57%</td>
<td>0.097†</td>
</tr>
<tr>
<td>VLPFC</td>
<td>0.18 ± 0.09</td>
<td>0.08 ± 0.06</td>
<td>53%</td>
<td>0.047*</td>
</tr>
<tr>
<td>OFC</td>
<td>0.15 ± 0.06</td>
<td>0.04 ± 0.07</td>
<td>76%</td>
<td>0.008*</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.05 ± 0.05</td>
<td>0.03 ± 0.05</td>
<td>2%</td>
<td>0.162</td>
</tr>
<tr>
<td>Parietal</td>
<td>0.11 ± 0.08</td>
<td>0.03 ± 0.05</td>
<td>73%</td>
<td>0.061†</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.07 ± 0.05</td>
<td>0.03 ± 0.03</td>
<td>59%</td>
<td>0.093†</td>
</tr>
<tr>
<td>ACC</td>
<td>0.20 ± 0.06</td>
<td>0.09 ± 0.06</td>
<td>54%</td>
<td>0.005*</td>
</tr>
<tr>
<td>PCC</td>
<td>0.13 ± 0.06</td>
<td>0.06 ± 0.04</td>
<td>52%</td>
<td>0.048*</td>
</tr>
<tr>
<td>Insula</td>
<td>0.18 ± 0.08</td>
<td>0.05 ± 0.09</td>
<td>71%</td>
<td>0.022*</td>
</tr>
</tbody>
</table>

Values presented as mean±SD

$^a$Significant at $p<0.05$ threshold. †Trend significance ($p<0.1$)
Figure 5.5 $\text{BP}_{\text{ND}}$ values across cortical ROIs for MDD patients with and without suicidal thoughts

*significant ($p<0.05$)

Figure 5.6 $\text{BP}_{\text{ND}}$ maps from MDD patients with and without suicidal thoughts

A: MDD patient with suicidal thoughts (male, age 20). B: MDD patient without suicidal thoughts (male, age 22). Colour bar represents $\text{BP}_{\text{ND}}$ values.
Patients were also divided into two groups based on their previous use of antidepressants. There was significantly higher $[^{11}C](R)$-PK11195 binding in the patients that had taken antidepressants in the past (n=7) compared to those that were antidepressant-naïve (n=7) ($F_{1,12}=8.77, p=0.01$) (figure 5.7). Mean $B_{\text{ND}}$ values across all ROIs were 60% higher in the patients that had previously taken antidepressants compared to antidepressant-naïve patients. The group difference remained significant after controlling for age, duration of illness and symptom severity. The main reason that patients stopped taking antidepressants was lack of efficacy.

![Figure 5.7 BP_{ND} values across cortical ROIs for MDD patients who had previously taken antidepressants vs antidepressant-naïve patients](image.png)
A: Patient 5 (female, age 29) who had been on antidepressants in the past (sertraline, citalopram, fluoxetine, venlafaxine) but stopped taking them due to lack of efficacy. B: Patient 14 (female, age 24) who was antidepressant-naïve
5.5. Peripheral markers of inflammation

Below are mean±SD values of the peripheral inflammatory markers for MDD patients (n=14) and healthy volunteers (n=5). Data for peripheral inflammatory markers were only available for 5 of the healthy volunteers.

Table 5.6 Peripheral inflammatory markers measured in MDD patients & healthy volunteers

<table>
<thead>
<tr>
<th>Inflammatory marker*</th>
<th>MDD patients (n=14)</th>
<th>Healthy Volunteers (n=5)</th>
<th>Mean interassay % Covariance b</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>3.41 ± 1.22</td>
<td>3.76 ± 1.02</td>
<td>33%</td>
<td>0.58</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3.65 ± 0.31</td>
<td>3.71 ± 0.37</td>
<td>9%</td>
<td>0.70</td>
</tr>
<tr>
<td>IL-6</td>
<td>6.49 ± 0.55</td>
<td>6.70 ± 0.36</td>
<td>8%</td>
<td>0.44</td>
</tr>
<tr>
<td>IL-8</td>
<td>4.76 ± 1.65</td>
<td>4.83 ± 1.13</td>
<td>5%</td>
<td>0.94</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5.85 ± 0.79</td>
<td>6.31 ± 0.57</td>
<td>13%</td>
<td>0.25</td>
</tr>
<tr>
<td>CXCL-10</td>
<td>67.72 ± 28.67</td>
<td>70.10 ± 53.56</td>
<td>51%</td>
<td>0.90</td>
</tr>
<tr>
<td>BDNF</td>
<td>1884 ± 706</td>
<td>3218 ± 900</td>
<td>43%</td>
<td>0.004*</td>
</tr>
<tr>
<td>RAGE</td>
<td>2238 ± 705</td>
<td>1834 ± 395</td>
<td>31%</td>
<td>0.24</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>399 ± 504</td>
<td>294 ± 283</td>
<td>123%</td>
<td>0.68</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>148 ± 65</td>
<td>89 ± 65</td>
<td>53%</td>
<td>0.31</td>
</tr>
<tr>
<td>CRP</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IL-2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IL-4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Inflammatory markers given as pg/ml unless stated otherwise
bInterassay covariance obtained from replicate values for each sample
%CV=SD of mean x 100 / Mean
*Significant at threshold p<0.05. p-values obtained from independent-samples t-tests.

Data was not available for CRP, IL-2 or IL-4 due to signal detection failure (values outside assay range). There were no significant differences in any of the remaining peripheral inflammatory markers except BDNF, whose levels were significantly lower in MDD patients compared to controls (see figure 5.9). Whilst BDNF is not a marker of inflammation per se, it has been shown to be related to inflammatory processes.
There were few significant correlations with peripheral inflammatory markers and PET data, demographic, clinical or questionnaire data. There was one significant negative correlation with scores on the HAM-D and TNFα levels (figure 5.10). There was a significant positive correlation between IL-8 and BMI, as well as between CXCL-10 and $^{[11]}C(\text{R})$-PK11195 binding in the Occipital cortex (figure 5.10). It must be noted that 24 variables were entered into this correlation matrix (including demographic, clinical, questionnaire, peripheral inflammatory data and PET data from all ROIs – see figure A-5 in appendix). Therefore the occurrence of false positives cannot be excluded with any certainty. Furthermore, the high coefficients of variation between assays (provided in table 5.6) limit the validity of the correlations between the assessed cytokines and regional PET data indicating inflammation in the CNS. Peripheral cytokine data will be re-analysed using the remaining aliquots of plasma which are available for each subject.

![Figure 5.10 Correlations of peripheral inflammatory markers in MDD patients](image)

5.6. Cognitive functioning

The main purpose of collecting cognitive data was to assess whether cognitive functioning was associated with peripheral or central inflammation. Cognitive data were only available for seven of the controls and such unequal group sizes undermine any statistical assessment in differences between patients and controls. However the group results are presented nonetheless as an indication of levels of emotional facial recognition and memory function in the patients compared to controls. Full patient data sets are not available for certain tests either due to software failure or patients failing to complete the task.

**Emotional facial recognition**

The main outcome measures used for emotional facial recognition were hit rate and false alarm rate. Figure 5.11 illustrates the hit rates for each emotion for healthy volunteers (n=7)
and MDD patients (n=11). Although not statistically significant, the results indicate a possible reduction in hit rate (ability to correctly recognise emotions) in patients compared to controls, who scored higher on every emotion except fear. Interestingly the false alarm rate was higher in patients in response to sad, angry and fearful but the reverse was true for happy faces, indicating a possible bias towards negative emotions. The difference between FA rates reached trend significance in the fear condition ($p=0.07$).

There were no significant correlations between emotional facial recognition and central inflammation, peripheral inflammation or symptom severity scores.

**Recall memory**

MDD patients remembered fewer words (mean±SD=6±3) than controls (8±3) but this failed to reach significance. Figure 5.12 illustrates the number of positively valenced, negatively valenced and neutral words remembered by patients and controls (A), as well as the number of intrusions according to valence (B). Although not statistically different, MDD patients had more intrusion words (words not on the encoding list) on average than healthy volunteers, with more negatively valenced intrusions.

![Figure 5.11 Hit and false alarm rates for emotional facial recognition across MDD patients and controls](image)

![Figure 5.12 Mean number of correctly remembered words and intrusions according to valence for MDD patients and healthy volunteers](image)
There were significant negative correlations between the total number of recalled words and $[^{11}\text{C}]$(R)-PK11195 binding in the VLPFC and ACC (figure 5.13). There were no correlations between memory recall and peripheral markers of inflammation.

**Recognition memory**

Although not statistically significant, hit rates were lower for total and positive words but higher for negative words in MDD patients compared to controls, indicating a possible negative bias (figure 5.14). FA rates were higher in MDD patients overall (total) and for positively and negatively valenced words. Taken together, lower hit rates and higher FA rates in MDD patients may indicate some degree of impairment in recognition memory.

There were no significant correlations between performance on the recognition memory task and central or peripheral inflammation or symptom severity scores.
In this chapter I present the findings for the schizophrenia cohort. First I present the demographic and clinical data, before providing the main PET results, the peripheral inflammation data and the correlations with symptom severity, questionnaire and cognitive measures.

### 6.1 Demographic & clinical data

All patients were deemed medically healthy after medical screening. The majority of patients (13/16) were, however, classed as being overweight based on their BMI (see table 6.1). Furthermore, most of the patients (11/16) were smokers. As stated in the methods chapter, patients were age- and gender-matched to healthy volunteers but it was not possible to match on BMI \( p=0.02 \) or smoking status. In terms of alcohol use, all patients were within the recommended safe limits of alcohol consumption (523). Two patients scored highly on the AUDIT, indicating possible harmful use (scores between 8 and 15 warrant advice on reduction of potentially hazardous drinking). However, no patients met criteria for current drug or alcohol misuse based on DSM-IV criteria. Exercise levels varied widely across patients. All patients experienced some form of childhood adversity, with 7/16 patients exhibiting a score of 5 or more (see table 6.1).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>BMI</th>
<th>Smoke</th>
<th>Alcohol: units/wk</th>
<th>AUDIT</th>
<th>Exercise</th>
<th>Childhood adversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>F</td>
<td>23.8</td>
<td>N</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>M</td>
<td>37.0</td>
<td>N</td>
<td>10</td>
<td>9</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>M</td>
<td>25.0</td>
<td>Y</td>
<td>5</td>
<td>9</td>
<td>65</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>F</td>
<td>27.2</td>
<td>Y</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>M</td>
<td>34.5</td>
<td>Y</td>
<td>5</td>
<td>2</td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>F</td>
<td>26.3</td>
<td>N</td>
<td>8</td>
<td>8</td>
<td>112</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>F</td>
<td>20.1</td>
<td>Y</td>
<td>14</td>
<td>12</td>
<td>119</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>M</td>
<td>18.5</td>
<td>Y</td>
<td>18</td>
<td>14</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>M</td>
<td>34.7</td>
<td>Y</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>M</td>
<td>29.4</td>
<td>Y</td>
<td>0</td>
<td>0</td>
<td>74</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>M</td>
<td>29.1</td>
<td>Y</td>
<td>0</td>
<td>2</td>
<td>74</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>M</td>
<td>27.1</td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>119</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>M</td>
<td>26.3</td>
<td>Y</td>
<td>5</td>
<td>8</td>
<td>56</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
<td>M</td>
<td>31.9</td>
<td>Y</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>F</td>
<td>37.3</td>
<td>N</td>
<td>4</td>
<td>6</td>
<td>36</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>40</td>
<td>M</td>
<td>25.5</td>
<td>Y</td>
<td>8</td>
<td>6</td>
<td>81</td>
<td>4</td>
</tr>
</tbody>
</table>

**Mean ± SD**

<table>
<thead>
<tr>
<th>Alcohol: units/wk</th>
<th>AUDIT</th>
<th>Exercise</th>
<th>Childhood adversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(28 ± 6)</td>
<td>(5 ± 6)</td>
<td>(65 ± 38)</td>
<td>(5 ± 2)</td>
</tr>
</tbody>
</table>

All patients had moderate-to-severe symptom severity as measured by the PANSS, with all scores in the 70-105 range (see table 6.2). Eight of the patients were antipsychotic-free for at least 3 months prior to scanning and eight of the patients were on either risperidone (n=7) or
paliperidone (n=1) Long Acting Injection (LAI), received bi-weekly. Overall, mean age of onset was 23±5yrs and duration of illness was 9±7yrs (see table 6.2).

Table 6.2 Clinical data for all schizophrenia patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antipsychotic + dose*</th>
<th>PANSS Total</th>
<th>PANSS Positive</th>
<th>PANSS Negative</th>
<th>PANSS General</th>
<th>Age at onset</th>
<th>Duration of illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>79</td>
<td>28</td>
<td>12</td>
<td>39</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>97</td>
<td>26</td>
<td>20</td>
<td>51</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>76</td>
<td>22</td>
<td>14</td>
<td>40</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>102</td>
<td>26</td>
<td>22</td>
<td>54</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>86</td>
<td>19</td>
<td>23</td>
<td>44</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>78</td>
<td>21</td>
<td>16</td>
<td>41</td>
<td>34</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>83</td>
<td>21</td>
<td>16</td>
<td>46</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>84</td>
<td>20</td>
<td>17</td>
<td>47</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Risperidone 50mg</td>
<td>85</td>
<td>19</td>
<td>26</td>
<td>40</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>Risperidone 50mg</td>
<td>86</td>
<td>15</td>
<td>28</td>
<td>43</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>Risperidone 37.5mg</td>
<td>85</td>
<td>21</td>
<td>24</td>
<td>40</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>Paliperidone 75mg</td>
<td>70</td>
<td>14</td>
<td>17</td>
<td>39</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>Risperidone 50mg</td>
<td>81</td>
<td>21</td>
<td>13</td>
<td>47</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>Risperidone 50mg</td>
<td>84</td>
<td>20</td>
<td>20</td>
<td>44</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>Risperidone 25mg</td>
<td>86</td>
<td>20</td>
<td>20</td>
<td>44</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>16</td>
<td>Risperidone 37.5mg</td>
<td>105</td>
<td>26</td>
<td>27</td>
<td>52</td>
<td>26</td>
<td>14</td>
</tr>
</tbody>
</table>

Mean±SD  85±9 21±4 20±5 44±5 23±5 9±7

*All medication given as ‘depot’ (Long Acting Injection) every two weeks

Table 6.3 shows a comparison between demographic and clinical data in antipsychotic-free and medicated patients. The medicated patients were significantly older (mean±SD years=38±8) than the antipsychotic free patients (mean±SD=27±7). Duration of illness was also significantly longer in medicated patients (mean±SD years=15±7yrs) compared to antipsychotic-free patients (mean±SD=4±2). There were however no significant differences between the two groups in BMI, age at onset or PANSS scores (total or subscales), though PANSS negative scores were slightly higher in medicated (22 ± 5) compared to unmedicated (18 ± 4) patients.

Table 6.3 Comparison of clinical data across antipsychotic-free and medicated schizophrenia patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Antipsychotic-free patients (n=8)</th>
<th>Patients on antipsychotics (n=8)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27±7</td>
<td>38±8</td>
<td>0.01*</td>
</tr>
<tr>
<td>BMI</td>
<td>27±6</td>
<td>30±4</td>
<td>0.20</td>
</tr>
<tr>
<td>PANSS Total</td>
<td>86±9</td>
<td>85±10</td>
<td>0.94</td>
</tr>
<tr>
<td>PANSS Positive</td>
<td>23±3</td>
<td>20±4</td>
<td>0.08</td>
</tr>
<tr>
<td>PANSS Negative</td>
<td>18±4</td>
<td>22±5</td>
<td>0.08</td>
</tr>
<tr>
<td>PANSS General</td>
<td>45±5</td>
<td>44±4</td>
<td>0.51</td>
</tr>
<tr>
<td>Age at onset</td>
<td>24±6</td>
<td>23±5</td>
<td>0.90</td>
</tr>
<tr>
<td>Duration of illness</td>
<td>4±2</td>
<td>15±7</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Numbers presented as mean ± SD

*Significant at threshold p<0.05, **Significant at threshold p<0.001
Table 6.4 provides details of additional medications that patients were on, any antipsychotics they had been on in the past (if any) and how long they had been off antipsychotic medication for. Six of the sixteen patients were on an antidepressant at the time of the study. Six of the eight patients not on antipsychotic-free patients had never taken antipsychotics before and so were antipsychotic-naïve (see table 6.4). All of the medicated patients had been on antipsychotics in the past. Four of the eight medicated patients had taken antipsychotics other than oral risperidone before starting on LAI. Only one had a score under 80 so given the level of residual psychotic symptoms on the PANSS, 3/8 met criteria for treatment resistant schizophrenia and 4/8 had not progressed beyond a failed trial of one antipsychotic.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Other current medication</th>
<th>Past antipsychotic</th>
<th>No. of months without antipsychotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Propranolol</td>
<td>Quetiapine, amisulpride</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Fluoxetine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Duloxetine, oxycontin, pregabolin, lansoprazole</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Venlafaxine</td>
<td>Olanzapine</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>Venlafaxine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Venlafaxine, procyclidine</td>
<td>Quetiapine</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Sertraline</td>
<td>Risperidone</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Procyclidine</td>
<td>Risperidone</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>Aripiprazole, risperidone</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Diazepam</td>
<td>Olanzapine</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>Clozapine</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>Risperidone</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Mirtazapine, oxycontin</td>
<td>Risperidone</td>
<td>-</td>
</tr>
</tbody>
</table>

NB. Past antipsychotic medication status may not be accurate as medication fields are not always filled in accurately on the Trust medical records databases

6.2 PET data

A repeated-measures ANOVA indicated no significant main effect of group ($F_{1,30}=1.78$, $p=0.19$) or interaction between group and region ($F_{3,87,116}=2.38$, $p=0.06$). However, mean $BP_{ND}$ values were 27% higher in patients with schizophrenia (mean±SD=0.09±0.07) compared to healthy volunteers (mean±SD=0.07±0.03). A one-tailed independent-samples t-test indicated a group difference that reached trend significance ($p=0.09$). See table 6.5 for the output from the ANOVA. See figure 6.1 and table 6.5 (next page) for $BP_{ND}$ values for each ROI across patients and healthy volunteers.
Table 6.5 Output from repeated-measures ANOVA (schizophrenia vs controls)

<table>
<thead>
<tr>
<th>Within-subjects effects</th>
<th>( F )</th>
<th>( df )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region ( ^a )</td>
<td>39.716</td>
<td>3.867</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Region*Group ( ^a )</td>
<td>1.433</td>
<td>3.867</td>
<td>0.058</td>
</tr>
<tr>
<td>Error (region) ( ^a )</td>
<td></td>
<td></td>
<td>116.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Between-subjects effects</th>
<th>( F )</th>
<th>( df )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>1.784</td>
<td>1</td>
<td>0.192</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Greenhouse-Geisser correction used due to violation of the assumption of sphericity

Exploratory t-tests revealed a significant increase in the anterior cingulate cortex (ACC), with binding being 40% higher in the patients compared to the controls \((p=0.04)\). No other ROIs reached statistical significance at the \( p<0.05 \) level, but patients had higher mean \( \text{BP}_{\text{ND}} \) values in all regions except for the insula (see % differences in table 6.6, next page).
Table 6.6 Regional $BP_{ND}$ values for schizophrenia patients & HVs with % differences & $p$-values

<table>
<thead>
<tr>
<th>Region</th>
<th>Schizophrenia patients (n=16)</th>
<th>Healthy Volunteers (n=16)</th>
<th>% difference</th>
<th>$p$-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC</td>
<td>$0.10 \pm 0.09$</td>
<td>$0.06 \pm 0.05$</td>
<td>36%</td>
<td>0.16</td>
</tr>
<tr>
<td>VLPFC</td>
<td>$0.17 \pm 0.10$</td>
<td>$0.13 \pm 0.04$</td>
<td>26%</td>
<td>0.10</td>
</tr>
<tr>
<td>OFC</td>
<td>$0.11 \pm 0.10$</td>
<td>$0.08 \pm 0.06$</td>
<td>30%</td>
<td>0.24</td>
</tr>
<tr>
<td>Temporal</td>
<td>$0.00 \pm 0.05$</td>
<td>$-0.01 \pm 0.04$</td>
<td>-</td>
<td>0.39</td>
</tr>
<tr>
<td>Parietal</td>
<td>$0.08 \pm 0.08$</td>
<td>$0.07 \pm 0.03$</td>
<td>21%</td>
<td>0.39</td>
</tr>
<tr>
<td>Occipital</td>
<td>$0.05 \pm 0.05$</td>
<td>$0.05 \pm 0.03$</td>
<td>10%</td>
<td>0.72</td>
</tr>
<tr>
<td>ACC</td>
<td>$0.16 \pm 0.11$</td>
<td>$0.10 \pm 0.06$</td>
<td>40%</td>
<td>0.04*</td>
</tr>
<tr>
<td>PCC</td>
<td>$0.10 \pm 0.07$</td>
<td>$0.07 \pm 0.03$</td>
<td>28%</td>
<td>0.15</td>
</tr>
<tr>
<td>Insula</td>
<td>$0.10 \pm 0.10$</td>
<td>$0.11 \pm 0.06$</td>
<td>-6%</td>
<td>0.84</td>
</tr>
</tbody>
</table>

$^a$p-values obtained from independent-samples t-tests

*Significant at threshold $p<0.05$

6.3 Subgroup analysis

I now present data from the patient subgroups: the antipsychotic-free (n=8) and the medicated (risperidone/paliperidone) patients (n=8). Because the medicated patients were older than the unmedicated patients, the two subgroups are compared to their own age- and gender matched controls. See tables 6.7 & 6.8 below for comparisons between each subgroup and controls. There were no significant differences between either groups of patients and their respective controls in age, BMI or injected mass of radiotracer. There was a significantly higher injected dose of radiotracer in the antipsychotic-free group compared to their matched controls but as mentioned previously (p.120), binding potential ($BP_{ND}$) is independent of injected dose.

Table 6.7 Comparison of demographic & radiotracer data for antipsychotic-free patients vs HVs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Antipsychotic-free patients (n=8)</th>
<th>Healthy volunteers 1 (n=8)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$27 \pm 7$</td>
<td>$28 \pm 8$</td>
<td>0.80</td>
</tr>
<tr>
<td>Gender, m:f</td>
<td>4:4</td>
<td>4:4</td>
<td>NA</td>
</tr>
<tr>
<td>BMI</td>
<td>$27 \pm 6$</td>
<td>$23 \pm 2$</td>
<td>0.21</td>
</tr>
<tr>
<td>Injected dose</td>
<td>$690 \pm 64$</td>
<td>$533 \pm 109$</td>
<td>0.03*</td>
</tr>
<tr>
<td>Injected mass</td>
<td>$2.5 \pm 0.5$</td>
<td>$2.3 \pm 1.7$</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table 6.8 Comparison of demographic and radiotracer data for medicated patients vs controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Medicated patients (n=8)</th>
<th>Healthy volunteers 2 (n=8)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$38 \pm 8$</td>
<td>$39 \pm 9$</td>
<td>0.93</td>
</tr>
<tr>
<td>Gender, m:f</td>
<td>7:1</td>
<td>7:1</td>
<td>NA</td>
</tr>
<tr>
<td>BMI</td>
<td>$30 \pm 4$</td>
<td>$26 \pm 4$</td>
<td>0.05</td>
</tr>
<tr>
<td>Injected dose</td>
<td>$673 \pm 64$</td>
<td>$600 \pm 101$</td>
<td>0.22</td>
</tr>
<tr>
<td>Injected mass</td>
<td>$1.5 \pm 0.8$</td>
<td>$1.8 \pm 0.7$</td>
<td>0.80</td>
</tr>
</tbody>
</table>
There was no significant difference in $BP_{ND}$ values between antipsychotic-free patients (mean±SD=0.07±0.04) and healthy volunteers (mean±SD=0.08±0.03), as revealed by repeated-measures ANOVA ($F_{1,14}=0.14$, $p=0.72$). There was no significant interaction between group and region. See table 6.9 for the ANOVA table. See figure 6.2 for $BP_{ND}$ values across groups for each ROI.

| Table 6.9 Output from repeated-measures ANOVA for (unmedicated schizophrenia vs controls) |
|---------------------------------|------------|-----|-----|
| **Within-subjects effects**    | **F**     | **df** | **p** |
| Region$^a$                     | 15.888    | 3.373 | <0.001 |
| Region$^a$*Group$^a$           | 1.368     | 3.373 | 0.262  |
| Error (region)$^a$             |           |       | 47.226 |
| **Between-subjects effects**  | **F**     | **df** | **p** |
| Group                          | 0.138     | 1     | 0.716  |
| Error                          |           | 14    |  |

$^a$Greenhouse-Geisser correction used due to violation of the assumption of sphericity.

Exploratory t-tests revealed no significant differences between antipsychotic-free patients and controls. Mean patient $BP_{ND}$ values were lower than the controls in five of the nine ROIs. The largest difference, though, was higher $[^{11}\text{C}](\text{R})$-PK11195 binding in the ACC of the patients (30% increase), though this failed to reach significance ($p=0.30$) (see table 6.10).
This indicates that the comparison is underpowered, as an effect of the same size was significant in the whole sample.

Table 6.10 Regional BPND values for antipsychotic-free schizophrenia patients & HVs

<table>
<thead>
<tr>
<th>Region</th>
<th>Antipsychotic-free patients (n=8)</th>
<th>Healthy volunteers (n=8)</th>
<th>% difference</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC</td>
<td>0.07 ± 0.08</td>
<td>0.09 ± 0.06</td>
<td>-31%</td>
<td>0.55</td>
</tr>
<tr>
<td>VLPFC</td>
<td>0.13 ± 0.06</td>
<td>0.13 ± 0.04</td>
<td>0%</td>
<td>1.00</td>
</tr>
<tr>
<td>OFC</td>
<td>0.07 ± 0.05</td>
<td>0.08 ± 0.06</td>
<td>-18%</td>
<td>0.65</td>
</tr>
<tr>
<td>Temporal</td>
<td>-0.01 ± 0.02</td>
<td>0.00 ± 0.03</td>
<td>-</td>
<td>0.47</td>
</tr>
<tr>
<td>Parietal</td>
<td>0.06 ± 0.06</td>
<td>0.07 ± 0.02</td>
<td>-28%</td>
<td>0.51</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>-59%</td>
<td>0.08</td>
</tr>
<tr>
<td>ACC</td>
<td>0.13 ± 0.08</td>
<td>0.09 ± 0.07</td>
<td>30%</td>
<td>0.30</td>
</tr>
<tr>
<td>PCC</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>15%</td>
<td>0.28</td>
</tr>
<tr>
<td>Insula</td>
<td>0.07 ± 0.08</td>
<td>0.10 ± 0.06</td>
<td>-53%</td>
<td>0.35</td>
</tr>
</tbody>
</table>

In terms of the medicated (risperidone/paliperidone) patients versus their age and gender matched controls, there was no significant main effect of group (F1,14=3.42, p=0.09), though this did reach trend significance. There was no interaction between group and region (F3.4,48=1.80, p=0.15). Mean BPND levels were 48% higher in medicated patients (mean±SD=0.13±0.09) compared to controls (mean±SD=0.07±0.03), which was significant as shown by a one-tailed independent-samples t-test (p=0.04). See table 6.11 for ANOVA table and figure 6.3 for regional BPND values for medicated patients and controls.

Table 6.11 Output from repeated-measures ANOVA (medicated schizophrenia vs controls)

<table>
<thead>
<tr>
<th>Within-subjects effects</th>
<th>F</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regiona</td>
<td>24.54</td>
<td>3.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Region*Groupa</td>
<td>1.80</td>
<td>3.43</td>
<td>0.15</td>
</tr>
<tr>
<td>Error (region)a</td>
<td>48.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Between-subjects effects</th>
<th>F</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>3.42</td>
<td>1</td>
<td>0.09</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aGreenhouse-Geisser correction used due to violation of the assumption of sphericity
Exploratory t-tests revealed significantly higher \([^{11}C](R)-PK11195\) binding in the DLPFC in patients compared to controls, with a 70% increase in signal \((p=0.01)\). BP\(_{ND}\) values were higher in patients in all other ROIs, with differences in OFC and ACC trending towards significance with 52% and 48% increases in patients respectively (table 6.12).

Table 6.12 Regional BP\(_{ND}\) values for medicated schizophrenia patients and healthy volunteers

<table>
<thead>
<tr>
<th>Region</th>
<th>Medicated patients (n=8)</th>
<th>Healthy volunteers (n=8)</th>
<th>% difference</th>
<th>p-value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC</td>
<td>0.13 ± 0.09</td>
<td>0.04 ± 0.03</td>
<td>70%</td>
<td>0.01*</td>
</tr>
<tr>
<td>VLPFC</td>
<td>0.21 ± 0.11</td>
<td>0.12 ± 0.03</td>
<td>41%</td>
<td>0.05</td>
</tr>
<tr>
<td>OFC</td>
<td>0.16 ± 0.12</td>
<td>0.07 ± 0.06</td>
<td>52%</td>
<td>0.10(^\dagger)</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.02 ± 0.07</td>
<td>-0.02 ± 0.05</td>
<td>-</td>
<td>0.22</td>
</tr>
<tr>
<td>Parietal</td>
<td>0.11 ± 0.08</td>
<td>0.06 ± 0.03</td>
<td>48%</td>
<td>0.12</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.07 ± 0.06</td>
<td>0.04 ± 0.04</td>
<td>47%</td>
<td>0.23</td>
</tr>
<tr>
<td>ACC</td>
<td>0.19 ± 0.13</td>
<td>0.10 ± 0.06</td>
<td>48%</td>
<td>0.08(^\dagger)</td>
</tr>
<tr>
<td>PCC</td>
<td>0.13 ± 0.09</td>
<td>0.08 ± 0.04</td>
<td>36%</td>
<td>0.23</td>
</tr>
<tr>
<td>Insula</td>
<td>0.13 ± 0.11</td>
<td>0.11 ± 0.06</td>
<td>18%</td>
<td>0.60</td>
</tr>
</tbody>
</table>

\(^a\)p-values obtained from independent-samples t-tests

\(^*\)Significant at threshold \(p<0.05\)

\(^\dagger\)Trend significance \(p<0.1\)
Figure 6.4 Representative BP<sub>ND</sub> maps of medicated and unmedicated patients and healthy volunteer

BP<sub>ND</sub> maps overlaid onto T1-weighted MRIs for A: patient on risperidone (male, age 35), B: Antipsychotic-free patient (male, age 28) and C: healthy volunteer (male, age 26). Colour bar represents BP<sub>ND</sub>. 
6.4 Correlations

There were no significant correlations between $[^{11}C](R)$-PK11195 binding and PANSS total, positive or general scores. However, there were strong correlations between $[^{11}C](R)$-PK11195 binding and scores on the negative subscale of the PANSS in 8 of the 9 ROIs (fig 6.5). See figure A-6 in appendix for a correlation matrix of all variables assessed.

Figure 6.5 Correlations between PANSS negative scores and BPND in 8 ROIs

NB. Correlations remained significant after correcting for medication status.
There were no correlations between $[^{11}C](R)$-PK11195 binding and age, BMI, duration of illness, alcohol consumption (units/wk), AUDIT scores, sleep quality (SCI scores) or scores on the childhood adversity questionnaire. There were however significant negative correlations between amount of exercise (as measured by the Godin Leisure Time Exercise Questionnaire) and PANSS negative scores, BMI and $[^{11}C](R)$-PK11195 binding in the ACC, PCC, DLPFC and VLPFC (figure 6.6). See figure A-7 in appendix for all correlations.

![Figure 6.6](image-url)

**Figure 6.6** Negative correlations between amount of exercise, PANSS negative scores, BMI and BP$_{ND}$ values
6.5 Peripheral markers of inflammation

Similarly to the MDD cohort, peripheral inflammatory marker data was only available for five of the healthy volunteers. As all the samples were analysed in the same batch, data from CRP, IL-2 and IL-4 were unavailable for the schizophrenia patients also. There were no significant correlations between any of the peripheral markers of inflammation and $[^{11}C](R)$-PK11195 in any of the ROIs. As mentioned previously, due to high interassay covariance values (shown in table 6.13 below), the validity of any correlations/comparisons is undermined.

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>Schz patients (n=16)</th>
<th>Healthy Volunteers (n=5)</th>
<th>Mean interassay % Covariance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>4.22 ± 0.92</td>
<td>3.76 ± 1.02</td>
<td>23%</td>
<td>0.35</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3.60 ± 0.32</td>
<td>3.71 ± 0.37</td>
<td>9%</td>
<td>0.38</td>
</tr>
<tr>
<td>IL-6</td>
<td>7.02 ± 0.88</td>
<td>6.70 ± 0.36</td>
<td>13%</td>
<td>0.37</td>
</tr>
<tr>
<td>IL-8</td>
<td>5.10 ± 1.46</td>
<td>4.83 ± 1.13</td>
<td>27%</td>
<td>0.70</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5.34 ± 0.97</td>
<td>6.31 ± 0.57</td>
<td>17%</td>
<td>0.05</td>
</tr>
<tr>
<td>CXCL-10</td>
<td>58.12 ± 25.5</td>
<td>70.10 ± 53.56</td>
<td>54%</td>
<td>0.90</td>
</tr>
<tr>
<td>BDNF</td>
<td>2132 ± 573</td>
<td>3218 ± 900</td>
<td>33%</td>
<td>0.005*</td>
</tr>
<tr>
<td>RAGE</td>
<td>1954 ± 707</td>
<td>1834 ± 395</td>
<td>33%</td>
<td>0.72</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>529 ± 457</td>
<td>294 ± 283</td>
<td>92%</td>
<td>0.30</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>148 ± 65</td>
<td>NA</td>
<td>53%</td>
<td>0.31</td>
</tr>
<tr>
<td>CRP</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IL-2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IL-4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Interassay covariance obtained from replicate values for each sample
%CV=SD of mean x 100 / Mean
*Significant at threshold $p<0.05$

There were no significant differences in any of the remaining peripheral inflammatory markers except BDNF, whose levels were significantly lower in schizophrenia patients compared to controls (see figure 6.7). Whilst BDNF is not a marker of inflammation per se, it has been shown to be related to inflammatory processes.

Figure 6.7 Lower BDNF levels in schizophrenia patients compared to controls
There were significant positive correlations between IL-6 levels and total PANSS scores, as well as with the general subscale. There were also significant correlations between levels of IFNγ and total PANSS scores and scores on the positive and general symptoms subscale (see figure 6.8). See figure A-8 in appendix for a matrix of peripheral markers and the variables they were correlated with.

![Figure 6.8 Correlations between IL-6, IFNγ and PANSS scores](image)

There were no significant correlations between any of the peripheral inflammatory markers and age, BMI, duration of illness, alcohol consumption, sleep quality, childhood adversity or amount of exercise.

### 6.6 Cognitive functioning

Similarly to the MDD results, cognitive data were only available for seven of the controls and whilst a full comparison between patients and controls is not possible, the results are reported as an indication of possible differences in performance. The primary goal was to investigate associations between performance on cognitive tests and levels of inflammation.

#### Emotional facial recognition

Figure 6.9 illustrates the hit rates and false alarm rates for schizophrenia patients (n=14) and healthy volunteers (n=7). Hit rates were lower and false alarm rates were higher for each
emotion in patients compared to controls, reflecting poorer performance in the patients, possibly indicative of an impairment in emotional facial recognition. The largest group difference was an increase in 'sad' false alarms in the patients, indicating high levels of misattributing other emotions as being sad (trend significance: $p=0.08$).

There were no correlations between emotional facial recognition and central inflammation, but there were negative correlations between levels of IL-1β and hit rates for happy, angry and fearful faces (figure 6.10). There were no correlations with any other peripheral inflammatory marker.

Figure 6.9 Performance on emotional facial recognition task: schizophrenia patients vs controls

Figure 6.10 Negative correlations between IL-1β levels and hit rates for happy, angry and fearful faces
Recall memory

Schizophrenia patients remembered significantly fewer words (mean±SD=3±3) than controls (8±3) \((p=0.007)\). Figure 6.11 illustrates the mean number of total words remembered, positively valenced, negatively valenced and neutral words for patients and controls (A), as well as the mean number of intrusion words according to valence (B).

![Figure 6.11 Mean number of correctly remembered words and intrusions according to valence for MDD patients and healthy volunteers](image)

There were no significant correlations between memory recall and peripheral or central inflammation or symptom severity.

Recognition memory

Although not statistically significant, hit rates were lower for total and positive words but slightly higher for negative words in schizophrenia patients compared to controls, indicating a possible negative bias (figure 6.12). FA rates were higher in schizophrenia patients overall (total) and for positively and negatively valenced words. Taken together, lower hit rates and higher FA rates in schizophrenia patients indicate some degree of impairment in recognition memory.

![Figure 6.12 Performance on recognition memory task: schizophrenia patients vs controls](image)

Schz patients: n=14, controls: n=7. Total refers to all words regardless of valence. Positive refers to positively valenced words and negative to negatively valenced words.
Within the schizophrenia patients, there were significant correlations between false alarm rates for negative words and \(^{11}\text{C}\)(R)-PK11195 binding in the VLPFC and ACC, suggesting a possible relationship between bias towards negative words and microglial activation in these regions (figure 6.13).

**Figure 6.13** Correlations between false alarms for negative words and VLPFC & ACC \(^{11}\text{C}\)(R)-PK11195 binding
6.7 A shared mechanism?

There is a value in investigating MDD and schizophrenia together, as regional overlaps in \(^{11}\text{C}\)(R)-PK11195 binding can then be explored. There were striking similarities in the distribution and extent of increased inflammation in MDD and schizophrenia sufferers compared to controls (figures 5.1 and 6.1). The region that showed the greatest difference in \(^{11}\text{C}\)(R)-PK11195 binding in MDD and schizophrenia patients compared to healthy volunteers was the anterior cingulate cortex (ACC) (see figure 6.14 & 6.15). Whether this could reflect a shared mechanism between the two disorders is discussed in Chapter nine.

![Figure 6.14 Increased microglial activation in the ACC of MDD & schizophrenia patients compared to controls](image)

![Figure 6.15 Regional BP\(_{ND}\) values of MDD patients, schizophrenia patients and all controls](image)

Note that all 18 healthy volunteers are displayed for the purpose of visual comparison. Bars represent mean BP\(_{ND}\) and standard error of the mean.
As described in the methods (section 4.3), we carried out a pilot preclinical investigation into the effects of risperidone on microglial activation. Details of the risperidone and saline-treated animals are listed below, followed by the main PET findings. To support the PET findings, immunohistochemistry and autoradiography was carried out on the brain sections post-scanning. These images are yet to be quantified however.

### 7.1 Animals

There was a technical failure on one of the placebo scans, meaning that we only obtained data for 4 as opposed to 5 of the saline-treated animals. The two groups of animals, the saline-treated (n=4) and the risperidone-treated (n=5) did not differ in terms of weight, temperature or in characteristics of the administered $^{[18F]}$DPA-714 (table 7.1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risperidone-treated animals (n=5)</th>
<th>Saline-treated animals (n=4)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>331 ± 22</td>
<td>341 ± 33</td>
<td>0.61</td>
</tr>
<tr>
<td>Injected dose (MBq)</td>
<td>35.0 ± 10.5</td>
<td>27.1 ± 8.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Specific radioactivity (MBq/nmol)</td>
<td>123.5 ± 19.2</td>
<td>109.2 ± 29.6</td>
<td>0.41</td>
</tr>
<tr>
<td>Amount of radioligand injected (nmol)</td>
<td>0.9 ± 0.5</td>
<td>0.7 ± 0.5</td>
<td>0.48</td>
</tr>
</tbody>
</table>

A two way ANOVA indicated a main effect of group on SUV ($F_{1,17}=39.22$, $p<0.001$), with higher SUVs in risperidone-treated animals (mean±SD=0.31±0.09) compared to saline-treated animals (0.23±0.08). See figure 7.1 for SUVs of individual animals across ROIs (A) and mean values across groups (B).
7.2 PET data

Figure 7.1 SUV values for risperidone-treated vs saline-treated animals

A: Each dot represents an individual animal. Red lines represent mean values.
B: Mean SUVs for both animal groups. Error bars represent standard error of the mean.
Exploratory t-tests revealed a significant increase in the accumbens in risperidone vs saline-treated animals ($p=0.04$). SUVs were higher in risperidone animals in all regions. No other regions reached significance at the $p<0.05$ threshold but a number of other regions were trending towards significance (see table 7.2).

**Table 7.2 Mean SUVs for risperidone and saline-treated animals for each region**

<table>
<thead>
<tr>
<th>Region</th>
<th>Risperidone-treated SUVs (n=5)</th>
<th>Saline-treated SUVs (n=4)</th>
<th>% difference</th>
<th>$p$-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumbens</td>
<td>$0.32 \pm 0.08$</td>
<td>$0.19 \pm 0.07$</td>
<td>39%</td>
<td>0.04*</td>
</tr>
<tr>
<td>Amygdala</td>
<td>$0.33 \pm 0.06$</td>
<td>$0.24 \pm 0.08$</td>
<td>29%</td>
<td>0.07†</td>
</tr>
<tr>
<td>Caudate Putamen</td>
<td>$0.23 \pm 0.03$</td>
<td>$0.19 \pm 0.05$</td>
<td>16%</td>
<td>0.23</td>
</tr>
<tr>
<td>Corpus Callosum</td>
<td>$0.28 \pm 0.04$</td>
<td>$0.22 \pm 0.07$</td>
<td>19%</td>
<td>0.18</td>
</tr>
<tr>
<td>Retrosplenial Cingulate Cortex</td>
<td>$0.34 \pm 0.07$</td>
<td>$0.29 \pm 0.08$</td>
<td>14%</td>
<td>0.32</td>
</tr>
<tr>
<td>Piriform Entorhinal Cortex</td>
<td>$0.39 \pm 0.07$</td>
<td>$0.21 \pm 0.05$</td>
<td>25%</td>
<td>0.11</td>
</tr>
<tr>
<td>Frontal Cortex</td>
<td>$0.29 \pm 0.06$</td>
<td>$0.29 \pm 0.05$</td>
<td>28%</td>
<td>0.07†</td>
</tr>
<tr>
<td>Motor Cortex</td>
<td>$0.32 \pm 0.07$</td>
<td>$0.25 \pm 0.06$</td>
<td>9%</td>
<td>0.51</td>
</tr>
<tr>
<td>Somatosensory Cortex</td>
<td>$0.31 \pm 0.05$</td>
<td>$0.30 \pm 0.06$</td>
<td>19%</td>
<td>0.13</td>
</tr>
<tr>
<td>Temporal Cortex</td>
<td>$0.32 \pm 0.07$</td>
<td>$0.18 \pm 0.05$</td>
<td>5%</td>
<td>0.71</td>
</tr>
<tr>
<td>Globus Pallidus</td>
<td>$0.24 \pm 0.04$</td>
<td>$0.26 \pm 0.76$</td>
<td>26%</td>
<td>0.06</td>
</tr>
<tr>
<td>Hippocampus AnteroDorsal</td>
<td>$0.34 \pm 0.05$</td>
<td>$0.20 \pm 0.06$</td>
<td>23%</td>
<td>0.11</td>
</tr>
<tr>
<td>Hippocampus PosteroDorsal</td>
<td>$0.26 \pm 0.04$</td>
<td>$0.21 \pm 0.10$</td>
<td>23%</td>
<td>0.13</td>
</tr>
<tr>
<td>Hypothalamus Lateral</td>
<td>$0.35 \pm 0.09$</td>
<td>$0.31 \pm 0.17$</td>
<td>39%</td>
<td>0.06†</td>
</tr>
<tr>
<td>Hypothalamus Medial</td>
<td>$0.53 \pm 0.14$</td>
<td>$0.17 \pm 0.05$</td>
<td>42%</td>
<td>0.06†</td>
</tr>
<tr>
<td>Mesencephalic Region</td>
<td>$0.24 \pm 0.04$</td>
<td>$0.28 \pm 0.07$</td>
<td>27%</td>
<td>0.07†</td>
</tr>
<tr>
<td>Septum</td>
<td>$0.31 \pm 0.05$</td>
<td>$0.22 \pm 0.08$</td>
<td>8%</td>
<td>0.53</td>
</tr>
<tr>
<td>Substantia Nigra</td>
<td>$0.34 \pm 0.07$</td>
<td>$0.22 \pm 0.08$</td>
<td>34%</td>
<td>0.06†</td>
</tr>
<tr>
<td>Superior Colliculus</td>
<td>$0.23 \pm 0.05$</td>
<td>$0.49 \pm 0.06$</td>
<td>19%</td>
<td>0.23</td>
</tr>
<tr>
<td>Thalamus Midline Dorsal</td>
<td>$0.27 \pm 0.05$</td>
<td>$0.23 \pm 0.06$</td>
<td>15%</td>
<td>0.30</td>
</tr>
<tr>
<td>Thalamus Ventromedial</td>
<td>$0.23 \pm 0.04$</td>
<td>$0.18 \pm 0.04$</td>
<td>20%</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values given as mean±SD  
$^a$Obtained from independent-samples t-tests  
*Significant at $p<0.05$, †Trending toward significance ($p<0.1$)

**Figure 7.2 PET images (SUVs) of risperidone vs saline-treated animals**

A: Risperidone-treated animal. B: Saline-treated animal. PET images are summed (20-60mins) raw values and are overlaid on corresponding CT images
For the reasons mentioned previously we chose not to use a modelling method which required a metabolite-corrected arterial input function and used a reference tissue approach instead. The appropriate reference region to use in TSPO studies is a matter of debate due to the ubiquitous expression of TSPO in the brain. The cerebellum was used as a reference region for the primary analysis based on its consistent use in previous studies and good agreement with BP values obtained from the gold standard arterial input method (510). However, we cannot necessarily rely on the assumption that any neuroinflammation present in the patient cohorts would spare the cerebellum. Therefore we also analysed the PET data using a supervised cluster reference input function (section 4.2.1; p126). To recap, this is a data-driven approach where time activity curves (TACs) of each voxel are analysed and classified into one of 6 tissue classes. The voxels that exhibit kinetic behaviour closest to that of normal grey matter are extracted and used as a reference region.

We consistently found negative $BP_{ND}$ values in temporal and parietal regions, indicating lower TSPO expression in these regions compared to the cerebellum. This was observed in the healthy volunteers as well as the patients, suggesting that higher TSPO expression in the cerebellum is not related to any disease-related process. Partial volume effects may explain this. Grey matter signal in PET is often underestimated due to signal loss to adjacent white matter and CSF. Furthermore, spill-in of signal can also occur from adjacent hot areas (where radioactivity is high) such as blood vessels. This said, previous attempts at partial volume correction in a [$^{11}$C](R)-PK11195 study found that $BP_{ND}$ values were only modestly affected, suggesting that the effect is not big (515). The high resolution of the HRRT also reduces partial volume effects compared to other cameras. The SVC approach is independent of grey/white matter segmentation and anatomical features such as blood vessels. Therefore a SVC reference region may not be as affected by partial volume effects as an anatomically defined reference region.

In this chapter I compare the performance of two reference tissue input functions: a grey matter cerebellum input function and a supervised cluster input function. First I compare the $BP_{ND}$ values obtained from each method according to group. Next I assess the capability of each reference tissue approach in detecting group differences and investigate why outcomes may differ between each input function.
8.1 Reference tissue comparison: healthy volunteers

BP_{ND} values obtained from grey matter cerebellum and supervised cluster (SVC) reference region input functions were plotted against each other for each of the 18 healthy volunteers. Correlations between the two approaches were strong (mean±SD Pearson $r = 0.969±0.05$) and highly significant. However, in 13 of the 18 healthy volunteers BP_{ND} values obtained from the SVC reference region input function were consistently lower than BP_{ND} values obtained from the cerebellum input function. Figure 8.1 illustrates regional BP_{ND} values obtained from in a healthy volunteer in whom there was good agreement between the two input functions. There is near perfect correlation and a slope of 1. The PET images obtained from each input function (A and B) on figure below are almost identical.

Figure 8.2 shows BP_{ND} values from a representative healthy volunteer whose regional BP_{ND} values were lower using a SVC compared to a cerebellum input function. Whilst the correlation between the two approaches was highly significant, the slope was less than 1, indicating an underestimation with the SVC input function. As can be seen from the BP_{ND} maps there is visually less $[^{11}\text{C}](\text{R})$-PK11195 binding using the SVC reference input function (B) than using the cerebellum (A). The TAC for the SVC input function was higher than that of the cerebellum between 8 and 25 minutes of acquisition, which may explain this discrepancy.
MDD & schizophrenia patients

BP$_{ND}$ values obtained from cerebellum and SVC reference input functions were also highly correlated in MDD patients (mean±SD Pearson $r=0.976±0.03$). However, 9 out of the 14 MDD patients displayed consistently lower BP$_{ND}$ values using the SVC compared to the cerebellum input function. Similarly, in schizophrenia patients, cerebellum and SVC-obtained BP$_{ND}$ values were highly correlated (mean±SD Pearson $r=0.975±0.03$) and 11 of the 16 patients displayed lower BP$_{ND}$ values when using a SVC compared to cerebellum reference input function.

8.3 Detecting group differences

As presented in the results for the MDD cohort in chapter five, using the cerebellum as a reference input function resulted in a main effect of group on [$^{11}$C](R)-PK11195 binding ($F_{1,8}=10.24$, $p=0.002$). This group difference was also detected using a SVC reference input function ($F_{1,8}=7.28$, $p=0.008$). Figure 8.3 displays dot plots of regional BP$_{ND}$ values obtained from a cerebellum reference input function (A) and regional BP$_{ND}$ values obtained from a supervised cluster reference input function (B). As can be seen higher [$^{11}$C](R)-PK11195 binding remains apparent in the MDD group when using SVC across most regions, consistent with the findings obtained from using the cerebellum as reference region. However, as illustrated by the error bars, the variance is increased when using SVC compared to cerebellum, with greater a greater spread of data points in both patients and controls.
In the schizophrenia cohort, however, whilst a group difference was detected using cerebellum as reference region \((F_{1,8}=11.36, p=0.001)\), this was not the case using a SVC reference region \((F_{1,8}=1.40, p=0.24)\). This seems to be due to a large increase in variance in between-subject \(BP_{ND}\) values in the SVC method in both patients and controls. See figure 8.4 for dot plots of regional \(BP_{ND}\) values obtained from (A) a cerebellum reference input function (B) a supervised cluster reference input function. As can be seen, the spread of data points is larger using the SVC method, perhaps explaining its inability to detect group differences. Note
that the SVC BP\textsubscript{ND} values are also consistently lower than the cerebellum values but display the same pattern of a greater mean value for ACC in patients.

![Graph A](image)

![Graph B](image)

Figure 8.4 Comparison of BP\textsubscript{ND} values obtained from both reference approaches in schizophrenia patients

### 8.4 Cerebellum BP\textsubscript{ND} values across groups

It is not possible to assess BP\textsubscript{ND} values in the cerebellum using the cerebellum as a reference input function. Therefore, to assess differences in cerebellar binding in patients vs controls, a supervised cluster input function was used. Figure 8.5 displays BP\textsubscript{ND} values in the cerebellum.
of MDD patients compared to controls, indicating no mean difference. Likewise, figure 8.6 demonstrates no difference in mean cerebellar binding in schizophrenia patients compared to controls. This is important as it demonstrates that the differences in ROI BP\textsubscript{ND} values across groups do not reflect differences in TSPO expression in the reference region and supports the use of the cerebellum as an anatomically defined reference region in this study. The increased variability in both the patient groups is widely observed across PET studies.

![Figure 8.5 No mean difference in cerebellum BP\textsubscript{ND} between MDD patients and healthy volunteers](image1)

![Figure 8.6 No mean difference in cerebellum BP\textsubscript{ND} between schizophrenia patients and healthy volunteers](image2)

To summarise, using the supervised cluster reference region approach resulted in a larger amount of negative BP\textsubscript{ND} values, increased variance and lower BP\textsubscript{ND} values. Using the SVC approach, the cerebellum binding was found to be the same across patients and healthy volunteers. Taking all this in to account, the cerebellum is preferable as a reference region.
I now address the research questions posed at the beginning of the thesis and interpret the findings in relation to the existing literature. I discuss the relevance of the findings to theory as well as to practice and consider implications for treatment strategies. Finally I highlight gaps which the research fills but also point out limitations of the study and gaps that still remain. Each research question is addressed and explored in turn. I begin with interpreting the findings from chapter five and discuss the presence of neuroinflammation in MDD. Next I discuss the results of chapter six and evaluate the presence of neuroinflammation in schizophrenia in relation to antipsychotic medication. I then consider the preclinical findings presented in chapter seven and discuss the effects of risperidone on microglia in rats, before assessing the most appropriate reference region approach based on the findings from chapter eight. Finally, I tie together all of the findings and conclude with how this thesis has provided new insights into the pathophysiology underlying MDD and schizophrenia, and how these insights could contribute to the development of novel, more effective treatment strategies.

9.1 Is there evidence of neuroinflammation in MDD?

The results presented in chapter five demonstrate some evidence for neuroinflammation, as indexed by increased TSPO expression, in MDD. A 26% increase in TSPO expression trending towards significance suggests that neuroinflammation is present in MDD and that with a larger sample size and greater power, the increase would reach statistical significance at the \( p<0.05 \) level. Our findings point to the presence of neuroinflammation in a cohort of antidepressant-free, non-smoking and medically healthy patients in a MDE of moderate-to-severe severity. The increase in TSPO expression seen in these patients compared to age- and gender-matched healthy volunteers was global, evident across all cortical regions (figure 5.1). The greatest increase though, which reached statistical significance following exploratory, secondary analysis, was in the anterior cingulate cortex (ACC). These findings are highly consistent with the recent findings of Setiawan et al., which is the only other PET study to date that has investigated TSPO expression in moderate-to-severe MDD specifically (524). Setiawan et al., using the second-generation TSPO tracer \(^{18}\text{F}\)FEPPA, also found an overall increase in TSPO expression. They also recruited a cohort of antidepressant-free, non-smoking medically healthy patients in a MDE of moderate-to-severe severity, and also used the HRRT PET camera. They found significant increases in patient TSPO expression in the prefrontal cortex, insula and ACC. Although we did not find significant differences in PFC or insula, we did find a highly significant difference in the ACC. Indeed the magnitudes of TSPO elevation in ACC were similar in Setiawan et al.’s study and ours (32% and 37%,...
respectively). That we have replicated this finding of increased TSPO expression in the ACC suggests that inflammation, or specifically, microglial activation, could play a role in the pathophysiology of MDD. Indeed the ACC plays a crucial role in regulation of cognitive and emotional processing. Functional imaging studies demonstrate a dorsal cognitive subdivision and a ventral-rostral affective subdivision of the ACC, with the cognitive division being activated by Stroop-like divided attention tasks and the emotion division being activated by tasks with emotional content (525). The ACC has strong connectivity with core emotion-processing regions such as the amygdala and hypothalamus. Lesions of ACC produce symptoms such as apathy, inattention and emotional instability, highlighting its role in the experience of emotion (525). Furthermore, as mentioned in chapter three, the ACC is a key region thought to be involved in MDD, with evidence for decreased grey matter volume (526) and abnormal activation patterns (527)(91). Therefore, our finding of increased microglial activation in the ACC is consistent with the existing literature that implicates its involvement in the pathophysiology of MDD. Whether inflammation in the ACC is related to the aetiology of the disorder or is merely a consequence of another pathological process is not clear. Importantly though, the subgenual ACC (sACC) is a target for DBS in treatment-resistant depression (106), providing some evidence for its aetiological involvement. Further support for the link between inflammation, the ACC and depressed mood comes from an fMRI study which found that healthy volunteers injected with typhoid but not placebo experienced an increase in peripheral inflammation and a deterioration in mood which correlated with enhanced activity in the sACC (528).

Whilst we reproduced the finding of increased microglial activation in the ACC in MDD, we did not find statistically significant increases in the PFC or insula. We did, however, find 20% and 17% increases in these regions, respectively. These findings are at least consistent with an underlying effect in these areas, that with a larger sample size might have reached significance. On the other hand, Setiawan et al. found a significant positive correlation between scores on the HAM-D and ACC TSPO expression which we were unable to replicate. In fact, in the parietal cortex and DLPFC we found negative correlations between HAM-D scores and TSPO expression, though these did not survive correction for multiple comparisons. That we failed to detect a correlation between inflammation and symptom severity might seem surprising based on the literature that suggests that inflammation has a causal role in the development of symptoms, and on studies citing an association between peripheral inflammation and symptom severity (136)(137). However, equally, many of the studies investigating peripheral inflammation in MDD have failed to detect correlations with symptom severity despite finding group differences. There might not be a direct proportionality between symptoms and TSPO expression. Microglia behave transiently – fluctuating between resting and activated states. The level of activation may not be directly proportional to the manifestation of symptoms so whilst inflammation may be causal in the
development of depression, it does not necessarily follow that more inflammation results in a worsening of symptoms. Indeed measuring symptom severity as a whole may not be the best approach in elucidating the role of inflammation in MDD. It could be that inflammation is related to certain symptoms or symptom profiles. Given the wide variation in symptoms across patients with MDD this is difficult to assess but warrants further research in a large sample where patients can be stratified into subgroups based on their symptom profiles. Along these lines, it could be that inflammation is apparent only in a subset of people with depression. Even if depression of an inflammatory aetiology produced symptoms similar to other aetiologies, there could still be distinct routes to generating the final syndrome. In any of these cases, a lack of correlation between inflammation and symptom severity would not be surprising.

As mentioned in chapter three, the first PET study investigating microglial activation in MDD found no differences in TSPO levels in patients compared to controls (248). That ours and Setiawan et al.’s study have since found evidence for increased TSPO expression in MDD patients suggests that their negative finding reflects methodological or severity differences. Hannestad et al. used the second-generation TSPO tracer [11C]PBR28, whose quantification is problematic. The very high affinity of the second-generation tracers disproportionally increases the signal from the TSPO on the BBB compared with the signal from the tissue (249), meaning that binding of tracer to TSPO in the endothelium obscures the binding of the tracer to the TSPO in the tissue. In other words, using a high affinity tracer could mean that the true extent of microglial activation is masked by tracer binding in the endothelium, limiting the ability to detect group differences. Several approaches to deal with the problem of the binding of second-generation TSPO tracers have since been proposed (529). Furthermore, patients in their study were exhibiting mild-to-moderate symptoms so one possibility is that any inflammation present in milder forms of depression may not be detectable with PET. Furthermore, a number of their patients were taking antidepressants at the time of scanning whereas the patients in our study were antidepressant-free for at least 8 months prior to scanning. As described previously (section 2.2.11), antidepressants are thought to have immunomodulatory properties.

Therefore, in terms of hypotheses, I can confirm that our findings support the primary hypothesis, that neuroinflammation is increased in major depressive disorder. The secondary hypothesis, that the extent of [11C](R)-PK11195 binding is correlated with symptom severity, however, is not supported by the findings of this study.

### 9.1.1 Subgroup analysis

Major Depressive Disorder is not a homogenous entity. Whilst the core symptoms common to everyone suffering from depression are persistent low mood and/or anhedonia, the rest of
the symptoms are highly variable between individuals. Whilst one patient can have psychomotor retardation, weight gain and hypersomnia, another patient could have the complete opposite; psychomotor agitation, weight loss and insomnia. It is possible that these dramatic differences in syndrome reflect different aetiological processes. Inflammation could be related to a certain symptomatology rather than an entire diagnostic category. If this were the case a dimensional approach to symptoms potentially allows these distinctions to be teased out. The caveat here is that our understanding of the complexity of the links between aetiology and symptoms is poor. Different pathological processes may therefore combine in unpredictable ways to drive differences in syndromes. Nonetheless, trying to differentiate processes and symptoms like this has potential to yield important insights.

A larger sample would be required for a stratification of patients into subtypes based on symptom clusters. But it was possible to investigate clinical subtypes based on whether patients were experiencing suicidal thoughts or not and on whether they had previously taken antidepressants or were antidepressant-naïve. I first discuss the findings relating to suicidality. The nine patients who were experiencing current thoughts of suicide exhibited greater levels of microglial activation than the five patients who were free of suicidal thoughts (figure 5.5). This difference remained significant after controlling for severity, suggesting that this is not simply a severity effect. Of these nine patients, five had previously attempted suicide. Several studies have investigated inflammatory markers in post-mortem brains of depressed patients who had committed suicide. Steiner et al. found elevated microglial density in depressed suicide victims (530). Tonelli et al. found elevated cytokine expression in the orbitofrontal cortex of suicide victims, though these findings were not restricted to those with MDD (531). Signs of inflammation in post-mortem brains of suicide victims could be reflective of pre-suicidal stress. Alternatively there could be a causal link between microglial activation and suicidal behaviour. Activated microglia release an array of neuroendocrine factors, cytokines and free radicals that can modulate neurotransmission which might in turn trigger suicidality. Other studies have looked at inflammatory markers in those that have attempted suicide. Lindqvist et al. found significantly higher levels of CSF IL-6 in suicide attempters compared to healthy controls (532). They did not find raised levels of IL-6 in the periphery and concluded that the elevated IL-6 in CSF originated from microglia and astrocytes in the CNS. Another study found significantly higher plasma levels of kynurenine (produced from the cytokine-stimulated conversion of tryptophan described in section 2.26) in a subgroup of suicide attempters MDD compared to non-suicide-attempters (533), suggesting the involvement of inflammatory processes in suicidality specifically. Furthermore, another study investigating post-mortem brains of individuals with severe depression who had committed suicide found an increased density of microglia positive for quinolinic acid (QA), the NMDA agonist associated with oxidative stress (534). Interestingly, the greatest increase in density was in the ACC, consistent with our in-vivo findings. Suicide
has also been found to be associated with hyperactivity of the HPA axis, as evidenced by a longitudinal study which found an increased suicide risk in patients that exhibited non-suppression of cortisol following the dexamethasone test (535).

Our findings of increased microglial activation in MDD patients with current thoughts of suicide are consistent with these findings. No evidence of increased peripheral inflammation was found in the suicidal thoughts subgroup, though small numbers and high variability will have limited the ability to detect a group difference. Whether microglial activation is a consequence of stress or causally related to suicidal thoughts/behaviour needs further investigation. If suicidality does reflect a specific biological subtype, patients experiencing suicidal thoughts might benefit from interventions that reduce inflammation. Indeed a heightened inflammatory state may be a risk factor in the development of suicidal thoughts. If the inflammation is specific to the brain though, a peripheral biomarker for suicidality may not exist.

In a separate within-patient comparison I compared levels of inflammation in patients who had previously taken antidepressants (n=7) and antidepressant-naïve patients (n=7). Patients who had been on antidepressants in the past had significantly higher levels of TSPO expression than patients who had never taken antidepressants before (figure 5.7). There was a marked increase across all regions in the previous antidepressant subgroup, with ACC, PCC, insula, temporal cortex, OFC and VLPFC reaching significance post-hoc. The main reason that the patients had stopped taking antidepressants was lack of efficacy. It is possible that the increased microglial activation seen in these patients reflects treatment resistance. Indeed the patients that had previously been on two or more antidepressants in the past exhibited the highest levels of $[^{11}C](R)$-PK11195 binding. A limitation to this line of theorising based on the current findings, however, is that we have no way of telling whether the antidepressant-naïve subgroup would be responsive or resistant to antidepressant treatment. But it is still possible to speculate that a subgroup of MDD patients with a specific biological underpinning involving heightened inflammation exists. Such a subgroup may be less responsive to antidepressants that target monoamine transmission. Rather, these patients might respond favourably to anti-inflammatory treatments. This would be consistent with Raison et al.’s RCT of the anti-inflammatory (TNFα antagonist) Infliximab in treatment-resistant depression (240). Although they found no overall difference in symptom severity between treatment groups over a 12 week period, they found a significant improvement in those patients with high levels of baseline CRP. Therefore, those patients with high levels of inflammation may represent a subgroup who will respond to anti-inflammatory drugs or alternative interventions that target inflammation. Inflammation may therefore provide a biomarker that informs us which treatment would provide the best response, giving way to a personalised treatment approach. A recent meta-analysis investigated biological differences between responders and non-responders based on inflammatory profiles (536). It found treatment
resistance to be associated with persistently elevated TNFα, supporting Raison et al.’s study and the possibility of a heightened inflammatory state contributing to treatment resistance. Although there is no way of confirming that the increased microglial activation seen in the previous antidepressant subgroup compared to the antidepressant-naïve group reflects treatment resistance, I tentatively suggest this as a possibility. Our findings partially give support to the existence of a subgroup of MDD patients with a specific biological underpinning involving heightened inflammation who may not respond to conventional antidepressants.

9.1.2 Peripheral inflammation

We found no correlations between any of the peripheral markers of inflammation and [11C](R)-PK11195 binding. Our secondary hypothesis that there would be a correlation between peripheral and central inflammation, therefore, was not supported. An association between peripheral and central inflammation might be expected based on our knowledge of cross-talk between the peripheral immune system and the CNS (see chapter 1), on the sickness behaviour research indicating an association between peripheral and central inflammation (155) and on the literature surrounding cytokine-induced stimulation of the TRYCAT pathway and subsequent neuroinflammatory effects (209). However, neither of the previous PET studies have found an association between peripheral and central inflammation either. As mentioned in chapter one the historical assumption was that the brain was an immune-privileged organ due to the presence of the BBB, but we now know that systemic inflammatory molecules can enter the brain. Indeed a recent PET study using [11C]PBR28 has shown for the first time in humans that a systemic immune challenge (LPS) induces a robust increase in microglial activation (152). However they did not find a direct correlation between TSPO expression and peripheral markers of inflammation either. Therefore although there is clearly a relationship between peripheral and central inflammation, there does not seem to be a direct coupling possibly accounting for the lack of correlations seen in ours and the previous PET studies. One possible explanation for the lack of association between peripheral and PET measures of inflammation is that peripheral markers of inflammation are highly variable. They fluctuate widely due to their sensitivity to numerous variables including time of day, exercise, dietary intake, stress and amount of sleep. Such fluctuations are therefore likely to mask the true association between peripheral and central inflammation. Another possibility is that inflammation in MDD originates in the brain and is independent of inflammation in the periphery. It could be that microglia become activated independently of infiltrating cytokines from the periphery, possibly in response to stress. In support of this, rats subjected to chronic stress expressed increased activated microglia (537). Another preclinical study found an association between psychological stress, microglial activation in the PFC and impaired working memory. The effect of stress on
microglia and working memory was reversed following treatment with minocycline (538). Therefore it seems plausible that stress, which often precedes depression, is directly associated with microglial activation and that an inflammatory response can begin in the CNS. The causal role of microglia, however, remains to be established. Is microglial activation a cause of neuronal dysfunction and behavioural changes associated with depression? Or is it a consequence of stress or an independent pathological process? That minocycline, a potent inhibitor of microglia, has been shown to have antidepressant effects in preclinical models and in preliminary clinical studies, suggests a causal role for microglia in the pathogenesis of depression (539). However, more concrete clinical evidence and a greater understanding of the mechanisms of minocycline is needed.

The implication of a lack of association between peripheral and central inflammation is that if microglial activation is confirmed as being a biomarker for MDD, or for a subset of patients with MDD, then it might not be possible to simply perform a blood test to identify these patients. In an ideal world, there would be a strong correlation between peripheral and central inflammatory biomarkers so that the presence of microglial activation could be elucidated from markers in the blood. But without such an association, PET may be the only way of identifying these patients at present. Due to the expensive and technical nature of PET this may not be feasible at present. However, the growth of PET-MR means that PET scanning is likely to become more accessible. Coupled with the advent of $^{18}$F-labelled TSPO tracers which overcomes the need for a cyclotron on-site, using PET as a diagnostic tool to image neuroinflammation in MDD could be on the horizon.

A limitation of this study is that not we did not have peripheral inflammatory marker data for all of the healthy volunteers, as the majority of them were taken from an existing dataset. Therefore, there was not sufficient power to detect differences in peripheral inflammatory markers between patients and healthy volunteers. Despite small numbers, we did detect a highly significant reduction in levels of BDNF in MDD patients compared to healthy volunteers. A reduction in BDNF is one of the most consistent findings in MDD, confirmed in a number of meta-analyses (215). BDNF is a crucial mediator of neurogenesis and neuronal plasticity. A consistent finding is that stress can halt hippocampal neurogenesis, which is thought to reflect the reduction in hippocampal volume seen in MDD (100). Inflammation reduces the expression of BDNF in the brain. It is therefore thought that the decreased neurogenesis associated with stress and inflammation exists in parallel to changes in BDNF expression and reduced neuronal plasticity. A causal role for BDNF in the pathophysiology of depression is supported by preclinical and clinical evidence that antidepressants stimulate BDNF expression (540). As described in chapter three, it has been hypothesised that a pro-inflammatory state in MDD results in microglial activation and the subsequent release of neurotoxic substances such as reactive oxygen species (ROS). Neurotoxic substances are thought to be in excess of neurotrophic factors in MDD, resulting in a loss of plasticity,
oxidative stress and excitotoxicity (30) (209). That we have observed increased microglial activation and decreased BDNF expression is consistent with this theory. Furthermore, it points to the activated microglia we have imaged having a pro-inflammatory phenotype, as neuroprotective ('M2') microglia produce BDNF (541). Therefore if we were imaging a neuroprotective phenotype, we would perhaps not expect to find reduced levels of BDNF.

9.1.3 Cognitive functioning

MDD is often associated with deficits in cognition function (see (542) for a recent review). The sickness behaviour literature and clinical evidence from patients undergoing immunotherapy strongly implicate inflammation as being able to induce cognitive impairment (27)(543). Therefore we carried out a preliminary investigation into the relationship between cognitive functioning and neuroinflammation using PET for the first time. Cognitive data were only available for seven of the healthy volunteers, limiting the validity of any comparison in performance on cognitive tasks with patients. However the results of the emotional recognition task leaned towards a bias towards negative emotions (sadness, anger and fear) in the depressed patients, consistent with a wealth of research illustrating an attentional bias toward negative emotional cues in depression (544). There were no correlations with performance on this task or measures of peripheral or central inflammation, suggesting that negative bias, in this emotional facial recognition task at least, is not associated with inflammation. Numerous functional imaging studies have implicated abnormal activity in prefrontal regions in MDD, particularly the DLPFC, during memory tasks (545). Although many brain regions have been implicated in executive function, three main subdivisions are generally thought to play key roles: the DLPFC, VLPFC and ACC (546). Problems in other domains may arise that rely heavily on aspects of executive functioning and prefrontal function such as memory, attention and problem solving. Our results point to impairments in recall and recognition memory in MDD, consistent with previous research (547). We found significant negative correlations between recall memory and inflammation in prefrontal regions (figures 5.13). Our results are consistent with the wealth of preclinical and clinical research indicating a relationship between inflammation and cognitive deficits. Furthermore, that the correlations were apparent in prefrontal regions is consistent with the established role of the PFC in regulating cognitive processes and with the abnormal activation patterns found in PFC in MDD. We found no significant correlations with cognitive functioning and microglial activation in the ACC. However, the fact that we found greatest signal increase in this region deserves highlighting due to its role in cognitive and emotional processing. Our preliminary investigation into cognitive function in this study may not have been able to tap into an association with an inflammatory process in the ACC.

Indeed one limitation of this study is that we were not able to administer a more comprehensive assessment of cognitive function. Ideally we would have fully assessed
executive function, sustained attention, memory and emotional processing bias in all patients and healthy volunteers. This was not possible due to time constraints of the screening session and because the majority of healthy volunteers were taken from an existing dataset. A large study assessing the relationship between inflammation and cognitive functioning in MDD is necessary to investigate this fully. If the relationship between inflammation and cognitive impairments were confirmed in MDD, there would be important treatment implications. It is thought that cognitive impairment is an important mediator of functional impairment and thus affects the ability of individuals with MDD to work. Clearly then, cognitive deficits are likely to contribute significantly to the burden of MDD to society as a whole. This has led to the development of antidepressants such as vortioxetine, with claims that they target the cognitive as well as mood symptoms in MDD (548). If it were confirmed that the cognitive deficits were related to peripheral/neuroinflammation, then interventions targeting inflammation might specifically improve cognitive and psychosocial functioning. Our study is the first to our knowledge to assess the relationship between microglial activation and cognitive function in MDD using PET, and the first to provide preliminary evidence of an association between microglial activation and memory function in MDD.

9.1.4 Demographic & questionnaire data

As mentioned in the introductory chapters, many variables can have effects on inflammatory processes, including age, BMI, medical illness, antidepressant use, alcohol use, dietary intake, smoking, drug use, amount of exercise, stress, sleep, time of day and season. We tried to control for, or at least assess, as many of these variables as possible. We found no significant correlations between [11C](R)-PK11195 binding and BMI, age, alcohol intake or sleep quality. Furthermore, the patients and healthy volunteers did not differ on any of these measures. None of the patients had taken antidepressants for at least 8 months. None of them smoked, abused drugs or had any signs or symptoms of medical illness. We found no differences in [11C](R)-PK11195 binding in participants who were scanned in the morning or the afternoon. Furthermore, there was no effect of season on [11C](R)-PK11195 binding. This means that we can be fairly confident that the increased [11C](R)-PK11195 binding we have seen in our patient cohort is related to the pathophysiology of depression itself rather than to any of the mentioned confounding factors which are known to affect inflammation.

Almost all patients experienced some form of childhood adversity, with half of patients reporting a substantial number of adverse experiences during childhood. This is consistent with one of the most replicated findings in the field: the link between early life stress and the later development of depression (549). There is strong evidence suggesting that inflammation is a mediator of this link (144) (see section 2.2.10). Based on this, an association between childhood adversity and microglial activation was expected. However we found no correlation between [11C](R)-PK11195 binding and scores on the Childhood
Adversity Questionnaire (CAQ). It could be that our sample size was too small to detect a correlation with overall scores. We had insufficient power to look at specific types of early of early life stress (such as emotional or physical abuse). Alternatively the CAQ may have been too limited a measure to tap into any association with inflammation. For example, the CAQ scale only measures adverse experiences perpetrated by parents. Furthermore, other significant stressors such as death of a close family member are not assessed. There are also likely to be moderating processes such as chronology of abuse; the length of abuse in childhood as well as the presence of adult victimisation and other exacerbating or ameliorating environmental variables. A more detailed assessment of early life stress would have therefore been preferable. The Childhood Trauma Questionnaire (550), for example, is a more comprehensive assessment of early life stress. It would have also been desirable to quantify more recent and current levels of stress to fully assess the relationship between stress, microglial activation and depression. Such a study would provide valuable insight into whether stress is associated with microglia specifically, as preclinical models have found. Taking this further, assessing whether microglial activation is a state or a trait marker would provide further insight to the aetiological role of stress and inflammation in depression.

We found a significant correlation between duration of illness and $[^{11}\text{C}](\text{R})\text{-PK11195}$ binding in the PCC. However this was driven by the patient who had a much longer duration of illness than the rest of the patients. Still, that the patient with the longest duration of illness displayed the highest amount of microglial activation deserves comment. It has been theorised that a maladaptive response to chronic stress in MDD leads to inflammatory response that can culminate in neurodegeneration (209). According to the inflammatory & neurodegenerative hypothesis of depression, excessive inflammation, glucocorticoid resistance and microglial activation leads to oxidative stress and excitotoxicity. Such a process would be chronic and would perhaps only manifest after many years. It would make sense that the longer an individual has been depressed, the more exposure they’ve had to stress and the greater the perpetuation of the inflammatory response. Further research is needed to determine which patients might exhibit such an out-of-control inflammatory response and why? One theory is that some individuals have a vulnerability to the physiological effects of stress. Indeed people with depression exhibit an exaggerated stress-induced inflammatory response (221). It is thought that both genetic and environmental factors affect an individual’s vulnerability to stress. As described in (section 2.2.10), numerous studies have linked early life stress with a sensitisation of the central stress response system and long-term disturbances of the HPA axis in depressed patients (226). Interestingly, the patient (patient 7) with the longest duration of illness and the highest levels of $[^{11}\text{C}](\text{R})\text{-PK11195}$ binding also experienced a high level of childhood adversity, with the second highest score on the CAQ.
We found a negative correlation between $[^{11}C](R)$-PK11195 binding in the ACC and amount of exercise, implying that the less exercise a patient did, the more microglial activation was present. The health benefits of exercise are well known. But is now believed that the effects of exercise on inflammation contributes to these health benefits, with mounting evidence suggesting that physical activity reduces inflammation (551). Our finding is consistent with this picture, with lower levels of neuroinflammation found in the patients who did more exercise. As far as is known this is the first study to show a relationship between amount of exercise and neuroinflammation in humans *in-vivo*. It is well known that exercise has therapeutic and preventive effects in depression (120). Exercise has also been found to improve symptoms of depression in neurodegenerative diseases where inflammation plays a key role, such as Alzheimer's Disease (552) and in patients with a chronic illness (553). Indeed an abundance of animal research has demonstrated that exercise has neuroprotective effects: it enhances plasticity, neurogenesis and resilience to injury, largely through stimulating growth factors (BDNF, IGF-1 and VEGF) (554). Given this research, a negative correlation between amount of exercise and symptom severity might be expected. Although no correlations reached significance at the $p<0.05$ level, we found negative correlations between exercise and scores on the MADRS and scores on the PHQ-9 which reached trend significance. This suggests that those patients who exercised more had lower levels of neuroinflammation and less severe symptoms of depression. Conversely, those who exercised less had higher levels of neuroinflammation and more severe symptoms of depression. Our findings therefore provide support for the mounting preclinical and clinical evidence demonstrating the neuroprotective and antidepressant effects of exercise in depression. Another explanation that exists in parallel to this is that the patients who exercise less could lead a sedentary lifestyle, possibly alongside unhealthy eating, which could result in a metabolic syndrome that would potentiate an inflammatory response. Therefore the correlation we observe between inflammation and exercise could reflect both the positive anti-inflammatory effects of exercise and the distinct pro-inflammatory effects of pervasive physical inactivity. By analogy, there is evidence that moderate exercise and a generally sedentary lifestyle have independent effects on longevity (555). This highlights the potential of exercise interventions and initiatives that encourage a healthier lifestyle in reducing inflammation and improving symptoms of depression.

### 9.1.5 Theoretical considerations

I have interpreted my results in light of the previous research and now give consideration to how these findings fit in with the theoretical framework of depression. The general consensus regarding the cause of depression is that it arises from a complex interaction between genetic and environmental factors. It is also now accepted that such a complex and heterogeneous disorder is unlikely to have a sole pathophysiology. As such multiple theories
of depression exist, discussed in chapter three. My findings are compatible with many of the existing theories, which I will briefly run through.

The monoamine hypothesis has remained the dominant framework for the treatment of depression for sixty years. Despite the numerous limitations of current antidepressants it would be difficult to deny the association between monoamines and depression. Over half a decade of preclinical and clinical research has confirmed that serotonin is associated with mood disturbances. However, whether reduced serotonergic transmission is the primary aetiological source of depression, and whether this is the primary pathology in all types of depression is less clear. Nonetheless, our findings of increased microglial activation in MDD are compatible with the monoamine hypothesis. Increased microglial activation is suggestive of a pro-inflammatory state. As previously described, inflammatory cytokines stimulate the enzyme IDO to break down TRP into kynurenine KYN and then QA, thus reducing the availability of TRP for serotonin (191). Therefore a pro-inflammatory state is consistent with a reduction in serotonin. But reductions in serotonergic functioning might be secondary to inflammatory processes, which may explain the limited efficacy of antidepressants in some patients. Indeed serotonin abnormalities may be part of the pathophysiology of depression as opposed to the aetiology. More and larger RCTs of anti-inflammatories are needed to assess this. It would be also interesting, using PET, to investigate the association between neuroinflammation and serotonin function in the same patients.

Our findings are also compatible with the wealth of research implicating glucocorticoid resistance and hyperactivity of the HPA axis in MDD (see section 2.2.10). A hyperactive HPA axis is thought to be secondary to impaired glucocorticoid receptor functioning following chronic exposure to inflammatory cytokines during stress or medical illness (197). Activated microglia might then be expected in patients with an overactive HPA axis and a potentially unregulated inflammatory response. But whether microglia become activated as a result of HPA axis hyperactivity or whether microglia have a causal role in HPA functioning is not clear. Alternatively the HPA axis and microglia could be functioning in parallel to create an abnormal reaction to stress, which together might be the driving force of depression. Indeed an abundance of research suggests that inflammation mediates the well-established link between stress and depression. As highlighted earlier though, most people experience stress of some sort but not everyone develops depression. Genetic and environmental factors are thought to create an at-risk state where subsequent stressors result in an exaggerated inflammatory response. Microglia may well become primed before the onset of depression. Indeed animal studies have shown that high levels of glucocorticoids produce a persisting sensitisation of microglia so that they will generate a potentiated pro-inflammatory response to subsequent immune stimuli or stressors (556). The authors speculated that this priming effect acts as a warning signal which sensitises the immune response to potential danger. There is clearly a link between stress, inflammation and depression, however the exact role...
that microglia play in this has not been investigated in humans yet. This is discussed further in future work in chapter ten.

Our findings are also in line with the cognitive theory of depression, which holds that depression arises from abnormal cognitive processing, namely negative thoughts surrounding the self, the world and the future (the cognitive triad) (111). An abundance of behavioural research has demonstrated a bias towards negative and aversive stimuli in people with depression (557). Accordingly it is thought that people with depression are more likely to perceive external stressors as posing a threat (89). This has been linked to an overly sensitised stress response system and hence the involvement of inflammation. A sensitised stress response system and inflammation could therefore contribute to the negative bias seen in depression.

Our findings are also consistent with the neuroimaging studies which have implicated abnormal activation patterns in the ACC (527)(102). Structural imaging studies indicate reduced grey matter volumes in a number of structures including the amygdala, hippocampus and ACC (see section 2.1.6). The presence of microglial activation might reflect a neurodegenerative process as proposed by the inflammatory and neurodegenerative hypothesis (209). Further analysis of the data in this study will explore whether microglial activation is associated with any structural changes, discussed in chapter ten. That our findings are compatible with many of the major theories and most replicated findings in MDD indicates that this research fits in comfortably with the existing knowledge base.

9.1.6 Conclusion: MDD

To conclude, I have replicated the recent PET finding of neuroinflammation in a cohort of antidepressant-free, non-smoking, medically healthy patients in a MDE of moderate-to-severe severity. A strength of our study is that patients were antidepressant-free for at least 8 months, compared to 6 weeks in the Setiawan et al. study (250), minimising the confound of antidepressant medication on our PET results. We have also investigated the association between neuroinflammation and alcohol use, childhood adversity, sleep quality, amount of exercise, suicidal thoughts and previous antidepressant use for the first time. Specifically, we found an association between microglial activation and suicidal thoughts, previous antidepressant use, reduced expression of BDNF and recall memory. Therefore, I have reproduced the first research evidence for neuroinflammation in moderate-to-severe MDD and made a further contribution to the existing knowledge base.

As discussed, the findings are consistent with the various lines of evidence implicating the involvement of inflammation in depression. There are still a number of unanswered questions though. Perhaps the most pressing question is the direction of causality. Causality cannot be inferred from correlational studies like this. However, based on the mounting
evidence suggesting that inflammation is causal in the development of depression, hypothesising that neuroinflammation has an aetiological role in depression is justified. Evidence for an aetiological role for inflammation in MDD comes from the sickness behaviour and immunotherapy literature and the potential of anti-inflammatory agents in reducing symptoms of depression, demonstrated both preclinically and clinically. Another unanswered question is which microglial phenotype is being measured with PET? This question cannot be answered at present. But based on the research indicating a causal role for stress in inducing a pro-inflammatory microglial phenotype and the preclinical and clinical effects of minocycline, which specifically suppresses a pro-inflammatory phenotype, I hypothesise that we are imaging a pro-inflammatory phenotype. Furthermore, reduced levels of BDNF are associated with a pro-inflammatory microglial phenotype. Another question that still needs addressing is whether microglial activation in MDD is a state or a trait marker? All patients were in a moderate-to-severe MDE at the time of scanning. It would be interesting to know whether microglia remain activated in periods of remission.

A final question, partially addressed by this research, is whether inflammation is involved in all types of depression? As mentioned previously, it is unlikely that there is one causal factor to such a heterogenous disorder. Rather it is more feasible that each person's depression is caused by a distinct and complex interaction between genes and environment. Due to such heterogeneity in the manifestation, course and symptom profiles across patients, it may not be possible to pinpoint the exact aetiology of depression as a whole. Breaking the disorder down into subtypes or symptom dimensions may be a more fruitful way of investigating and elucidating the mechanisms of depression. We found increased microglial activation in patients who were experiencing suicidal thoughts and in patients who had previously been on antidepressants. Raised inflammation could therefore act as a biomarker for these subsets of patients, which would have important implications for treatment. The findings from this and Setiawan et al.'s study point to the existence of a subset of patients who exhibit a heightened inflammatory state. Whether these patients would respond to interventions targeting inflammation needs further investigation, but Raison et al.'s preliminary findings are promising (240). The crucial questions then, are whether inflammation can be used as a reliable biomarker for stratifying patients into treatment groups and whether MDD can be separated into distinct clusters of aetiology and symptoms based in part by inflammatory markers.
Is there evidence of neuroinflammation in schizophrenia?

We found a 27% increase in global TSPO expression, indicative of microglial activation, in schizophrenia patients compared to age and gender-matched healthy volunteers (figure 6.1), pointing to evidence of neuroinflammation in schizophrenia. Using one-tailed statistical tests, this group difference reached trend significance. It is likely that a lack of power meant that this group difference did not reach significance at the p<0.05 level. Our primary hypothesis that $[^{11}C](R)$-PK11195 would be increased in schizophrenia patients compared to healthy volunteers is therefore partially supported. We found significant correlations between $[^{11}C](R)$-PK11195 binding and negative symptoms (figure 6.5). However we found no correlations between PANSS total, positive or general scores. Our secondary hypothesis that $[^{11}C](R)$-PK11195 binding would be correlated with symptom severity is therefore partially supported. Our findings of group differences are consistent with two of the previous four PET studies investigating neuroinflammation and schizophrenia. Methodological differences may go some way to explain the diversity of findings in these studies. Some of our sensitivity analyses showed that factors such as age, antipsychotic use and stage of illness were important predictors of the extent of microglial activation.

The first PET study, by Van Berckel and colleagues in Amsterdam, scanned ten patients with recent-onset schizophrenia, also using $[^{11}C](R)$-PK11195 and the HRRT (470). They found a significant increase in total grey matter BP $_p$ values in schizophrenia patients compared to controls. Similarly we found a significant increase in $[^{11}C](R)$-PK11195 binding in cortical regions, though the increase we detected was of a larger magnitude (25% increase compared to their 14%). The authors found no significant correlations between $[^{11}C](R)$-PK11195 binding and symptom severity. Although the authors did not publish patient PANSS scores, they state that patients were only moderately symptomatic. The difference in magnitude could therefore be explained by clinically different patient populations. Furthermore, their patients were in the first five years of onset, whereas we recruited patients with a range of duration of illness. As mentioned previously, a limitation to Van Berckel et al.'s study is that all patients were on antipsychotics at the time of scanning. Our findings in relation to antipsychotic use are discussed shortly.

The second PET study, by Doorduin and colleagues in Groningen, was a small investigation in seven acutely psychotic patients, also using $[^{11}C](R)$-PK11195 but on a full body clinical PET camera with lower resolution than the HRRT (467). They found significantly higher binding in the hippocampus and a non-significant 30% increase in grey matter. Patients were symptomatic and clinically similar to the patients in this study. However, all patients were on an antipsychotic at the time of scanning. They did not find any correlations between $[^{11}C](R)$-PK11195 and symptom severity either. We did not directly assess BP $_{ND}$ values in the hippocampus due to its propensity for negative BP $_{ND}$ values, partial volume effects and poor
delination using the Hammers brain atlas. However, we did find a grey matter increase of a similar magnitude (25%). The increase in their study may not have reached significance due to such small numbers (i.e. Type II error due to lack of power). Our results are therefore partially consistent with this PET study also.

The third PET study, from Takano and colleagues in Chiba, Japan, scanned fourteen patients with schizophrenia, this time using the second-generation TSPO tracer $[^{11}C]$DAA1106 on a clinical PET scanner (468). They found no significant differences in $[^{11}C]$DAA1106 $BP_{ND}$ values between patients and controls. Interestingly they found significant correlations between $[^{11}C]$DAA1106 binding and scores on the positive subscale of the PANSS. However, the findings of this study need to be interpreted with caution. Importantly, this study was published two years before the discovery of the nucleotide polymorphism in the TSPO gene (rs6971) which affects the binding affinity of the second-generation TSPO tracers (50). Accordingly, they did not genotype their patients or controls and so there is no way of knowing the distribution of non-binders, mixed-affinity binders, or high affinity binders across groups.

The fourth and most recent PET study, from Kenk and colleagues in Toronto, used another second-generation TSPO tracer, $[^{18}F]$FEPPA, to scan sixteen patients with schizophrenia on the HRRT (469). All patients and controls were genotyped and classified as high-, medium- or low-affinity $[^{18}F]$FEPPA binders. They found no group difference between patients and controls when controlling for genetics and no correlations between $[^{18}F]$FEPPA binding and PANSS scores. Differences in radioligand and in patient populations could explain their lack of group difference which contradicts the findings of the first two PET studies and ours. In terms of radioligand, $[^{18}F]$FEPPA is a second-generation TSPO ligand. As mentioned previously, the quantification of the second-generation TSPO tracers is problematic due to excessive binding to TSPO in the vasculature masking the signal from activated microglia in brain tissue. However, $[^{18}F]$FEPPA was used in the MDD study described previously and was able to detect a significant group difference (250). Therefore perhaps a more likely explanation for the discrepancy in findings is that their patient population was slightly different from ours. Firstly, their patients had lower PANSS scores than ours (a mean of 70, compared to our 85). Secondly, their patients were older than ours (mean age 43 compared to 33) with most in a chronic stage of illness. Finally, as with all of the previous PET studies, all of the patients were taking antipsychotics (2 on typical and 14 on atypical; the authors do not specify types).

Our findings are therefore partially consistent with the previous PET research assessing neuroinflammation in schizophrenia in-vivo. We have confirmed the first two reports of neuroinflammation in schizophrenia. Our findings are inconsistent with the most recent PET study, but this is likely to be reflected by important methodological differences (radiotracer...
and patient population). To answer the question of whether neuroinflammation is present in schizophrenia, I conclude that it is but that antipsychotic medication might have an effect on this association, as discussed below.

### 9.2.1 Antipsychotics & neuroinflammation

A limitation common to the previous PET studies is that all patients were taking antipsychotics, which are thought to have immunomodulatory properties. We attempted to address this by recruiting patients who were antipsychotic-free and patients who were taking the antipsychotic risperidone. Of note, six of the eight antipsychotic-free patients were in fact antipsychotic-naïve. A direct comparison between the two patient groups was not feasible as they were not matched for age, gender or duration of illness. Therefore we compared each subgroup to their own age and gender-matched healthy volunteers. A limitation to this subgroup analysis, however, is that a sample size of 8 may not have been sufficiently powered to detect a significant group difference.

Comparing antipsychotic-free patients to healthy volunteers, we found no significant group difference (figure 6.2). That this negative finding may be due to a lack of power is supported by the fact that we did find a 30% mean increase in antipsychotic-free patients compared to healthy volunteers. Whilst the antipsychotic-free subgroup were not all first-episode psychosis (FEP) patients, they were all within the first six years of onset, and distinguishable from chronic patients. A recent meta-analysis demonstrated that first episode psychosis is associated with increased peripheral inflammation, specifically raised levels of IL-6, TNFα and IL-1β (403). In line with this, we would have expected to see an increase in microglial activation in our sample of largely drug-naïve recent-onset patients. There are a possible number of explanations for this difference: i) our sample was underpowered; ii) our sample was unrepresentative of those with inflammation. Given that we demonstrated an effect in the ACC of comparable size to the medicated group and to the existing literature, I hypothesise that our sample was underpowered. This is the likely explanation for failing to find an increase in peripheral inflammation too.

In contrast, when comparing patients on risperidone (or paliperidone) and healthy volunteers, we found a 48% increase in mean TSPO expression, with mean increases in \( \text{BP}_{\text{ND}} \) across all regions in the medicated patients (figure 6.3). Exploratory t-tests showed that the increase was statistically significant in the DLPFC of the medicated patients. Increases reached trend significance in the VLPFC, OFC and ACC. This suggests that those patients on antipsychotics may be driving the main group difference. However these patients had a more chronic course and a longer duration of illness than the antipsychotic free patients who were all scanned within the first six years of onset. So whether increased microglial activation is reflective of antipsychotic medication or a chronic course is not clear and needs discussion.
First I discuss how these findings fit in with the existing literature surrounding antipsychotics and inflammation.

As described in the literature review, studies investigating the effect of antipsychotics on inflammation have given mixed results, showing an increase, a decrease or unchanged levels following antipsychotic treatment (see section 3.2.7). Furthermore, the effects seem to differ across antipsychotics (558). Also, antipsychotics are known to induce metabolic changes and weight gain, which in turn lead to an increase in inflammation. Accordingly antipsychotics have been found to have differential effects on metabolic liabilities as measured by changes in peripheral inflammation (559). Song et al. observed an initial decrease in IL-6 and IL-1β following risperidone treatment, but then an increase alongside steady weight gain (560). The increased neuroinflammation we have observed in the antipsychotic-treated patients could therefore be related to antipsychotic-induced metabolic changes and subsequent increases in systemic inflammation. Indeed a preclinical PET study has shown that obesity induces neuroinflammation in rats (561). A medication-mediated systemic inflammatory response may induce microglial activation and a central inflammatory response. The BMI of the medicated patients was higher, but not significantly higher than the unmedicated patients and healthy volunteers. There was no correlation between BMI and peripheral or central measures of inflammation, however the metabolic and associated inflammatory changes can occur without excessive weight gain.

Clearly then, the effects of antipsychotics on inflammation are not clear and are likely to be affected by the propensity of antipsychotics to induce weight gain and metabolic dysregulation. As mentioned in section 3.2.7, numerous in-vitro studies have shown that antipsychotics have anti-inflammatory properties. Indeed risperidone specifically has been shown to suppress microglial activation in in-vitro models (562). Based on this we would expect a decrease in microglial activation in the patients on risperidone. However, it is well known that the extrapolation of in-vitro findings to the clinic is not straightforward.

In contrast to the research suggesting that antipsychotics have anti-inflammatory properties, another line of evidence suggests that antipsychotics can in fact have damaging effects on the brain. For example, first-generation antipsychotics have been found to have neurotoxic effects, inducing neuronal loss and gliosis (563). A primate study found that chronic exposure to both first and second generation antipsychotics was associated with glial proliferation in the prefrontal cortex (564). Multiple human studies have found either progressive loss of grey matter (565)(566) or decreased grey matter/enlarged ventricles compared to controls (327) (319). It is thought that this reduction in grey matter reflects the disease process of schizophrenia. However, most if not all patients recruited to these studies had been on some form of antipsychotic medication. Thus it is unclear whether this reduction in grey matter reflects the disease process itself or is related to the antipsychotic medication. For example, a
systematic review (567) found that 14 out of 26 longitudinal studies showed a decline in brain volume or an increase in ventricular volume during the course of antipsychotic treatment. Furthermore, a larger cumulative dosage of antipsychotic medication has been related to progressive decreases in frontal lobe volume (568). Therefore it could be that chronic antipsychotic use can have neurotoxic properties, in which case microglia would be expected to be activated.

To complement the clinical study we carried out a small preclinical study investigating the effect of risperidone on microglia in-vivo, using PET and the second-generation TSPO tracer $[^{18}F]$DPA-714. We found significantly higher $[^{18}F]$DPA-714 SUV values in risperidone-treated animals compared to saline-treated animals (figure 7.1), indicating a possible upregulation of TSPO and microglial activation in the risperidone animals. As far as is known this is the first study to investigate the effects of antipsychotic on microglia in-vivo using PET. Interestingly we found small but significant global upregulation of TSPO expression, evident across all regions. There are two possible explanations for this. The first is that there is increased microglial activation in the risperidone-treated animals, which would support our clinical findings where we also found an upregulation of TSPO across ROIs. However we cannot rule out the possibility that risperidone is altering the free fraction of radiotracer in blood. By altering the chemical milieu, risperidone may be affecting the amount of protein-bound radioligand in plasma, which would in turn affect the amount of free tracer delivered to the brain. Without kinetic modelling (which is problematic in preclinical TSPO studies due to a lack of reference region), we cannot be certain that the SUVs reflect radioligand specifically bound to TSPO. The preclinical arm of the study is ongoing. We plan to measure the free fraction of radiotracer in the blood of risperidone vs placebo-treated animals to explore whether our findings are a true reflection of increased microglial activation. We also plan to carry out immunohistochemistry and autoradiography on the brain sections of the scanned animals to determine whether our PET findings are confirmed in-vitro.

It is difficult to place our findings into context of the previous research due to a lack of previous in-vivo research. Previous preclinical studies have focussed on the effects of antipsychotics on LPS-induced microglial activation in culture, with the majority of work indicating that antipsychotics either directly suppress microglial activation (449) or attenuate LPS-induced neurotoxicity (569). Our findings are therefore in contradiction with the in-vitro work. However, extrapolating of in-vitro to in-vivo models is problematic. In-vitro models are unable to account for the true biological complexity of a living animal. Clearly more in-vivo preclinical and clinical research is needed to further explore the effect of antipsychotics on microglia. Limitations of the preclinical study include a small sample and the use of a different radiotracer from our clinical study. Furthermore, animals were only treated for three weeks and the correspondence of the dose we chose to the clinical dosing may not be accurate. Future work needs to investigate chronic antipsychotic treatment to
reflect the nature of antipsychotic treatment in the clinic and explore the effect of different doses. Furthermore in this pilot study we were not able to carry out behavioural testing on the animals to assess side effects. Still, our preliminary findings suggest that risperidone might be associated with upregulated TSPO and microglial activation, though more work is needed to determine whether differences in free fraction of radiotracer has mediated the observed group difference.

A strength of our study is that all our medicated patients were on one type of antipsychotic, eliminating the confound of differential effects of different antipsychotics on microglia. Furthermore, we only recruited those who were on a long-acting or ‘depot’ injection of risperidone or paliperidone, overcoming the common problem of medication incompliance. However, this could also present a potential confound. There might be something specific about the patients on a depot injection meaning that they might not be representative of schizophrenia as a whole. One likely reason for these patients being on a depot injection is that they were non-compliant with their medication. Related to this, they might have had less insight into their illness and the need for medication.

Based on the previous literature we hypothesised that that the medicated patients and the risperidone-treated animals would exhibit decreased microglial activation. Our results were not supportive of this. The existing research indicates that the relationship between antipsychotics is far from clear. Our results are consistent with findings from some of the previous studies but not with others. It seems clear that more research is needed to assess the effect of antipsychotics on microglia in-vivo. If our finding that microglial activation is associated with antipsychotic use is confirmed, this would have important implications in terms of treatment but also in the interpretation of the previous PET studies. Another possibility though, is that our finding of increased microglial activation in the medicated patients but not in the unmedicated patients is due to other clinical differences between the two samples. The medicated patients experienced more negative symptoms than the unmedicated patients. Increased inflammation might be related to the more severe negative symptoms rather than antipsychotic use. Saying this, antipsychotics may lead to an increase in negative symptoms, for example due to sedative side effects. Another difference between the two patient samples that could explain increased microglial activation in the medicated patients is duration of illness. The medicated patients had a mean duration of illness of 15 years, compared to just 4 years in the unmedicated patients. It could be that a central inflammatory process worsens with time. This said, we did not find a correlation between microglial activation and duration of illness. The majority of the medicated patients had chronic schizophrenia so whilst peripheral inflammation may be apparent at onset, inflammation in the CNS may only manifest later on in the course of the illness. But duration of illness is often a proxy for something else, such as changes in pathophysiology over time, chronic antipsychotic treatment, cumulative effect of continuous symptoms and effects of
repeated relapses. Therefore studying these aspects directly may be more fruitful in elucidating the true role of inflammation in schizophrenia. Another possibility is that the inflammation seen in the medicated patients is related to some form of treatment resistance, as these patients were still unwell, with moderate-to-severe symptom severity despite being on a LAI of risperidone or paliperidone. It could be that conventional antipsychotics are not targeting the primary pathology in these patients. Perhaps an anti-inflammatory agent would then treat these patients more effectively.

9.2.2 Negative symptoms & neuroinflammation

To the best of my knowledge, we have demonstrated a direct association between neuroinflammation and the negative symptoms of schizophrenia for the first time (figure 6.5). This is consistent with the minocycline trials which have shown significant improvements in the negative symptoms specifically (462–466). Although these trials assessed minocycline as an add-on to antipsychotic treatment, they are highly suggestive that minocycline is able to specifically target and alleviate the negative symptoms of schizophrenia. Moreover, they are consistent with the inability of conventional antipsychotics to modify negative symptoms substantially, as they do not target this pathology. Taking the results of these trials together with our finding of correlations between microglial activation and scores on the PANSS negative scale across multiple brain regions and with the knowledge that minocycline potently inhibits activated microglia, it is highly conceivable that microglial activation plays a causal role in the negative symptoms of schizophrenia. Furthermore, it has recently been shown that minocycline selectively inhibits the M1 polarisation (the pro-inflammatory phenotype) of microglia (570), suggesting that the microglia we have imaged have a pro-inflammatory phenotype.

The correlation between negative symptoms and extent of microglial activation across multiple brain regions remained significant after controlling for medication status, indicating that the correlation is not being driven by the medicated patients. However, the medicated patients did exhibit high scores on the negative subscale of the PANSS. Whether these are true negative symptoms or ‘pseudo’ symptoms associated with antipsychotic medication is not clear. For example the sedative and extrapyramidal effects of antipsychotics could contribute to a lack of motivation, social withdrawal and a flat affect. One possibility is that there is an interaction between negative symptoms, antipsychotic medication and inflammation. If the negative symptoms are being caused by the medication, and if the medication is associated with inflammation, as our findings suggest, then inflammation might mediate the link between antipsychotics and negative symptoms.

An association between inflammation and the negative symptoms of schizophrenia also fits in with the sickness behaviour and immunotherapy literature. Negative symptoms of
schizophrenia include lack of motivation, inability to feel pleasure, lack of interest and social withdrawal. Most people have experienced such symptoms during illness. From an abundance of animal and clinical research, we know that these symptoms are induced by pro-inflammatory cytokines infiltrating the brain and affecting our behaviour. A link between inflammation and the negative symptoms of schizophrenia would have important treatment implications. As described in section 3.1.8, antipsychotics are largely ineffective in treating the cognitive and negative symptoms of schizophrenia, despite emerging evidence that it is actually these symptom domains that are the most important predictors of quality of life (352). Treatments that specifically target these up until now largely ignored symptom domains are therefore vital. Our findings, along with the research cited above, suggest that inflammation is a promising target for treatments that specifically target the negative, and possibly cognitive, symptoms of schizophrenia. The obvious candidate is minocycline, but other anti-inflammatories and non-pharmacological interventions are worth investigating too. Non-pharmacological interventions that can reduce inflammation include exercise and encouraging a healthier lifestyle. Indeed a Cochrane review assessing the effects of exercise in three RCTs found significant improvements in negative symptoms of mental state and PANSS negative scores (571), highlighting the potential of exercise in alleviating the negative symptoms of schizophrenia.

9.2.3 Peripheral inflammation

We found no correlations between central and peripheral measures of inflammation. This is consistent with the previous four PET studies, none of which found associations between microglial activation and peripheral markers of inflammation either. As described in the MDD section of the discussion, the lack of correlation could reflect that the microglial activation we have observed in schizophrenia is independent of systemic inflammation. Or, and perhaps more plausibly, the variation in peripheral markers of inflammation is so high and affected by so many factors that correlations are difficult to detect. This said, we did detect significant positive correlations between IL-6 and total PANSS scores, and between IFNγ and PANSS total, positive and general scales (figure 6.8). Interestingly there were no correlations with PANSS negative scores, which might be expected given the correlations with inflammation in the CNS. It is possible that there is a distinction between peripheral and central inflammation and the symptom domains affected by each, but this would need to be investigated further in a larger sample. Still, that we found a correlation between symptom severity and inflammatory markers IL-6 and IFNγ suggests that peripheral inflammation might reflect a state marker for schizophrenia. It must be noted, however, that the interassay variance in our peripheral inflammatory marker data was high, limiting the validity of any conclusions that can be drawn from this data.
As mentioned previously, a lack of control data for peripheral inflammation means that a comparison between patients and healthy volunteers is not statistically viable. High variance is a likely explanation for a lack of difference in many of the peripheral inflammatory markers between patients and healthy volunteers, which was expected given the literature indicating consistent increases in peripheral inflammation in schizophrenia (section 3.2.4). However, even with such small numbers we did find a significant reduction in BDNF levels in the schizophrenia patients (figure 6.7). This supports the consistent finding of reduced blood BDNF levels in both drug-naïve and medicated patients with schizophrenia, as demonstrated by meta-analysis (572). Reduced BDNF is consistent with neurodevelopmental models of schizophrenia, which implicate a role for reduced BDNF in underlying impairments in synaptic connectivity. Some studies suggest increases in BDNF levels following antipsychotic treatment (573) but conflicting evidence indicating no change has also been reported (574). We found no difference in BDNF levels between medicated and unmedicated patients. Reduced BDNF has also been reported in the DLPFC of post-mortem tissue of patients with schizophrenia (575). As mentioned earlier, reduced levels of BDNF are associated with a heightened inflammatory state, consistent with our overall finding of increased microglial activation in schizophrenia. BDNF, as mentioned previously, plays a crucial role in neuronal plasticity and neurogenesis and so could be associated with the structural changes seen in schizophrenia (see section 3.1.7). One possibility is that stress and inflammation reduce BDNF expression, which together can result in neurodegeneration/decreased neurogenesis. Consistent with this, one study demonstrated that a history of childhood trauma and high levels of recent stress predicted lower BDNF expression through an inflammation-mediated pathway in FEP patients. Furthermore they found that reduced BDNF and increased IL-6 and cortisol were associated with a smaller hippocampal volume (576). Indeed an abundance of research links stress to the onset of psychosis/schizophrenia (section 3.2.6). Whilst we did not find a direct association between childhood adversity and inflammation, we did find that the patients experienced high levels of childhood adversity, consistent with the link between early life stress and schizophrenia. A limitation of our study is that we did not assess recent life stress, which would have given us more insight into whether microglial activation might be a state or trait marker in schizophrenia.

**9.2.4 Cognitive functioning**

Although not all our findings from the cognitive tasks reached significance, the direction of findings are consistent with the abundance of evidence of general neurocognitive deficits from the earliest stages of schizophrenia with particular evidence of executive, memory and social task performance being impaired (577). In terms of the emotional facial recognition task, our findings are suggestive of poorer performance in the patients, who had a lower hit rate and a higher false alarm rate than healthy volunteers across all emotions (figure 6.9).
This is consistent with robust findings of impaired facial emotional perception in patients with schizophrenia, as demonstrated by meta-analysis previous research indicating impaired emotional facial recognition in schizophrenia (578). Interestingly the largest group difference (which reached trend significance) was an increase in false alarms for sad faces in the patients, reflecting an increased tendency to misattribute other emotions as being sad. This suggests that a negative bias may exist in schizophrenia. There were no correlations between performance on the emotional recognition task and central inflammation. However, there were negative correlations between levels of IL-1β and hit rates for happy, angry and fearful faces (figure 6.10), suggesting a possible relationship between inflammation and emotional recognition.

The patients recalled significantly fewer words than the controls, suggestive of an impairment in recall memory (figure 6.11). This is consistent with a meta-analysis of memory performance in schizophrenia indicating large effect size for impairments in recall memory (579). There were, however, no correlations between recall memory performance and measures of peripheral or central inflammation. On the recognition memory task, patients had a slightly lower total hit rate than healthy volunteers and higher false alarm rates (figure 6.12). Although not significant, these results point to a possible impairment in recognition memory in our patients, which has been demonstrated in schizophrenia consistently (579).

We found significant correlations between false alarm rates for negatively valenced words and [11C](R)-PK11195 binding in the VLPFC and ACC (figure 6.13), suggesting a possible relationship between a memory bias towards negatively valenced and microglial activation in these regions. This is largely consistent with fMRI findings of emotion recognition impairment associated with dysfunctions in the affective division of the ACC and dorsomedial prefrontal cortex (580). The ACC has also been implicated in an impaired ability to infer the feelings of others in schizophrenia (581). Abnormalities in the ACC might therefore be associated with impaired social cognition in schizophrenia.

9.2.5 Demographic & questionnaire data

As mentioned in the MDD section above, we tried to control for as many of the variables that can affect inflammation as possible. However, because such a large proportion of schizophrenia patients smoke (582) and because schizophrenia is associated with an unhealthy lifestyle (583) and antipsychotic-induced weight gain (342), we were not able to match for smoking status or BMI. As mentioned in the literature review, both smoking and a high BMI can induce inflammation, presenting a possible confound to our results. However, we found no significant differences in peripheral or central inflammation between smokers and non-smokers. Furthermore we found no correlation between BMI and peripheral inflammatory markers or [11C](R)-PK11195 binding, suggesting that our group differences are not being driven by these factors. None of the patients or healthy volunteers had abused
drugs or alcohol within the previous year and none had any signs or symptoms of medical illness. Four of the patients were taking an antidepressant at the time of scanning. As mentioned antidepressants are thought to have immunomodulatory properties, thus introducing a potential confound. However, the majority of the evidence suggests that antidepressants either have no effect on cytokine levels or they suppress cytokine levels (126), meaning that if anything antidepressants would inhibit microglial activation and minimise a group difference. We found no differences in $[^{11}C](R)$-PK11195 binding in participants who were scanned in the morning or the afternoon. Furthermore, there was no effect of season on $[^{11}C](R)$-PK11195 binding. We found no significant correlations between $[^{11}C](R)$-PK11195 and age, alcohol intake or sleep quality. This means that we can be fairly confident that the microglial activation seen in the schizophrenia patients is related either to the pathophysiology of schizophrenia or to antipsychotic medication.

All patients exhibited some form of childhood adversity, with almost half exhibiting high levels of adverse experiences in childhood. This is consistent with the wealth of literature demonstrating that childhood adversity is a risk factor for schizophrenia (584). We did not however find a link between childhood adversity and inflammation, which was expected given the previous research (see section 3.2.6). One reason for this could be limitations of the Childhood Adversity Questionnaire, which is a self-report questionnaire and does not provide an in-depth assessment of early life stress, discussed earlier. An association between stress and schizophrenia is one of the most robust and replicated findings in the field. We did not assess recent or current levels of stress due to time constraints of the screening session, but future work should investigate the relationship between stress and microglial activation in schizophrenia in more depth.

We found significant negative correlations between amount of exercise and $[^{11}C](R)$-PK11195 binding across multiple ROIs (ACC, PCC, DLPFC and VLPFC) (figure 6.6). Interestingly we found the same negative correlation with exercise and neuroinflammation in the MDD cohort. Finding the effect in both cohorts lends further support to the mounting evidence suggesting that the anti-inflammatory effects of exercise contribute to its physical and mental health benefits. We also found a negative correlation between amount of exercise and BMI, which makes sense given that exercise results in weight loss and physical inactivity results in weight gain. Perhaps most interestingly, we also found a highly significant negative correlation between amount of exercise and severity of negative symptoms (figure 6.6). A wealth of anecdotal, preclinical and clinical evidence demonstrates without a doubt that exercise promotes physical and mental well-being. Our findings are highly consistent with this, given that we have shown that the patients in our study who did the least amount of exercise had the highest expression of microglial activation and the most severe negative symptoms. Furthermore, they support the findings from the Cochrane review of exercise interventions in schizophrenia, which showed significant improvements specifically in the
negative symptoms (571). We demonstrate a link between exercise, neuroinflammation and negative symptoms of schizophrenia for the first time, a finding that is highly consistent with the existing research. The implications of this are obvious: exercise can be used as an effective means of reducing inflammation and improving the often highly debilitating negative symptoms of schizophrenia. Exercise is a very accessible means of improving quality of life in those with schizophrenia. It would also have important effects on physical health which is often poor in people with schizophrenia and is linked to antipsychotic medication. It could therefore also contribute to minimising the huge discrepancy in life expectancy between people with schizophrenia and the general population.

9.2.6 Theoretical considerations

After interpreting the main findings of the thesis I now consider their theoretical relevance. Although the relationship between antipsychotics and microglial activation needs further investigation, we have demonstrated an overall overexpression of activated microglia in schizophrenia, suggesting that neuroinflammation may be involved in its pathophysiology. This finding is compatible with much of the existing knowledge of the aetiology and pathophysiology of schizophrenia. Whilst its exact aetiology remains largely unknown, it is becoming clear that a complex interaction between genetics and environment underlie an increased vulnerability to schizophrenia. As described in the literature review, the strongest evidence for the involvement of specific genes comes from three large GWAS studies which have implicated the MHC region in schizophrenia (408)(410)(411). The MHC region encodes hundreds of genes that control immune function. That the MHC finding remains the most significant and consistent across GWAS studies in schizophrenia (413) provides compelling evidence for the involvement of immune function in the aetiology of schizophrenia. It is generally accepted that schizophrenia arises from a complex interaction between genes and environment. It could be that we do not all have an equal response to environmental risk factors such as stress and infection. Rather, a genetic predisposition to a heightened inflammatory response might underlie the disorder. In this case, we might expect to find evidence of neuroinflammation at symptom onset and possibly in the prodromal phase. We did not find significantly elevated microglial activation in a small group of antipsychotic-free patients who were in the early stages of the disorder, though there was a non-significant 30% increase in TSPO expression in the ACC. The lack of power makes it impossible to rule out such inflammatory activity. Alternatively it could be that neuroinflammation only becomes apparent at later stages of the disorder. But further support for the aetiological role of inflammation in the development of schizophrenia comes from the fact that so many of the established risk factors for schizophrenia are associated with inflammation (see figure 9.1).
On top of this is evidence of increased peripheral inflammation, the potential of anti-inflammatory drugs in alleviating symptoms and, of course, the previous PET studies. Our finding of an overall increase in microglial activation in schizophrenia is also compatible with the existing theories of schizophrenia described in the literature review (chapter three). Activated microglia can release excessive glutamate, possibly contributing to the glutamate abnormalities seen in schizophrenia (310). Microglial-induced excitotoxicity and oxidative stress might also contribute to the structural abnormalities apparent in schizophrenia (see section 3.1.7). Furthermore, microglia and a heightened inflammatory state are thought to contribute to the white matter abnormalities seen in schizophrenia and the resultant functional disconnectivity (585). Excessive glutamate signalling has also been hypothesised to overstimulate dopamine neurons (312). Dopamine hyperactivity is clearly associated with the positive symptoms of schizophrenia and this could be the final common pathway for psychosis (288). However it could be secondary to glutamate abnormalities which could in turn be induced by inflammatory processes. Inflammation and glutamate abnormalities may therefore represent more upstream pathologies. Whilst dopamine may be the final common pathway for psychosis, inflammation may underlie the negative and possibly cognitive symptoms of schizophrenia. This would also account for the fact that despite the overwhelming evidence suggesting a link between inflammation and schizophrenia, heightened inflammation is only apparent in a subset of patients. It could be that inflammation reflects a certain symptomatology, with our findings pointing to an association between inflammation and the negative symptoms of schizophrenia. Our research poses a number of questions which are yet to be addressed, such as the effect of antipsychotics on
inflammation. However, we have demonstrated an overall increase of microglial activation in schizophrenia, consistent with previous PET research and compatible with existing theories.

9.2.7 Conclusion: schizophrenia

To conclude, our findings point to the presence of increased microglial activation in schizophrenia, consistent with two of the previous $^{11}$C(R)-PK1195 PET studies. What sets our study apart from the previous research is that our patient sample included patients who were antipsychotic-free and patients who were on just one type of antipsychotic drug (risperidone/paliperidone). We investigated the presence of neuroinflammation in a group of antipsychotic-free, largely antipsychotic-naïve patients for the first time, and found no significant difference with age and gender-matched healthy volunteers. However we did find a 30% increase in ACC of the patient group, which might have reached significance with more power. These patients were at an early stage of the disorder and given the literature that demonstrates a heightened inflammatory state at onset, and the literature suggesting a causal role for inflammation, neuroinflammation would be expected. One possibility is that a systemic inflammatory response is apparent at the onset and possibly in the prodromal phase, and that only later does neuroinflammation manifest itself.

In contrast, the medicated patients, who were in a later stage of the disorder, exhibited a highly significant increase in microglial activation compared to age and gender matched healthy volunteers. Whether this increase reflects the fact that they were on antipsychotic medication, the longer duration of illness or a certain symptomatology is not clear. It could be that the neuroinflammation we have imaged reflects an interaction between all these factors. The effects of antipsychotic medication might be more pronounced in chronic patients who have taken them for many years. Furthermore, the medication could be worsening certain symptoms (negative and cognitive). Further research into the interaction between antipsychotics, duration of illness, symptoms and microglial activation is needed. Unanswered questions still remain regarding microglial phenotype, direction of causality, whether microglia act as state or trait markers, heterogeneity and the existence of a subgroup with heightened inflammation. But continued research in the emerging field of immunopsychiatry should result in a greater understanding of the role of inflammation in schizophrenia.

In conclusion, we have investigated neuroinflammation in antipsychotic-free patients for the first time and shown a possible relationship between antipsychotics and microglial activation. We have also demonstrated a relationship between microglial activation and the negative symptoms of schizophrenia for the first time, and a link between exercise, neuroinflammation and negative symptoms. Although there are still many unanswered questions, we have provided additional and novel knowledge to the field, which could have
real-world implications for treating and improving the quality of life of those with schizophrenia.

9.3 Reference tissue comparison

As described previously, the choice of reference region in TSPO studies where an arterial input function is not available is a matter of debate. We compared the $B_{ND}$ values obtained from the most common anatomically defined reference region, the cerebellum, and from the data-driven supervised cluster (SVC) method (513). $B_{ND}$ values obtained from the two reference tissue input functions were highly correlated in all healthy volunteers ($n=18$), all MDD patients ($n=14$) and all schizophrenia patients ($n=16$). However, in 13 of the healthy volunteers, 9 of the MDD patients and 11 of the schizophrenia patients (in over half of all participants), $B_{ND}$ values obtained from the supervised cluster reference tissue input were systematically smaller than those obtained from the cerebellar reference tissue input, with many of the ROIs displaying negative $B_{ND}$ values. In these participants, the TAC from the SVC reference input was higher than that of the cerebellum TAC for a longer duration of time (see figures 8.1 and 8.2 for plots of regional $B_{ND}$ values obtained from each input function with corresponding TACs and $B_{ND}$ maps in two representative healthy volunteers). Therefore it is possible that in some instances the voxels classed as ‘normal grey matter’ are contaminated by high signal from connective tissue outside the brain or from venous sinuses. See figure 4.13 (p126) for an example of spill-in of signal from sinuses and connective tissue outside the brain.

Looking at group comparisons using each reference input function, we found a significant group difference between MDD patients and healthy volunteers using both cerebellum and SVC reference input functions (figure 8.3). However, the variance was increased in both healthy volunteers and MDD patients when using the SVC input function. In terms of the schizophrenia cohort, whilst we found a significant group difference using the cerebellum as a reference input function, we were unable to detect a group difference using the SVC input function (figure 8.4). As can be seen from figure 8.4-B, the variance was greatly increased particularly in the healthy volunteers but also in the patients when using the SVC input function. It is clear that the increase in variance with the SVC input function has rendered the group difference between patient and healthy volunteer $B_{ND}$ values non-significant.

A likely explanation for this increase in variance with the SVC input function is that the standard SVC method clusters voxels into six kinetic classes (normal grey matter, white matter, pathologic TSPO binding, blood pool, muscle and skull). The six-tissue SVC method therefore includes signal from outside the brain, from soft tissue and bone where TSPO signal is high. It is possible then, that the SVC TACs were contaminated by spill-over from outside the brain, resulting in increased variance across subjects as well as an underestimation of
BP$_{ND}$ in the target regions. An alternative approach has been developed in an attempt to address the variability from the six-class SVC method. Applying a brain mask to the dynamic $[^{11}C](R)$-PK11195 image prior to performing supervised cluster analysis strips out the soft tissue and bone, resulting in four tissues and removing signal from outside the brain (586). In a comparison with the gold-standard two tissue compartment model with a metabolite-corrected arterial input function, the four-tissue SVC input function provided the best agreement as well as the best contrast between subject groups (in comparison to cerebellum and six-tissue SVC input functions) (586). However, this method has not been validated for use with the HRRT.

Nonetheless, our choice of cerebellum as reference region seems justified given its ability to detect group differences in both our patient cohorts. The concern with using the cerebellum as a reference region in case-control studies is that it might be affected by the disease process. In other words the cerebellums of the patient groups might include higher levels of specific binding than the healthy volunteers, resulting in an underestimation of BP$_{ND}$ values in the target regions. This would increase the likelihood of false negatives and mean that we may be missing pathology in the target regions. However, our results suggest that this has not been the case. We have detected group differences in both patient cohorts using a cerebellar input function, suggesting that the cerebellum specific binding is not increased in the cerebellum of the patient cohorts. Furthermore, cerebellum BP$_{ND}$ values obtained from the SVC input function indicate no mean difference between $[^{11}C](R)$-PK11195 patients and healthy volunteers in either cohort (figures 8.5 & 8.6). We have demonstrated that the cerebellum input function is more sensitive in detecting group differences than the SVC input function, which greatly increases variability and produces a higher number of negative BP$_{ND}$ values. We confirm that cerebellum should be the reference region of choice in $[^{11}C](R)$-PK11195 studies on the HRRT, consistent with previous studies which came to the same conclusion (514)(518).
I now offer a conclusion to the thesis as a whole and suggest avenues of future work that will further unravel the relationship between inflammation, depression and schizophrenia. The value in studying depression and schizophrenia together is that we can observe overlaps in findings between the two disorders. Indeed we have found a striking resemblance between the extent and regional expression of microglial activation in the two disorders. Figure 6.15 illustrates mean regional BP\textsubscript{ND} values for MDD patients, schizophrenia patients and all healthy volunteers. As can be seen the magnitude of the signal increase is similar in both groups across most regions. Although statistical tests were not carried out between the two patient groups, visually there are two regions that show distinct patterns between patient groups; mean BP\textsubscript{ND} values are higher in the VLPFC in the schizophrenia patients whereas mean BP\textsubscript{ND} values are higher in the insula in the MDD patients. Apart from these exceptions though, the PET findings are remarkably similar across patient groups. The most striking resemblance is that in both patient groups, the greatest increase in microglial activation was in the anterior cingulate (see figure 6.14). Indeed the magnitude of the increase was almost identical across patient groups (37% in MDD and 40% in schizophrenia). The increase in signal in the ACC was also seen in the antipsychotic-free patients, suggesting that this is not solely related to antipsychotic medication. I hypothesise that the neuroinflammation we have imaged, perhaps focal to the ACC, is a shared mechanism reflecting a symptomatic overlap between MDD and schizophrenia. There is some overlap between symptoms of depression and the negative symptoms of schizophrenia, for example anhedonia, apathy, avolition, asociality and attentional impairment. As already discussed we have demonstrated an association between neuroinflammation and the negative symptoms of schizophrenia. Given the overlap in symptomatology and in our PET findings, it is highly plausible that neuroinflammation reflects a shared mechanism.

However, neuroinflammation is not just specific to these two disorders. Indeed there is evidence of neuroinflammation in other psychiatric disorders. A recent PET study, also using $[^{11}\text{C}]$(R)-PK11195, demonstrated increased neuroinflammation in the hippocampus of patients with bipolar disorder (587). A systematic review concluded that inflammation is likely to play a role in the pathophysiology of bipolar disorder (588). The symptoms of depression that characterise bipolar disorder could therefore be reflective of an inflammatory process. This highlights that psychopathology probably lies on a continuum and that distinct pathologies underlying the diagnostic categories may not exist.

As mentioned previously, neuroinflammation is also apparent in neurodegenerative disease, such as Parkinson’s Disease, Alzheimer’s Disease, Multiple Sclerosis, as well as in Stroke, HIV.
and traumatic brain injury. Symptoms of depression are common in all of these conditions. Inflammation might therefore be a shared mechanism common across multiple psychiatric and neurological conditions, contributing to the symptoms of depression that so commonly arise in them. Inflammation could be the driving force behind certain symptoms such as lack of motivation, anhedonia, apathy, social withdrawal and low mood. Our findings also point to a possible link between neuroinflammation and cognitive symptoms. Clearly this needs investigation, but if confirmed, there would be important implications for targeting symptoms of depression across neurological and psychiatric diagnostic categories.

Another finding apparent in both disorders is that neuroinflammation was lower in patients that did more exercise and higher in those that did less. Furthermore we found a correlation between amount of exercise and negative symptoms of schizophrenia. This highlights the potential of exercise to reduce neuroinflammation and possibly alleviate symptoms. This can be extrapolated to other conditions where neuroinflammation is present, mentioned above. For example exercise has been found to have neuroprotective effects in Mild Cognitive Impairment (MCI) and Alzheimer’s Disease (589) and to alleviate symptoms of depression (552). Another overlapping finding is that we found reduced levels of BDNF in both MDD and schizophrenia, which could be reflective of a pro-inflammatory and possible neurodegenerative process in both disorders. Stress is known to reduce the level of BDNF in the brain (590). Stress is also a known risk factor for both disorders. Indeed both disorders are characterised by a hyperactive HPA axis (197)(420). It is therefore plausible that stress induces an overactive immune response in both disorders. Compounded by a reduction in BDNF and microglial activation, this might then lead to a neurodegenerative process and structural changes. Indeed volumetric reductions are seen in both disorders. A consistent finding in both disorders is reduced grey matter in the hippocampus (204) (321), which is particularly sensitive to stress and to glucocorticoids (208).

In conclusion, I suggest that inflammation is not necessarily specific to MDD or schizophrenia, or to any other psychiatric disorder. Rather, inflammation seems to underlie a spectrum of disorders. It is thought that an at-risk mental state can turn into an affective or psychotic syndrome longitudinally. Indeed anxiety and depressive symptoms often mark the onset of the prodromal phase of psychosis (591). Risk factors for depression, psychosis and bipolar disorder are largely overlapping. Childhood adversity for example is a risk factor for all classes of mental disorder (592). Moreover, there is a striking overlap between the genes predisposing individuals to schizophrenia and to bipolar disorder, with the MHC region strongly implicated for both disorders (408). Therefore, it is highly plausible that immunogenetic factors and environmental risk factors give rise to a spectrum of psychiatric disorders. Inflammation may then be a common aetiological factor to quite different syndromes. The question that then arises is at what point do the pathological mechanisms divide, giving rise to the final syndromes of psychiatric disorders?
Consequently, neuroinflammation might not be useful as a diagnostic biomarker due to its commonality across diagnostic categories and resultant lack of specificity. However it seems highly promising as a biomarker for stratifying patients into phenotypes which would inform Clinicians which treatment approach would be most beneficial. This ‘personalised medicine’ approach stratifies patients into subpopulations based on comprehensive genotyping and phenotyping. Using neuroinflammation as a biomarker could be an important part of this approach. It seems as though a move towards a data-driven approach to determine new treatment categories using a combination of genetic, environmental, behavioural and biomarker data is on the horizon (see figure 9.2). Such a tailored approach is highly promising. Attempting to treat patients with one blanket treatment will inevitably fail some people. But matching patients to the treatment which is most likely to work for them based on genetics, biomarkers, environmental factors and symptom profiles could genuinely result in more efficacious treatments for psychiatric disorders such as MDD and schizophrenia.

![Diagram](image)

**Figure 10.1** Data-driven approach to clustering patients for personalised treatment approaches

Whilst the findings of this study have provided novel insight into the role of inflammation in MDD and schizophrenia, more research is needed to further elucidate its involvement. In terms of MDD, I tentatively suggested a link between treatment resistance and increased levels of microglial activation. However without knowing whether the antidepressant-naïve patients would or would not respond to antidepressants we could not fully investigate this. A PET study could be designed to specifically look into this. Ideally, three patient groups would be scanned: treatment-resistant patients, treatment-responsive patients not currently on antidepressants and treatment-responsive patients on antidepressants. This would also provide insight into the effect of antidepressants on microglia/peripheral inflammation. Another study could scan patients at first onset and then longitudinally follow the same patients, scanning them in episodes of remission also. This would help answer the question of
whether neuroinflammation might be a state or trait marker. In such studies it would also be interesting to measure cortisol levels and assess recent stressors to explore the relationship between stress, HPA axis function and peripheral/neuroinflammation. A comprehensive assessment of cognitive functioning, for example using the CANTAB®, would give further insight into the relationship between inflammation and cognitive function.

Activated microglia release glutamate and the NMDA agonist quinolinic acid and so it is thought that microglial activation in MDD might be associated with heightened glutamatergic transmission (192). Using PET to measure microglial activation and MRS to measure glutamate in the same patients would provide novel insight into their relationship. Similarly, we could investigate the relationship between peripheral/neuroinflammation and serotonergic function in the same patients by for example scanning them with $^{11}$C(R)-PK11195 and the serotonin transporter radioligand $^{11}$C-DASB. There have been few RCTs using anti-inflammatory agents in MDD and more are clearly needed. It would be interesting, similar to Raison et al.’s study (593) to stratify patients into those with and without an initial heightened inflammatory state and compare responsiveness. Indeed PET scanning patients pre- and post-treatment would allow us to determine whether anti-inflammatories target a central inflammatory response. Minocycline would be the obvious choice of anti-inflammatory agent in such a study, due to promising preliminary findings but also because it is able to cross the BBB and directly inhibit microglia.

In terms of schizophrenia, future research is clearly needed to explore the effect of antipsychotics on microglia in-vivo. Including this study, three $^{11}$C(R)-PK11195 PET studies have now demonstrated an increase in expression of activated microglia in patients with schizophrenia who have been on antipsychotics. Whether this is related to the disease process or the medication is still unclear. To address this, an ideal study would recruit a sample of first episode patients and scan them with $^{11}$C(R)-PK11195 PET. Half of these patients would then receive antipsychotic medication and half, through choice, would not. Ideally the patients would be followed longitudinally and scanned say each year. This would help untangle the relationship between neuroinflammation, antipsychotic use and the natural progression of the illness. Such a study would, however, be practically difficult to implement, due to the challenges of recruiting and engaging with this patient population. Indeed, perhaps the most challenging part of this PhD was recruiting patients, specifically patients who were not on antipsychotics and often disengaged from services. A slow recruitment rate is a common obstruction to the research process and can significantly hinder progress. Future work could look into ways of facilitating recruitment of patients to research studies and clinical trials, making the research process more efficient and accessible. One way of doing this is to carry out public engagement to make service-users more aware of the benefits of research and what studies are available. A user-friendly website detailing all Trust research studies, the inclusion criteria and how to self-refer could increase recruitment rates.
Another study could investigate the presence of neuroinflammation in patients in a psychotic episode and patients in remission. Ideally this would be done in the same patients in a longitudinal study. This would provide insight into whether microglial activation reflects a state marker. It would be interesting to measure the presence of recent stressors in these patients to assess whether stress is associated with an exacerbation of symptoms/inflammation. Cortisol levels could also be measured in relapse and remission to assess HPA activity. To explore whether microglial activation could reflect a trait marker, individuals in an at-risk mental state (showing prodromal signs of psychosis) could be scanned and then longitudinally followed up. This would inform us whether a heightened peripheral/central inflammatory state predicted a transition to psychosis. Further work could investigate the relationship between inflammation and neurotransmitter functioning. Abnormalities in NDMA receptor and glutamatergic functioning are now thought to play a role in schizophrenia. Given the propensity of activated microglia to release glutamate, it might be expected that neuroinflammation is associated with elevated levels of glutamate. Therefore $^{11}$C(R)-PK11195 PET and MRS could also be used in schizophrenia to explore the relationship between inflammation and glutamate. Taking this further, we could investigate whether reducing inflammation would also reduce glutamate levels. Indeed more RCTs investigating the efficacy of minocycline and other anti-inflammatory agents are needed in schizophrenia. RCTs of these agents on their own, not in conjunction with antipsychotics would be desirable, though in practise this would be challenging.

Non-pharmacological interventions aimed at targeting inflammation could also be trialled in both MDD and schizophrenia. Indeed our findings point to a relationship between exercise and neuroinflammation. Assessing the ability of exercise to reduce symptoms and peripheral/neuroinflammation could provide further support for the beneficial role of exercise in improving mental health. Interventions that encourage a healthier lifestyle could also have an impact on inflammation and symptoms, as well as on medical health. Encouraging a healthier diet and not smoking or drinking excessively would reduce levels of inflammation. Furthermore, targeting stress, a risk factor for both MDD and schizophrenia known to induce inflammation, is also likely to be beneficial. Initiatives educating people on how to manage stress may even reduce the incidence of depression. Whilst the government’s IAPT initiative has made psychological therapy more accessible, waiting lists are still long and more can be done to offer people psychological support. It would be interesting to investigate the role of psychological interventions such as CBT in reducing inflammation, stress levels and symptoms.

The role that inflammation plays in influencing our behaviour and in underlying psychiatric disorders such as MDD and schizophrenia is receiving growing interest and has given rise to the field of immunopsychiatry. It is an exciting research area due to the genuine possibilities that it offers in terms of treating mental disorders more effectively. With continued research,
I am hopeful that there will be a move away from attempting to treat all patients with one blanket treatment and a move towards using convergent data for a more targeted treatment approach that will result in improving quality of life in people with MDD and schizophrenia.
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Volunteers needed for Brain Imaging Research at the University of Manchester

Are you experiencing symptoms of depression and interested in taking part in brain imaging research?

We are investigating whether depression is associated with any signs of inflammation in the brain. We can measure this with a brain imaging technique called positron emission tomography (PET). Ultimately our findings will further our knowledge of the neurobiology underlying depression, possibly leading to more effective treatment.

To take part volunteers must be aged 18-60, be experiencing symptoms of depression but not be taking antidepressants (for 3 months minimum), and be medically healthy.

Taking part in the study would involve:

- A screening session
- An MRI scan (Magnetic Resonance Imaging)
- A PET scan (Positron Emission Tomography)

The total time spent on the study would be approximately 5 hours. Volunteers will be reimbursed for time (£10 per hour) and travel.

If you are interested in taking part in this research or want to find out more please email sophie.holmes@manchester.ac.uk or call 07974 877597.

For more information visit www.bbmh.manchester.ac.uk/inflammationindepression

REC approval number:09/H1013/78

Figure A-1 Poster advertisement for recruiting individuals with depression
Figure A-2 Image analysis flowchart for $[^{11}{\text{C}}](R)$-PK11195 studies at WMIC

Key

- File (type, contents)
- Software Process

- VH1
- T1
- MR
- In PET space
- SPM Segmentation
  - Create GM map
  - Warp atlas into individual space
  - Normalization
  - SPM
  - Analyze Create GM atlas
- Hammers Atlas
- Binary brain mask
- Analyze Edit spillover and sample values
- Brain atlas (whole & GM in individual space)
- Analyze Create whole brain & GM object maps
- Parametric map $k_2$
- Parametric map $R_w$
- Parametric map $k_3$

- SUPER PK
  - Supervised cluster analysis
- Reference tissue TAC Normal GM
- Reference tissue TAC Cerebellum GM
- MICE PMI kinetic modelling (GRIMM)
- Dynamic PET image (18 frames, 4mm FWHM)
- Parametric map $R_c$
- Parametric map $k_2$
- Parametric map $k_3$

- Dynamic PET image (16 frames, 2mm FWHM)
- Sample dynamic PET with object map
- Summed PET image (Frames 2 to 18, 2mm FWHM)
- ROC object map
- Adding Frames 2 to 18 Decay corrected
- Reconstructed PET image (18 frames)
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* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Figure A-3 Correlation matrix: symptom severity and regional PET data for MDD patients
|----------|-------------|---------------------------|---------------------------|-----------------------------|----------------------------|------------------------|-----------------|----------------|---------------------------|-------------------------------|---------------------------------|-----------------------------|------------------------------|-------------------------------|-----------------------------|-------------------------------|-----------------
|           |             | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Age       | -0.236      | -0.455 | 0.428 | -0.162 | 0.734 | -0.307 | 0.351 | 0.211 | 0.108 | 0.151 | 0.392 | 0.201 | 0.302 | 0.283 | 0.194 | 0.357 |
| BMI       | 0.140       | 0.277 | 0.095 | 0.303 | 0.386 | 0.765 | -0.352 | 0.487 | 0.786 | 0.535 | 0.617 | 0.701 | 0.634 | 0.517 | 0.500 | 0.539 |
| AUDIT     | 0.161       | 0.083 | 0.338 | 0.508 | 0.735 | 0.239 | 0.378 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 |
| Sleep     | 0.297       | 0.185 | 0.178 | 0.192 | 0.372 | 0.285 | 0.482 | 0.414 | 0.327 | 0.374 | 0.327 | 0.374 | 0.327 | 0.374 | 0.327 | 0.374 |
| ChildHood_Absentee | 0.158 | 0.393 | 0.348 | 0.248 | 0.256 | 0.570 | 0.381 | 0.425 | 0.308 | 0.343 | 0.308 | 0.343 | 0.308 | 0.343 | 0.308 | 0.343 |
| Exercise_score | 0.049 | 0.192 | 0.176 | 0.188 | 0.187 | 0.177 | 0.177 | 0.177 | 0.177 | 0.177 | 0.177 | 0.177 | 0.177 | 0.177 | 0.177 | 0.177 |
| Duration_of_sick | 0.015 | 0.143 | 0.139 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 | 0.144 |
| ACC_LR_CER | 0.120 | 0.277 | 0.192 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 | 0.277 |
| PCQ_LR_CER | 0.185       | 0.083 | 0.338 | 0.508 | 0.735 | 0.239 | 0.378 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 |
| Insula_LR_CER | 0.158 | 0.393 | 0.348 | 0.248 | 0.256 | 0.570 | 0.381 | 0.425 | 0.308 | 0.343 | 0.308 | 0.343 | 0.308 | 0.343 | 0.308 | 0.343 |
| TEM_LR_CER | -0.043 | -0.117 | -0.038 | -0.063 | -0.613 | 0.391 | 0.724 | 0.459 | 0.782 | 0.179 | 0.371 | 0.442 | 0.431 | 0.371 | 0.442 | 0.431 |
| PAR_LR_CER | -0.101 | -0.285 | -0.048 | -0.131 | -0.472 | 0.391 | 0.724 | 0.459 | 0.782 | 0.179 | 0.371 | 0.442 | 0.431 | 0.371 | 0.442 | 0.431 |
| OFC_LR_CER | 0.070 | 0.277 | 0.185 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 | 0.187 |
| DLFC     | 0.015       | 0.083 | 0.338 | 0.508 | 0.735 | 0.239 | 0.378 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 | 0.304 | 0.212 |
| VLPFC    | 0.158       | 0.393 | 0.348 | 0.248 | 0.256 | 0.570 | 0.381 | 0.425 | 0.308 | 0.343 | 0.308 | 0.343 | 0.308 | 0.343 | 0.308 | 0.343 |

**Correlation** is significant at the 0.01 level (2-tailed). **Correlation** is significant at the 0.05 level (2-tailed).
Figure A-5 Correlation matrix: peripheral inflammatory markers, demographic, clinical and PET data for MDD patients

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* Correlations are significant at the 0.05 level (two-tailed).
** Correlations are significant at the 0.01 level (two-tailed).
### Correlations

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* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Figure A-6 Correlation matrix: symptom severity and regional PET data for schizophrenia patients
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| **Correlation Matrix: demographic/questionnaire and regional PET data for schizophrenia patients**

**Figure A-7** Correlation matrix: demographic/questionnaire and regional PET data for schizophrenia patients

**Note:** Correlation is significant at the 0.05 level (2-tailed).
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Figure A-8 Correlation matrix: peripheral inflammatory markers, demographic, clinical and PET data for schizophrenia patients.