A STUDY OF DEPRESSION AND THE ANTIDEPRESSANT EFFECTS OF KETAMINE FROM AN IMMUNOINFLAMMATORY PERSPECTIVE

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A THESIS SUBMITTED FOR THE JOINT DEGREE OF DOCTOR OF PHILOSOPHY

NATIONAL UNIVERSITY OF SINGAPORE

IMPERIAL COLLEGE LONDON

2018

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

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30 March 2018

ACKNOWLEGEMENTS

I would like to thank my supervisors Gavin Dawe and Paul Matthews for their guidance, and my family, colleagues and God for all the support.

Overview

Depression is a leading cause of disability. The World Health Organisation estimates that over 320 million people around the world are affected by depression. To date, scientists and clinicians are still finding ways to better understand and treat depression. One hypothesis suggests that depression is a disorder of the inflammatory system, and that treatment will involve modulating the immunoinflammatory response. This thesis will delve into whether specific inflammatory insults are associated with depression, and whether the treatment of depression is immunomodulatory in nature.

The first chapter examined the relationship between depression and episodes of infection and injury, using data from a large population database and hospital-linked records. It also examined the relationship between depression and the use of anti-inflammatory agents. The second chapter examined whether the treatment of depression with a potent and rapid-acting antidepressant drug, ketamine, was associated with immunomodulatory changes in rodents. This chapter also examined the role of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) in mediating ketamine's antidepressant effect.

Hospitalisation for either infection or injury was associated with higher odds of recurrent depression. The consumption of fish oil supplements was associated with lower odds of recurrent depression, but the use of non-steroidal anti-inflammatory drugs was associated with higher odds of recurrent depression. In rodent models of depression, ketamine treatment altered the morphology of microglia as well as the messenger ribonucleic acid expressions of microglial activity. Further experiments determined that the AMPA pathway was crucial to ketamine's antidepressant effect.

Key findings in this thesis support the immunoinflammatory hypothesis of depression. Future research should place emphasis on identifying inflammatory risk factors for depression and developing novel treatment approaches that target inflammation signalling pathways.

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Chapter I

Depression as a model of immunoinflammatory disease

A) Introduction

Depression

Major depressive disorder (MDD) is a leading contributor to global burden of disease. It is estimated that over 350 million people worldwide suffer from depression[1,2]. MDD is not merely transient low mood in response to life's challenges but a mental disorder that can be chronic, debilitating and life-threatening.

The standardised diagnostic criteria in the International Statistical Classification of Diseases and Related Health Problems (ICD)[3] or Diagnostic and Statistical Manual of Mental Disorders (DSM)[4] can be used to diagnose MDD. A major depressive episode is characterised by core symptoms of persistent low mood and/or anhedonia, accompanied by other symptoms such as changes to appetite and sleep, behavioural

activation, fatigue, impaired cognition, feelings of excessive guilt or worthlessness, and suicidality. MDD, or clinical depression, differs from temporary feelings of sadness that one might experience at some point in life. In MDD, this sadness is more intense and prolonged, and co-occurs with many of the other depressive symptoms mentioned above, resulting in significant distress and functional impairment.

A patient with MDD may have one or more major depressive episodes in their lifetime, and each episode can last from weeks, months to even years[5,6]. Recurrent depression is defined as having two or more lifetime episodes of major depression. In community samples, 35-76% have a subsequent major depressive episode after the first episode[7,8], while up to 85% in clinical settings experience a recurrent episode after recovery from the index episode[9]. The hallmark of a recurrent pattern is cycle acceleration, where with each new episode of depression, the next episode tends to occur sooner and have a more severe course than the preceding episode[10]. After the first lifetime episode of MDD, approximately 60% of patients suffer a second episode; 75% of those with a second episode will suffer a third, and 90% of those with three or more episodes will experience further recurrences[11]. In a landmark

longitudinal study, the median time to the first recurrence was 150 weeks, following that 83 weeks to the second recurrence, 77 weeks to the third, 68 weeks to the fourth, and 57 weeks to the fifth episode[12]. Compared to single episode/non-recurrent depression, recurrent depression is associated with higher suicide risk, more psychiatric comorbidities, lower social and occupational function, greater loss of personal productivity, and increased healthcare costs[13-16].

Urgent strategies are needed to mitigate depression recurrence. A thorough understanding of factors that contribute to the recurrence of depression is paramount to the development of effective interventions. The following discourse will focus on an area that has been gaining increasing attention and research – inflammation.

Inflammation and Behaviour

Inflammation can be broadly understood as a body's innate biological response to harmful stimuli. It is a self-protective mechanism that aims to remove or neutralise the injurious stimuli and promote tissue healing. The inflammatory response involves complex changes to immune cells,

cellular mediators, blood vessels and tissues. When the immune system senses invading pathogens or damaged tissues, immune molecules are produced to direct the inflammatory process towards the area of damage. These changes are aimed at resisting the pathogens, removing cell debris and facilitating wound recovery[17]. Infection and physical injury, among others causes, are the most common precipitants of inflammation.

Inflammation, through pro-inflammatory molecules like cytokines, activates behavioural responses that are collectively known as sickness behaviour. Sickness behaviour includes symptoms such as lethargy, depression, anorexia, loss of interest in activities and body care, social withdrawal, disturbed sleep, hyperalgesia and impaired cognition[18-20]. Hart[18] conceptualised sickness behaviour as a coordinated and adaptive response to enhance survival. He hypothesised that this behaviour helped conserve energy in order to meet the metabolic demands of fever and inflammation. Lethargy, reduced mobility and increased sleep would encourage rest and reduce energy consumption. Increased sensitivity to pain could help prevent further damage to sensitive tissues or organs. Decreased appetite and food intake are believed to reduce

iron consumption, which deprives certain infective pathogens of an essential growth nutrient. Fatigue, increased sleep, and the lack of social and sexual interest could all act to limit physical contact. This could help reduce the risk of disease transmission or prevent the contracting of new infections. The decreased motivation to forage for food or search for a mate could protect a weakened individual from further injury by reducing exposure to the elements. From an evolutionary perspective, sickness behaviour may have conferred a form of defence against infections in early man.

Infectious diseases have been the biggest causes of death throughout history. Hundreds of millions worldwide perished from pandemics like the plague, smallpox, influenza and cholera. Up till the early 20th century, the leading causes of death were still infections. In 1900, the top three causes of death in the United States were pneumonia/influenza, tuberculosis and gastrointestinal infections[21]. As recent as 2015, lower respiratory tract infections, tuberculosis and diarrhoeal diseases were still among the top 10 causes of death globally[22].

Death from injury may come in any way, shape or form. Humans though the ages have succumbed to war, violence, accidents and natural disasters. Today, road injury ranks among the top 10 causes of death globally – 1.3 million people died from road accidents in 2015 alone[22]. In the United States, unintentional injury is the number one cause of death among people aged one to 44[23]. Injuries are common - approximately one-third of all emergency department admissions are due to injury, and an estimated 10% of the population will require an emergency department visit for injury[24-25].

Infection and injury, through inflammation, may have shaped the evolution of sickness behaviour over time. Sickness behaviour, like the fight or flight response, has been viewed as a physiological behaviour that enhances survival in critical situations. However, in the same way prolonged fear and anxiety are detrimental to health, excessive sickness behaviour can be debilitating. Could major depressive disorder, as we know today, be a form of sickness behaviour that is protracted and maladaptive?

Depression - An Inflammatory Model of Illness

There are striking similarities between clinical depression and sickness behaviour. Common to both are vegetative symptoms that affect appetite, sleep and energy levels. Cognitively, both can present with deficits such as impaired concentration. Functionally, both can lead to lower physical activity and social withdrawal. Could they possibly share a common inflammatory aetiology?

When an infection or injury occurs, our immune system acutely reacts by releasing molecular mediators from immune cells to coordinate an inflammatory response against the threat. These mediators, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), are known as pro-inflammatory cytokines. These cytokines can act directly or indirectly on the brain to effect behavioural changes. Many animal experiments have shown that sickness behaviour can be directly elicited through peripheral or central administration of IL-1β[26-30] or TNF-α[29-31]. These cytokines can dose-dependently cause lower locomotor activity, fewer explorations of their environment, poorer grooming and self-care, lesser food and water intake, and greater

impairment in learning and memory[26-35]. IL-6 on its own triggers a febrile response, but when combined with IL-1β and TNF-α they act synergistically to produce the above behaviours[36-38].

In humans, meta-analyses of multiple cross-sectional studies have consistently shown elevated plasma levels of pro-inflammatory cytokines (especially IL-6 and TNF-α) in depressed patients relative to controls[39-42]. A meta-analysis of prospective intervention studies has also shown that antidepressant medications lower plasma levels of IL-6 and TNF-α[43]. Even with these associations, one would still question whether pro-inflammatory cytokines are a direct cause of depressive symptoms in humans. Fortunately, the use of interferon-alpha (IFN-α) in the treatment of various forms of cancer and hepatitis has given us the opportunity to examine this relationship.

IFN-α is an endogenous cytokine that can potently activate other cytokines in the cytokine network. It has been shown to increase expressions of IL-1, IL-6, and TNF-α in cell cultures and in vivo[44]. Patients given IFN-α therapy have acutely elevated plasma levels of IL-6

and TNF-α[45-48]. Studies have further shown positive correlations between increased depression severity scores and higher IL-6/TNF-α plasma levels[47-49]. Investigators have sought to analyse the temporal sequence of symptom emergence with progressive IFN-α use. A course of IFN-α treatment typically involves multiple injections per week for three to six months, depending on the indication. Neurovegetative symptoms (fatigue, appetite loss, sleep disturbance, psychomotor slowing) appear at their highest frequencies within the first one to two weeks of treatment, while depressive symptoms (depressed mood/sadness, anhedonia, guilt, suicidal thoughts) significantly increase at week eight and peak by week 12 of treatment[50-53]. Antidepressant use during IFN-α treatment had no effect on early onset vegetative symptoms but could diminish vegetative symptoms from week eight onwards[50]. Unlike the phased treatment effects on vegetative symptoms, antidepressant medications (selective serotonin reuptake inhibitors) were able to reduce depressive symptoms throughout the length of IFN-α therapy, relative to no treatment[50,54]. This interesting observation implies that early onset vegetative symptoms induced by cytokines were likely sickness / flu-like symptoms, not clinical depression symptoms, as they were not amenable to antidepressant treatment. It was only from

eight weeks onwards, when vegetative and depressive symptoms combined to form the whole clinical depression construct, that both symptom clusters responded to antidepressants. This gives supporting evidence that cytokines may induce sickness symptoms initially, but when protracted, these symptoms develop into a depressive disorder.

Depressive symptoms are commonly experienced during IFN-α therapy, with some reporting frequencies of up to 96% during active IFN-α treatment[55]. Recent systematic reviews and meta-analyses of hepatitis C studies indicate that 28% to 35% of patients, without depression at baseline, go on to develop a full major depressive episode (MDE) that meets DSM/ICD criteria by the end of an IFN-α treatment course[56,57]. The frequency and severity of IFN-associated depression is significantly increased with higher induction doses of IFN-α[58]. Long-term follow-up of post-treatment patients showed no statistically significant difference in the risk of recurrent depression after an index IFN-α induced depressive episode versus the risk of recurrent depression after first-episode depression in the general population[59]. Using IFN-α as an inflammatory model of depression, we learn that the direct administration of a pro-inflammation cytokine could 1) induce major depressive symptoms, 2)

dose-dependently increase depression frequency and severity, 3) induce depressive symptoms that respond to conventional antidepressant drug therapy, 4) give rise to a longitudinal course of depressive illness that is similar to de novo recurrent major depressive disorder.

Inflammation is a two-edged sword – what is meant to be a protective mechanism may turn destructive if unbridled. Major depressive illness could be the result of a maladaptive inflammatory response in vulnerable individuals, where a chronic waxing and waning inflammation persists beyond the initial insult. Indeed, there is evidence of persistent low-grade inflammation in the form of elevated blood levels of pro-inflammatory cytokines[39-42] and C-reactive protein (CRP)[60,61] in depressed patients. But what might have triggered this?

Sources of inflammation are often encountered early in life. Infections that require hospitalisation during childhood increases the risk of subsequent behavioural and emotional disorders [Hazard Risk Ratio (HRR)=2.03; 95% Confidence Interval (CI)=1.80 to 2.30]; even less severe infections treated in primary care increases this risk (HRR=1.46;

95% CI=1.30 to 1.63)[62]. The association between childhood infection and subsequent emotional disorder could be mediated by pro-inflammatory cytokines. In longitudinal cohort studies, children with higher IL-6 levels measured during childhood were more likely to have persistent depressive symptoms as adolescents and young adults[63,64]. Similarly, childhood maltreatment also leaves a pro-inflammatory mark that persists into adulthood. A meta-analysis of retrospective and prospective studies found that those who suffered childhood trauma were more likely to have elevated pro-inflammatory markers (CRP, IL-6 and TNF-α) in adulthood[65].

Stress to the body, whether physical or psychological, presents an immunoinflammatory challenge to the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a potent modulator of inflammation in the body. Stress induces hypothalamic secretion of corticotropin-releasing hormone (CRH), which suppresses immune responses through the release of glucocorticoids from the adrenal glands to bring about homeostasis[66]. The HPA axis is a neuroendocrine system that is highly sensitive to environmental stressors and could become dysfunctional when exposed to repeated adversity. Chronic and repeated episodes of

stress could cause HPA axis dysregulation in many ways: depletion of cortisol, impaired CRH function, glucocorticoid receptor resistance or down-regulation, increased affinity for mineralocorticoid receptors, or hypersensitivity of the negative feedback system[67-68]. The end result, through one or all the mechanisms above, is a blunted cortisol (anti-inflammatory) response to stress[66]. With an impaired HPA axis, the body's inflammatory response becomes unmodulated when faced with further stress. The ensuing release of pro-inflammatory cytokines could be heightened and protracted with cumulative episodes of stress, consequently precipitating or perpetuating symptoms of depression.

The central focus of this chapter is to investigate whether episodes of inflammatory stress are associated with clinical depression, and more specifically, whether multiple episodes of inflammation are associated with a recurrent form of depression. As described earlier, infection and injury are potent triggers of inflammation, therefore hospitalisations for infection or injury will be used as markers of severe inflammation.

Few large epidemiological studies have reliably examined the role of infection in major depressive disorder. Various small studies have examined the relationship between specific infections and depression, but their methodologies and findings have been inconsistent[69]. Larger studies used national health records that contained a broad base of infection diagnoses identified through clinic/hospital visits, and depression diagnoses were identified through psychiatric records. The longitudinal Danish cohort study (n=29,194) used data from the Danish Psychiatric Central Research Register and Danish National Hospital Registry. Subjects aged 16 to 65 with at least one hospital contact for infection were followed up until they had a mood disorder (including bipolar disorder, depressive disorder, other unspecified mood disorders) diagnosed by a psychiatrist. A history of hospitalisation for infection increased the risk of mood disorders [Incidence Rate Ratio (IRR)=1.62, 95% CI=1.60-1.64][70]. The risk of mood disorders increased with the number of hospitalisations for infections[70]. A more recent longitudinal cohort study (n=63,597) used data from the Taiwan National Health Insurance Research Database. Subjects aged 18 to 50 with repeated low-grade upper respiratory infections were followed up until they had major depression diagnosed by a psychiatrist (bipolar disorders and affective psychoses were excluded). Those with frequent low-grade

upper respiratory infections had increased risk of MDD [Hazard Ratio (HR)=1.37, 95% CI=1.19-1.58 for Cohort 2002; HR=1.91, 95% CI=1.53-2.39 for Cohort 2004][71]. These two studies however did not examine which class of infective agents were associated depression. They also did not examine whether different depressive subtypes were associated with infection.

With regards to injury, even fewer studies have sought to determine the relationship between physical injury and depression, and existing data is conflicting. In the Canadian National Population Health Survey (NPHS), analysis of prospective data on self-reported injury revealed an increased risk of developing a major depressive episode after injury (HR=1.4, 95% CI 1.1-1.8)[72]. In a better designed study that used hospital data instead of self-report, patients hospitalised for physical injury had a low mean depression symptom score [3.7 (3.1-4.3)] on the Hospital Anxiety and Depression Scale (HADS) upon hospital discharge. At 12 months post-discharge, the mean depression score [2.8 (2.2-3.4)] remained well below the cut-off score (>8) for clinical depression[73]. The latter study suggests that hospitalisation for injury may not be that psychologically distressing as to cause depression, therefore hospitalisation for injury

could be used as a marker of inflammatory load in this chapter's study, as the psychological effects of hospitalisation would probably not confound the association between inflammation and depression.

While hospitalisation for either infection or injury marks a definite episode of inflammation, there are instances when inflammatory events do not require hospitalisation. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the community to alleviate body pain or fever, symptoms which often indicate underlying tissue inflammation. NSAIDs work by inhibiting the enzyme cyclo-oxygenase, which reduces the production of prostaglandins – a class of secondary messenger molecules that are integral to the inflammation process[74]. NSAIDs like aspirin and ibuprofen are widely available in pharmacies as over-the-counter drugs. Aspirin is also used as an antithrombotic agent[75] to reduce the risk of recurrent ischemic stroke or myocardial infarction - both these diseases involve intravascular inflammation[76,77]. In general, NSAIDs are used only when there is some indication of prior or ongoing inflammation. Its use helps to reduce inflammation, but it does not eradicate the inflammatory process. Hence in this study, use of NSAIDs

will be considered a surrogate marker of inflammation, and will be examined as a risk factor for depression.

Dietary supplements are often taken to promote good health, as opposed to drugs which are taken in response to illness or disease. Fish oil is a commonly consumed dietary supplement that has anti-inflammatory properties. They are often consumed even when there are no active symptoms of inflammation, as a protective measure against ill-health. Fish oils contain two important omega-3 fatty acids – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA have inflammation-lowering properties through several mechanisms. They decrease the production of inflammatory eicosanoids that are derived from arachidonic acid (AA). EPA and DHA compete with AA for incorporation into membrane phospholipids, thereby lowering cellular and plasma availability of AA. EPA also competes with AA for the cyclo-oxygenase enzyme, thus inhibiting AA's downstream metabolism into inflammatory eicosanoids[78]. DHA and EPA both reduce the release of pro-inflammatory cytokines through directly inhibiting eicosanoid release[79]. In observational studies, individuals who had higher dietary EPA and DHA intake experienced less inflammation as they

aged[80-83]. In a meta-analysis of randomised controlled clinical trials, omega-3 fatty acid use was associated with lower levels of inflammation in cardiovascular and other diseases[84]. To further investigate the relationship between an anti-inflammatory supplement and depression, this chapter will explore whether the regular use of fish oil has protective effects against depression.

In summary, the main study aims of this chapter are to determine:

1) whether hospitalisation for infection is associated with single/recurrent episode major depression
2) whether hospitalisation for injury is associated with single/recurrent episode major depression
3) whether NSAIDs use is associated with single/recurrent episode major depression
4) whether hospitalisation for infection, hospitalisation for injury and NSAIDs use have cumulative effects on single/recurrent episode major depression
5) whether fish oil use is associated with single/recurrent episode major depression

The main hypothesis posits that depression is associated with inflammation – the more recurrent the type of depression, the stronger the association with inflammation. Specifically, it is hypothesised that hospitalisation for infection, hospitalisation for injury, and regular use of NSAIDs will each be associated with recurrent major depression, while such associations will be weaker with single episode major depression. It is further hypothesised that when more than one instance of inflammation is present, the association with recurrent major depression will be stronger. Lastly, it is hypothesised that regular fish oil supplementation will be associated with recurrent major depression, and the association will be weaker with single episode major depression.

The exploratory aims of the study are to investigate whether a specific class of infective agent or a particular type of injury is associated with recurrent major depression. These findings will help generate hypotheses for future studies.

Epidemiological data from the UK Biobank is used in this study. The UK Biobank is an open-access general health resource that aims to improve the prevention, diagnosis and treatment of diseases through research[85]. Funding is provided primarily by the Wellcome charity trust and the Medical Research Council, UK (https://www.ukbiobank.ac.uk/wp-content/uploads/2018/10/ Funding-UK-Biobank-summary.pdf). A large population-based cohort was recruited from across the UK and longitudinal follow-up is being planned[85]. The design of UK Biobank offers the opportunity to examine a wide range of risk factors and outcomes in a sample large enough to detect relatively small effects, making UK Biobank a highly efficient resource for epidemiological studies.

B) Methodology

This study used baseline data from the UK Biobank for analysis (http://www.ukbiobank.ac.uk)[85,86]. The Research Ethics Committee approval reference for UK Biobank is 11/NW/0382. The UK Biobank investigators sent postal invitations to approximately 9.2 million individuals, having been identified through the National Health Service

(NHS) register. These invitations targeted individuals aged 40–69 years who lived within 25 miles of a UK Biobank assessment centre located throughout England, Wales, and Scotland. Recruitment was conducted from 2006 to 2010. Responders were required to visit their nearest UK Biobank assessment centre, where written informed consent for participation was obtained. They then gave background information on their demographics, lifestyle and medical history; underwent cognitive, physical and psychosocial assessments; and provided biological samples for future analysis[85,86]. All data extracted were deidentified for analysis.

Defining Major Depressive Disorder

UK Biobank used the following questions on the touchscreen questionnaire to assess depression: (1) Ever felt depressed for a whole week; (2) Ever felt disinterested or unenthusiastic for a whole week; (3) Only one episode; (4) Two or more episodes; (5) Episode lasted at least two weeks; (6) Ever seen a GP for nerves, anxiety, tension or depression; (7) Ever seen a psychiatrist for nerves, anxiety, tension or depression. Each question prompted a response of either 'Yes', 'No', 'Do not know' or 'Prefer not to answer'. The latter two responses were

excluded from analysis. Data were collected from participants during their visit to one of the UK Biobank assessment centres at Stockport, Manchester, Oxford, Glasgow, Cardiff, Edinburgh, Stoke, Bury, Newcastle, Leeds, Reading, Bristol, Nottingham, Barts, Liverpool, Middlesborough, Hounslow, Sheffield, Croydon, Birmingham, Swansea, Wrexham and Cheadle.

UK Biobank used the following criteria groups to define Major Depressive Disorder (MDD): A) Probable MDD: [(1) or (2)] and (3) and (5) and [(6) or (7)] + [(1) or (2)] and (4) and (5) and [(6) or (7)]; B) Probable single episode MDD [(1) or (2)] and (3) and (5) and [(6) or (7)]; C) Probable severe recurrent episode MDD [(1) or (2)] and (4) and (5) and (7). The above definitions combined the core MDD diagnostic features based on the DSM-IV criteria[4] together with data on accessing professional mental health care. These definitions have been used to biologically phenotype MDD in previous studies[87,88]. For this study, lifetime MDD was coded positive if definition A) was met; lifetime single episode MDD was coded positive if definition B) was met; lifetime recurrent episode MDD was coded positive if definition C) was met. Subjects in single and recurrent episode depression groups were

mutually exclusive. It should be noted the questions that were used to ask about depression at baseline were only added to the assessment at later stages of recruitment, therefore only a quarter of the cohort underwent screening for depression at UK Biobank assessment centres.

Participants with schizophrenia and bipolar disorder were excluded from analysis. Subjects were excluded if there was a self-reported lifetime diagnosis of schizophrenia or bipolar disorder recorded on the touchscreen questionnaire, or during verbal interview at UK Biobank assessment centres.

Biodata

Sociodemographic information such as age, sex, ethnicity, education, employment status and household income were obtained from data collected at UK Biobank assessment centres using touchscreen questionnaires. Psychosocial, lifestyle, dietary and over-the-counter drug information were also obtained through touchscreen questionnaires administered at these centres. Social isolation was identified through a question on frequent feelings of loneliness. Tobacco smoking status was

given a binary outcome of 'Yes=Ever smoked in the past or present' and 'No=Never smoked before'. Alcohol drinking status was given a binary outcome of 'Yes=Ever consumed alcohol in the past or present' and 'No=Never consumed alcohol'. Chronic illness and disability were identified by the question: "Do you have any long-standing illness, disability or infirmity?", followed by a 'yes/no' choice. Use of NSAIDs was identified by the question: "Do you regularly take any of the following?", followed by a list of drugs to choose from, which included aspirin and ibuprofen. Fish oil consumption was identified by the question: "Do you regularly take any of the following?", followed by a list of dietary supplements to choose from, which included fish oil. These questions for NSAIDs and fish oil did not distinguish between past or present use, and details on dose, frequency and duration were not captured as the design of those data fields allowed only a 'yes/no' response.

Hospital data

Inpatient hospital admissions for infection or injury were obtained through UK Biobank's data linkage to the National Health Service

(NHS), which contain hospitalisation data from the Health Episodes Statistics, Scottish Morbidity Record and Patient Episode Database for Wales. These records-based systems respectively cover all NHS hospitals in England, Scotland and Wales. Clinical codes based on ICD 10th Revision (ICD-10) were standardised across these datasets. Inpatient primary diagnoses of infection and injury were extracted using ICD-10 codes. Below are examples of causative agents that were classified under these codes.

Bacterial infections were identified by ICD codes A00-A05.9 (*Vibrio cholerae/parahaemolyticus, Salmonella sp, Shigella sp, Escherichia coli, Campylobacter sp, Yersinia enterocolitica, Staphylococcal spp, Clostridium difficile/botulinum/perfringens/tetani, Bacillus cereus*); A15-A59.9 (*Mycobacterium spp, Yersinia pestis/enterocolitica, Francisella tularensis, Bacillus anthracis, Brucella spp, Burkholderia mallei, Spirillum minus, Streptobacillus moniliformis, Erysipelothrix rhusiopathiae, Leptospira spp, Pasteurella spp, Bartonella spp, Listeria monocytogenes, Corynebacterium diphtheriae, Bordetella pertussis/parapertussis, Streptococcal spp, Neisseria meningitidis/gonorrhoeae, Actinomyces spp, Nocardia spp, Legionella spp, Haemophilus influenzae/aegyptius/ducreyi, Treponema pallidum, Chlamydia*

trachomatis, Klebsiella granulomatis, Trichomonas vaginalis); A65-A79 (*Treponema pallidum pertenue/endemicum/carateum, Borrelia spp, Prevotella intermedia, Fusobacterium nucleatum/necrophorum, Peptostreptococcus spp, Selenomonas spp, Brachyspira spp, Chlamydophilia spp, Rickettsia spp*); B90-B90.9;B94.0 (bacterial infection sequelae); B95-B96.8;B98 (other bacterial infections).

Viral infections had ICD codes A08-A08.5 (*Rotavirus, Norwalk virus, Adenovirus, other viral enteritis*); A60-A63 (*Herpes simplex virus; Molluscum contagiosum virus, Human papillomavirus spp*); A80-B34.9 (*Poliovirus, Lyssavirus, Arthropod-borne viruses, Haemorrhagic fever viruses, Enterovirus spp, Adenovirus spp, Lymphocytic choriomeningitis virus, Varicella-zoster and other Human Herpesvirus spp, Poxviruses/Parapoxviruses, Measles virus, Rubella virus, Mumps virus, Parvovirus B19, Hepatitis viruses, Human immunodeficiency virus, Coronavirus spp, Human polyomavirus spp*); B94.1-94.2 (viral infection sequelae); B97-B97.8 (other viral infections).

Parasitic infections had ICD codes A06-A07.9 (*Entamoeba histolytica, Balantidium coli, Giardia lamblia, Cryptosporidium parvum, Isospora belli,*

Trichomonas spp, Sarcocystis spp); B50-B88.9 (*Plasmodium spp, Leishmania spp, Trypanosoma spp, Toxoplasma gondii, Pneumocystis spp, Babesia spp, Acanthamoeba spp, Naegleria fowleri, Microsporidia spp, Schistosoma spp, Clonorchis sinensis, Opisthorchis viverrini/felineus, Dicrocoelium dendriticum, Fasciola spp, Paragonimus spp, Fasciolopsis buski, Echinostoma spp, Heterophyes, Metagonimus spp, Nanophyetus spp, Watsonius watsoni, Echinococcus spp, Taenia spp, Diphyllobothrium spp, Spirometra spp, Hymenolepis spp, Dipylidium caninum, Dracunculus medinensis, Onchocerca volvulus, Wuchereria bancrofti, Brugia spp, Loa, Mansonella spp, Dirofilaria spp, Trichinella spp, Ancylostoma spp, Necator americanus, Ascaris lumbricoides, Strongyloides spp, Trichuris trichiura, Enterobius vermicularis, Anisakis spp, Capillaria philippinensis, Trichostrongylus spp, Angiostrongylus spp, Oesophagostomum spp, Ternidens deminutus, Toxocara spp, Gnathostoma spp, Mammomonogamus spp, Macracanthorhynchus hirudinaceous Moniliformis, Bolbosoma spp, Gongylonema spp, Metastrongylus spp, Capillaria hepatica, Thelazia spp, Pediculosis spp, Sarcoptes scabiei, Myiasis/Acariasis/Scarabiasis/ Hirudiniasis infestations, Tunga penetrans, Vandellia cirrhosa, Linguatula serrata, Porocephalus crotali, Armillifer moniliformis*).

Fungal infection had ICD codes B35-B49 (*Epidermophyton spp, Microsporum spp, Trichophyton spp,, Malassezia spp, Hortaea werneckii, Trichosporon spp, Piedraia hortae, Candida spp, Coccidioides spp, Histoplasma spp, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Sporothrix schenckii, Dematiaceous fungi, Aspergillus spp, Cryptococcus spp, Zygomycetes fungi, Eumycetes fungi, Lacazia loboi, Rhinosporidium seeberi, Pseudallescheria boydii, Geotrichum candidum, Penicillium marneffei, Emmonsia crescens/parva*).

Injuries from external causes were identified by ICD codes S00-S09 (head); S20-S39 (torso); S40-S69 (upper limb) and S70-S99 (lower limb).

Statistical Analysis

Chi-square test or t-test were used to identify differences in categorical or continuous baseline characteristics respectively. Multivariate logistic regression was used to examine for associations between depression and variables of interest. Adjustment for covariates like age, education, race, employment, income, social isolation, smoking status and chronic illness/disability was performed during regression analysis. Sensitivity

analysis was performed by stratification of covariates. Multicollinearity of covariates in regression models was examined using collinearity diagnostics in SPSS. All statistical analyses were performed using R-statistics and SPSS software version 24[89]. The size of the UK Biobank cohort was based on conventional power calculations for nested case-control studies, showing that 5,000–10,000 cases of any condition with 5-10% prevalence will have approximately 80% power to detect minimum main effect odds ratios (ORs) of 1.13 to 1.26[85].

C) Results

A total of 502,655 community-dwelling adults aged 37 to 73 years consented to participate in this study. Of these, 121,053 subjects were screened for depression at UK Biobank assessment centres via the DSM-IV based questions on the touchscreen questionnaire. Among them, 31,586 met the case definition of lifetime major depression; 7909 had single episode major depression and 23,677 had recurrent episode major depression. Figure 1 summarises the flow of participants in this study.

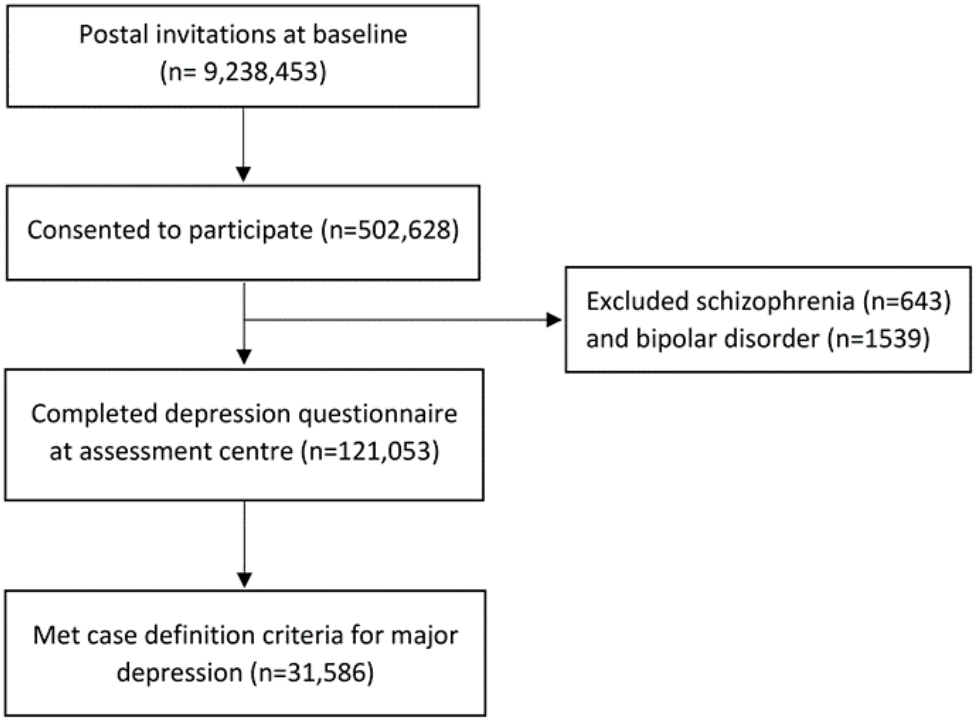

Fig.1 Flowchart of UK Biobank participants from invitation to case selection.

The characteristics of study participants are summarised in Table 1, which compared major depression subjects with non-depressed controls. The depressed group had younger mean age and proportionately more females and whites. Although they had a greater proportion with college/university education and employment, they also had below median total household income (<£31,000). A higher percentage of those depression experienced social isolation and chronic illness/disability. There were also more tobacco smokers and alcohol

drinkers in the depressed group. The depressed group had higher percentages of hospitalisation for infection/injury and NSAIDs use, but a lower percentage of fish oil supplementation. Characteristics with statistically significant difference between groups are labelled in Table 1.

Table 1. Characteristics of study participants

Characteristics	**Control** (n=89467)	**Depressed** (n=31586)
Age (years)* (mean ± SD)	*n=89467* 57.4 ± 8.1	*n=31586* 55.7 ± 8.0
Gender (%)* Male/Female	*n=89467* 50.1/49.9	*n=31586* 35.4/64.6
Ethnicity (%)*	*n=81509*	*n=31475*
White	91.5	94.5
Black/Asian/Others	8.5	5.5
Education (%)*	*n=74294*	*n=27423*
College/University	39.9	40.8
O/A Levels	45.7	45.8
Vocational/Others	14.4	13.4
Employment (%)*	*n=88590*	*n=31306*
No	44.0	42.8
Yes	56.0	57.2
Total Household Income (%)*	*n=76148*	*n=28338*
Below median (< £ 31,000)	46.3	49.8
Above median (≥ £ 31,000)	38.8	50.2

Social Isolation (%)*	*n=88436*	*n=31108*
No	88.2	70.7
Yes	11.8	29.3
Chronic Illness/Disability (%)*	*n=87708*	*n=30924*
No	71.8	58.2
Yes	28.2	41.8
Smoker (%)*	*n=89126*	*n=31515*
No	42.6	35.4
Yes	57.0	64.4
Alcohol drinker (%)*	*n=89421*	*n=31570*
No	5.1	3.9
Yes	94.9	96.1
Hospitalisation for Infection (%)	*n=67108*	*n=25990*
No	97.9	97.3
Yes	2.1	2.7
Hospitalisation for Injury (%)	*n=67108*	*n=25990*
No	90.3	89.4
Yes	9.7	10.6
NSAID Use (%)*	*n=88538*	*n=31312*
No	75.6	70.7
Yes	24.4	29.2
Fish Oil Use (%)*	*n=89282*	*n=31538*
No	84.3	86.0
Yes	15.7	14.0

NSAID, Non-steroidal anti-inflammatory drug. *p<0.01.

Tables 2a and 2b summarise the associations between inflammatory episodes (hospitalisation for infection, hospitalisation for injury, and use of NSAIDs) and the types of major depression (single episode and recurrent episode). A positive history of hospitalisation for infection was associated with higher odds of recurrent major depression (OR=1.38, 95% CI=1.25-1.52; adjusted OR=1.25, 95% CI=1.09-1.42). A positive history of hospitalisation for injury was associated with higher odds of recurrent major depression in the unadjusted model (OR=1.14, 95% CI=1.08-1.20). Regular use of NSAIDs was associated with both higher odds of single episode major depression (OR=1.12, 95% CI=1.06-1.18; adjusted OR=1.09, 95% CI=1.03-1.16), and higher odds of recurrent major depression (OR=1.33, 95% CI=1.29-1.38; adjusted OR=1.23, 95% CI=1.18-1.28), with a stronger association in the latter. By contrast, regular use of fish oil supplement was associated with lower odds of recurrent major depression (OR=0.87, 95% CI=0.83-0.90; adjusted OR=0.93, 95% CI=0.88-0.97).

Table 2a. Associations between inflammatory/anti-inflammatory variables and single/recurrent episode major depression

Category	**Control** (n=89467)	**Single episode major depression** (n=7909)			**Recurrent episode major depression** (n=23677)		
			OR	95% CI		OR	95% CI
Hospitalised for Infection (%)							
No	97.9	98.1	1.00	Reference	97.1	1.00	Reference
Yes	2.1	1.9	0.90	0.74-1.08	2.9	**1.38**	**1.25-1.52***
Hospitalised for Injury (%)							
No	90.3	90.6	1.00	Reference	89.1	1.00	Reference
Yes	9.7	9.4	0.97	0.89-1.06	1.9	**1.14**	**1.08-1.20***
NSAID Use (%)							
No	75.6	73.5	1.00	Reference	69.9	1.00	Reference
Yes	24.4	26.5	**1.12**	**1.06-1.18***	30.1	**1.33**	**1.29-1.38***
Fish Oil Use (%)							
No	84.3	85.6	1.00	Reference	86.1	1.00	Reference
Yes	15.7	14.4	**0.91**	**0.85-0.97***	13.9	**0.87**	**0.83-0.90***

OR, Odds Ratio; CI, Confidence Interval. *p<0.01.

Table 2b. Associations between inflammatory/anti-inflammatory variables and single/recurrent episode major depression (adjusted model)

Category	**Control** (n=89467)	**Single episode major depression** (n=7909)			**Recurrent episode major depression** (n=23677)		
			OR	95% CI		OR	95% CI
Hospitalised for Infection (%)							
No	97.9	98.1	1.00	Reference	97.1	1.00	Reference
Yes	2.1	1.9	0.95	0.76-1.18	2.9	**1.25**	**1.09-1.42***
Hospitalised for Injury (%)							
No	90.3	90.6	1.00	Reference	89.1	1.00	Reference
Yes	9.7	9.4	0.94	0.85-1.04	1.9	**1.06**	**1.00-1.13**
NSAID Use (%)							
No	75.6	73.5	1.00	Reference	69.9	1.00	Reference
Yes	24.4	26.5	**1.09**	**1.03-1.16***	30.1	**1.23**	**1.18-1.28***
Fish Oil Use (%)							
No	84.3	85.6	1.00	Reference	86.1	1.00	Reference
Yes	15.7	14.4	0.96	0.89-1.03	13.9	**0.93**	**0.88-0.97***

OR, Odds Ratio; CI, Confidence Interval. Regression model adjusted for age, education, ethnicity, income, employment, smoking and alcohol status, social isolation, chronic illness/disability. *p<0.01.

Tables 3a and 3b show the association between cumulative inflammatory load and lifetime experience of major depression. Hospitalisation for infection, hospitalisation for injury and use of NSAIDs each represented a marker of inflammation. Increasing the number of inflammatory markers did not appear to be associated with higher odds of single episode major depression. But for recurrent depression, the greater the number of inflammatory markers, the higher the odds of recurrent episode major depression. There was a positive trend between the level of inflammation and odds of recurrent major depression (p-value for linear trend <0.01). Subanalysis was performed for class of infective pathogen and type of injury diagnosed during hospitalisation. As the purpose was to identify whether a certain class of pathogen or injury type was associated with depression, those with mixed infections or injuries were not analysed. For infections, the two most common classes of pathogens were virus and bacteria. Parasite and fungal infection numbers were both relatively small, thus they were grouped as "other infections" for more meaningful statistical analysis. Viral infection was associated with recurrent major depression (OR=1.65, 95% CI=1.43-1.90; adjusted OR=1.42, 95% CI=1.18-1.71), but not the other infections. Head injury and torso injury were both associated with recurrent major depression (OR=1.33, 95% CI=1.18-1.49 and OR=1.28,

95% CI=1.08-1.51 respectively), but not in the adjusted model. Sensitivity analysis was performed by stratifying covariates individually for each analysis, and no significant impact on the main findings was found. For all regression analyses, multicollinearity was examined for in each model. The variable inflation factors for independent variables were all <2.5 (tolerance value >0.4), suggesting that multicollinearity was not an issue in the models used.

Table 3a. Associations between inflammation levels and single/recurrent episode major depression

Category	**Control**	**Single episode major depression**			**Recurrent episode major depression**		
	n	n	OR	95% CI	n	OR	95% CI
Levels							
0	43281	4085	1.00	Reference	11674	1.00	Reference
1	25388	2418	1.00	0.96-1.06	7950	**1.16**	**1.12-1.19***
2	2048	186	0.96	0.83-1.21	876	**1.59**	**1.46-1.72***
3	34	5	1.56	0.61-3.99	26	**2.84**	**1.70-4.72***

*p<0.01.

Definition of levels:

Level 0: No Hospitalisation for Injury, No Hospitalisation for Infection & No NSAIDs Use

Level 1: 1 out of Hospitalisation for Injury, Hospitalisation for Infection or NSAIDs Use

Level 2: 2 out of Hospitalisation for Injury, Hospitalisation for Infection or NSAIDs Use

Level 3: Hospitalisation for Injury & Hospitalisation for Infection & NSAIDs Use

Table 3b. Associations between inflammation levels and single/recurrent episode major depression (adjusted model)

Category	Control	Single episode major depression			Recurrent episode major depression		
	n	n	OR	95% CI	n	OR	95% CI
Levels							
0	43281	4085	1.00	Reference	11674	1.00	Reference
1	25388	2418	1.02	0.95-1.08	7950	**1.09**	**1.05-1.13***
2	2048	186	0.91	0.76-1.10	876	**1.36**	**1.23-1.51***
3	34	5	2.10	0.68-6.47	26	**2.63**	**1.32-5.27***

OR, Odds Ratio; CI, Confidence Interval. Regression model adjusted for age, education, ethnicity, income, employment, smoking and alcohol status, social isolation, chronic illness/disability. *p<0.01.

Definition of levels:

Level 0: No Hospitalisation for Injury, No Hospitalisation for Infection & No NSAIDs Use

Level 1: 1 out of Hospitalisation for Injury, Hospitalisation for Infection or NSAIDs Use

Level 2: 2 out of Hospitalisation for Injury, Hospitalisation for Infection or NSAIDs Use

Level 3: Hospitalisation for Injury & Hospitalisation for Infection & NSAIDs Use

Table 4a. Associations between hospital infection/injury subtype and recurrent episode major depression

Category	Control	Recurrent episode major depression		
	n	n	OR	95% CI
Infection Type				
No Infection	65680	19038	1.00	Reference
Viral Infection	597	286	**1.65**	**1.43-1.90***
Bacterial Infection	658	220	1.15	0.99-1.34
Other Infections	173	65	1.30	0.97-1.72
Injury Type				
No Injury	60568	17466	1.00	Reference
Head Injury	991	380	**1.33**	**1.18-1.49***
Torso Injury	529	195	**1.28**	**1.08-1.51***
Upper Limb Injury	2653	789	1.03	0.95-1.11
Lower Limb Injury	1923	598	1.08	0.98-1.18

OR, Odds Ratio; CI, Confidence Interval. *p<0.01.

Table 4b. Associations between hospital infection/injury subtype and recurrent episode major depression (adjusted model)

Category	Control	Recurrent episode major depression		
	n	n	OR	95% CI
Infection Type				
No Infection	65680	19038	1.00	Reference
Viral Infection	597	286	**1.42**	**1.18-1.71***
Bacterial Infection	658	220	1.05	0.86-1.28
Other Infections	173	65	1.30	0.90-1.88
Injury Type				
No Injury	60568	17466	1.00	Reference
Head Injury	991	380	1.11	0.96-1.30
Torso Injury	529	195	1.19	0.96-1.47
Upper Limb Injury	2653	789	1.08	0.98-1.19
Lower Limb Injury	1923	598	0.99	0.88-1.11

OR, Odds Ratio; CI, Confidence Interval. Regression model adjusted for age, education, ethnicity, income, employment, smoking and alcohol status, social isolation, chronic illness/disability. *p<0.01.

D) Discussion

In this UK Biobank (UKB) study, the lifetime prevalence of major depression was 26%. In the 2014 Health Survey for England (HSE)[90], 19% reported that they were ever diagnosed with depression by a healthcare professional. Considering only older adults aged 35-64 in the HSE, the lifetime prevalence of depression was 21-25%[90], which is comparable to UKB's prevalence of major depression. In the United States, a large nationwide study which used the DSM-V structured clinical interview for diagnosis found that 20.6% of the adult population had a lifetime

diagnosis of major depression[91]. Adults aged 30-64 had a lifetime prevalence of 22-23%[91], which is again comparable to those similarly aged in the UKB cohort.

There are some differences in sociodemographic and health-related characteristics between the UKB cohort and the general population of the same age. For example, comparisons made with data from the UK Census and Health Survey of England found that UKB participants were more likely to own property, were taller and leaner, had fewer current smokers, and had lower total cancer incidence rates[92]. There is some evidence of a "healthy volunteer" selection bias in the UKB sample. Although these differences exist, when investigating an association between exposure and disease, experts argue that it is more important that the sample is large and has good internal validity, than for the participants to be nationally representative[93]. The UKB may not be suited for generating national disease prevalence and incidence rates, but its large size and variety of exposures offer invaluable data for studying exposure and disease relationships.

The exposure of interest in this study was inflammation. Hospitalisation for infection and injury were two markers chosen to represent severe episodes of inflammation, while regular use of NSAIDs was chosen to represent more common but less severe inflammation in the community setting. The outcome of interest was major depression. The temporal relationship between exposure and disease could not be firmly established because the time periods of major depressive episodes were not captured in the UKB. Although causality could not be ascertained, this study did allow exploration into whether there was a 'dose-response' relationship between exposure and disease, and whether the observations were reproducible, coherent and biological plausible, as per Hill's criteria for causal inference[94].

It was shown that increasing levels of inflammation was associated with progressively higher odds of recurrent major depression. With two instances of inflammation, the odds of recurrent major depression increased by 130% and with three instances the odds increased by 240%. To our knowledge no epidemiological study has shown a dose-response relationship with this combination of variables. There was a previous study that affirmed this dose-dependent relationship between

inflammatory load and mood disorder, but it used a different set of variables. The longitudinal Danish study showed that autoimmune disease and hospitalisation for infection together increased the subsequent risk of mood disorder by 1.5 fold (IRR=2.35, 95% CI=2.25-2.46), compared to having either autoimmune disease (IRR=1.45, 95% CI=1.39-1.52) or hospitalisation for infection (IRR=1.62, 95% CI=1.60-1.64) alone[70]. The Danish results supported the findings of our UKB study – it added temporality to the dose-response association through prospective analysis. Both the UKB and Danish study observed a similar dose-response association, but through different inflammation variables and different cohorts, which adds reproducibility and consistency to the finding.

A strength of the UKB study was that it used an outcome variable that was more specific to major depression, and further distinguished single episode from recurrent episode major depression, whereas the Danish study used a non-specific group of mood disorders as a collective outcome. Having two forms of depression helped create another biological gradient, which added depth to analysis. The above results showed that as the level of inflammation rose, the odds of major

depression increased. However, the results also revealed that at each level of inflammation, the association with recurrent major depression was stronger than with single episode depression (which had mostly trace to no association). This suggests that recurrent major depression has an inflammatory aetiology, whereas single episode depression may have a more heterogeneous aetiology. If this is true, then taking products with anti-inflammatory properties should protect against recurrent major depression.

In this study, regular fish oil consumption was indeed associated with lower odds of recurrent major depression but had no association with single episode depression. These findings suggest that taking fish oil, which is anti-inflammatory, may be protective against an inflammatory type of depression (recurrent major depression), but not a non-inflammatory type of depression (single episode depression). The inability to resolve temporal relationships in this study also applies here but having an additional association that suggest that lowering inflammation benefits depression adds coherence to the other results and strengthens the biological plausibility of the inflammation hypothesis. NSAIDs did not show a protective effect as expected. This is because

NSAIDs are taken in response to underlying acute or chronic inflammation, and even when taken they do not eradicate the inflammatory processes entirely. They are also not taken on a long-term basis when not indicated because of common gastrointestinal adverse effects as well as other adverse effects[95]. Conversely, fish oil is a dietary supplement that is often taken regularly in good health and not in response to active symptoms of inflammation. It can also be taken over a long period with negligible adverse effects when taken in moderation[96]. Hence compared to NSAIDs, fish oil consumption is more likely to be used over the long term while in good health for it to confer prophylactic protective effects.

Thus far, the associations between exposure and outcome appear to support the inflammatory hypothesis of depression. But as with all epidemiological studies, confounding may exist. Not all sources of inflammation were included in the investigation model, for example less severe infections and injuries that did not require hospitalisation but were treated by general practitioners (GPs) in the outpatient setting, or infections and injuries that did not present to the healthcare system. Psychological stress or trauma from childhood to adult life, which is

known to also trigger inflammatory responses, were not accounted for in this study. These potential confounders could be controlled for by including them in the study in the future, when the data becomes available. The UKB piloted primary care data linkage for a subset of participants in 2016 and are in the process of expanding primary care linkage to the entire cohort (https://www.ukbiobank.ac.uk/wp-content/uploads/2018/11/EMIS.pdf). This would allow us to examine the impact of infections and injuries that are treated in primary care (likely markers of moderate inflammation) on major depression. For mild infections and injuries that do not require medical attention, the inflammatory load is probably low and may not produce associations that are strong enough to be observed. With regards to psychological stress, the UKB Mental Health Consortium devised a web-based questionnaire that was sent to existing UKB participants in 2017. This online questionnaire contained questions about childhood trauma and past psychological distress that impaired function. This additional data would allow us to adjust for the effects of major psychological stress in future which is currently absent in our regression model. Data from the online mental health questionnaire was still being accrued at the time of writing. As for known sociodemographic and lifestyle confounders, they were identified and adjusted for statistically with multivariate regression

analysis. All results were presented in crude and adjusted values for comparison.

Misclassification of the outcome may bias inferences drawn from the data. The UKB steering committee decided early on that it was prohibitively expensive and impractical to use the Structured Clinical Interview for DSM (SCID) as the 'gold standard' to diagnose major depression. Hence, the UKB used two core major depression symptoms instead of the full DSM criteria for case definition. This approach is akin to using the Patient Health Questionnaire-2 (PHQ-2), a widely validated screening instrument that targets the same core MDD symptoms[96]. The UKB case definition of MDD in this study is similar to scoring ≥3 on the PHQ-2, which has a test sensitivity of 0.83 and specificity of 0.90[97]. Besides symptoms, the UKB definition of MDD also require a past visit to a mental health professional for depression, which improved ecological validity for case definition. The caseness of recurrent episode MDD in UKB is arguably stronger than just using the DSM symptom criteria alone, because an individual is more likely to remember how many times he/she visited a psychiatrist for depression, than the number, frequency and severity of each symptom that fulfilled DSM criteria in

past depressive episodes (i.e. less prone to recall bias). The UKB case definition of MDD have been used to phenotype MDD in previous studies[87,88]. In the newer UKB online mental health questionnaire, the Composite International Diagnostic Interview Short Form (CIDI-SF)[98] is used for MDD diagnosis. The CIDI-SF has been validated against the SCID and showed good concordance (AUC 0.75-0.87)[99]. It remains to be seen whether the CIDI-SF will outperform the original method, and whether the findings of this study will differ.

In observational studies, though causality cannot be established, causal inference may still be exercised to explore the possible meanings behind the association. The introduction has described much evidence for forward causality, so here the evidence for reverse causality will be discussed. The questions to ask in relation to reverse causality would be whether recurrent major depression is likely to cause hospitalisation for infection, hospitalisation for injury, and regular use of NSAIDs. Few studies have reliably examined whether depression will lead to subsequent infection[100-102]. One large (n=976,398) prospective study reported that depression increased the risk of having any type of infection (IRR=1.61, 95% CI=1.49–1.74)[102]. The authors reasoned that

depression would chronically activate the neuroendocrine stress-signalling and neuro-immunological pathways, which over time could alter immunological gene expressions, thus increasing the susceptibility to infection[103-105]. Oddly, this study did not find a linear dose-dependent relationship; the risk of infection decreased for the second depressive episode (IRR=1.49, 95% CI=1.21–1.83) and by the third depressive episode there was no statistically significant risk of infection[102]. These findings displayed a level of incoherence, which casts doubt on whether depression could lead to infection. In terms of depression elevating the risk of physical injury, both negative and positive associations have been reported[106-110]. It is plausible that a depressed individual may engage in deliberate self-harm in response to suicidal thoughts or be involved in unintentional injury due to depressive symptoms like impaired attention, fatigue and poor sleep. But as it stands with current literature, the evidence for reverse causality is limited and unclear. Lastly, for NSAIDs in this study, it was used as a marker of inflammation. So, for reverse causality, the question would be whether depression could cause inflammation. Meta-analyses of longitudinal studies have consistently found that raised inflammatory markers (IL-6, CRP) *preceded* the development of depressive symptoms[111,112], and prospective clinical studies also found that inflammatory conditions preceded the onset of

depression[70,71]. Therefore in light of these findings, reverse causality would appear less likely.

Taking into account the above study limitations and assuming the associations hold true, what then are the biological underpinnings for the association between inflammation and recurrent major depression? Recurrent major depression is a chronic illness characterised by repeated episodes of severe exacerbations and lower-grade symptoms between exacerbations. This waxing and waning characteristic is common to other chronic inflammatory diseases. Chronic inflammatory disease may be the result of a poorly controlled host immune/inflammatory response to environmental stressors. Infection is one example of an environmental stressors. Microbes can cause chronic inflammation through persistent infection. They can continue to live in the host by evading immune surveillance through antigenic mutation/variation (e.g. Human immunodeficiency virus), coating themselves with host proteins (e.g. Treponema pallidum), living within host cells (e.g. Viruses, Mycobacteria tuberculosis), or residing in immunologic sanctuaries within the central nervous system (e.g. human herpesviruses)[113]. Viruses are particularly adept at surviving in the host

through various mechanisms of subverting the host's immune system. Viruses have three main patterns of persistence[113]: 1) Viruses can continue to actively replicate in high titres by being noncytocidal or by invading target cells that are rapidly replaced by cellular proliferation (e.g. Hepatitis B virus); 2) Viruses can become latent and the viral genome goes into non-replicating mode or is integrated into the host genome, allowing for intermittent activations when host conditions are optimal (e.g. Herpes simplex virus, Varicella-zoster virus); 3) Viruses can continue to be infective with low level replication and cell-to-cell transmission (e.g. Paramyxoviruses, Lentiviruses). In the above examples, the primary pathogen fails to get eliminated and the host continues to mount a low-grade or intermittent inflammatory response towards the agent.

Our UKB results appear to support this pathoaetiology. Hospitalisation for infection was associated with recurrent major depression, and in the subanalysis, viral infection was the only class of infection that had a significant association with recurrent major depression. Further supporting this observation is a meta-analysis of case-control studies that examined the relationship between microbial (viral, bacterial, fungal and

parasitic) infections and depression[69]. Of all the infective agents, only five had statistically significant associations with depression and four of them were viruses (Epstein-Barr virus, Herpes simplex virus-1, Varicella zoster virus and Borna disease virus)[69]. These infectious agents typically cause diseases that can be managed in the outpatient setting. It is interesting that in our study, viral infections in the inpatient setting were also associated with depression. Viruses that lead to acute hospitalisations are frequently respiratory and gastrointestinal infections and they typically have causative agents that are different from the ones identified in the above meta-analysis. In our study, due to sample size limitations, it was not possible to further stratify analysis for individual viruses and depression.

Now that we have identified a source of inflammation, how does inflammation affect mood? Inflammatory cytokines can alter the monoamine neurotransmitter pathways and influence the balance of serotonin, norepinephrine and dopamine in the brain. These neurotransmitter pathways have been the target of antidepressant drugs since the 1950s. Tryptophan is an amino acid used for the synthesis of serotonin. Tryptophan is metabolised through one of two competing

pathways – the serotonin pathway or the kynurenine pathway. Tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) are major enzymes in the kynurenine pathway. IDO activity is increased by pro-inflammatory cytokines such as IFN-α and TNF-α. TDO activity is increased by cortisol, which is released when cytokines activate the hypothalamic–pituitary-adrenal axis. More tryptophan gets metabolised though the kynurenine pathway activity and thus less tryptophan becomes available for serotonin synthesis through the serotonin pathway[114]. Cytokines like IL-6 and TNF-α also induce the subsequent enzymatic metabolism of serotonin[115,116]. Serotonin reuptake back into the neuron is increased by IL1-β, TNF-α and IFN-α through increased synthesis serotonin transporters[117]. Dopamine levels are lowered by IL-6, IL-2, and TNF-α[118,119] and IL-1β increases dopamine turnover in the hippocampus and hypothalamus[120,121]. The net effect of pro-inflammatory cytokine activity is reduced monoaminergic neurotransmission, and this may precipitate or prolong a depressive episode in vulnerable individuals. Glutamatergic neurotransmission has also been implicated in major depression, where overactivity is viewed as pathological[122]. Inflammation raises IDO expression and shunts tryptophan to the kynurenine pathway, increasing downstream

metabolites like kynurenic acid (KA) and quinolinic acid (QA) in the process[123]. KA is an antagonist while QA is an agonist of glutamate receptors. Inflammatory cytokines and QA together increase glutamate release and decrease glutamate reuptake[124,125], while KA also decreases glutamate reuptake[126]. Therefore, the net effect of inflammation on glutamatergic neurotransmission is still considered stimulatory[122,123]. Figure 2 summarises the inflammation-mediated pathways that have been implicated in depression.

Brain-derived neurotrophic factor (BDNF) is involved in neurogenesis and neuroplasticity. It is thought that BDNF plays a protective role in depression. Inflammatory cytokines reduce BDNF release and affects BDNF receptor phosphorylation[127,128]. However, BDNF is also increased by TH2-related cytokines, which underlines the complex relationship between cytokines and BDNF[129]. In the clinical setting, IFN-α administration has been shown to decrease BDNF levels[128] and having the Methionine allele of the BDNF gene (as opposed to the higher expressing Valine/Valine genotype) increases vulnerability to depression during IFN-α treatment[130]. BDNF Val66Met polymorphism highlights the fact that there are genetic variations which may confer either resilience

or susceptibility to depression, hence not everyone will respond similarly when stressed by inflammation.

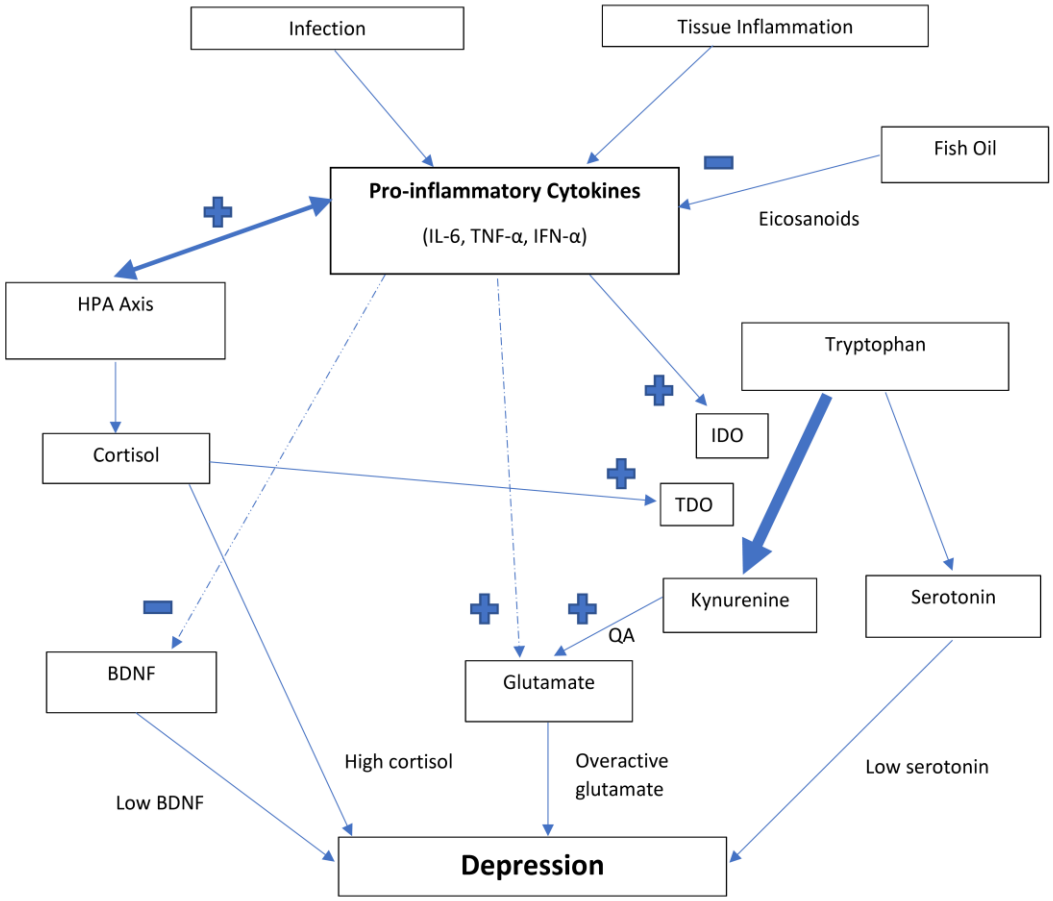

Fig 2. Schematic of the inflammation-mediated pathophysiological pathways in depression.

BDNF: Brain derived neurotrophic factor; HPA: Hypothalamic-pituitary-adrenal; IDO: indoleamine 2,3-dioxygenase; QA: Quinolinic acid; TPO: Tryptophan 2,3-dioxygenase.

The hypothalamic–pituitary-adrenal (HPA) axis exerts profound effects on the brain. Through glucocorticoids, it can regulate neuronal survival, neurogenesis, the formation of new memories and the emotional

appraisal of events. There is HPA dysfunction in depressed patients, with excess cortisol and dexamethasone test non-suppression being the most frequently reported[131,132]. Pro-inflammatory cytokines have stimulatory effects on HPA axis hormones[133] and can disrupt glucocorticoid receptor signalling to alter glucocorticoid-mediated feedback regulation[134], further enhancing pro-inflammatory cytokine release. The HPA axis is highly responsive to psychosocial stress and these stressors may activate the HPA axis in a similar way to inflammatory stress, eliciting similar neuro-immuno-endocrine effects[135]. It is likely that through this pathway, inflammatory processes and psychological factors interact to influence both cognitive and affective symptoms of depression.

Cytokines are usually expressed at very low levels in a healthy brain, but an infectious or inflammatory stimulus could rapidly increase its levels. Cytokines can gain access to the brain through various pathways. One way involves macrophage-like cells that are found in circumventricular organs and the choroid plexus. These choroid plexus macrophages surveil circulating pathogens and will respond to them by releasing inflammatory mediators that activate choroid plexus epithelial cells to

express trafficking molecules, and these molecules may leak cytokines to the cerebral spinal fluid[136]. Another way of gaining access is through areas of the blood brain barrier that are more permeable (e.g. the organum vasculosum laminae terminalis) or where there are active transport mechanisms[137]. Lastly, the integrity of the blood brain barrier may be compromised during pathological states (e.g. seizure)[138]. In addition to peripheral infiltration, cytokines are also centrally expressed at brain regions such as the circumventricular regions, hypothalamus, hippocampus, cerebellum and forebrain. They may be activated when there are severe systemic or central infections or when there is a brain injury or seizure. Cytokines can bind to neuronal receptors, modulate ion channels and activate intracellular second messenger systems[139]. Neurotransmitter function and vascular changes within the brain can also be effected[140].

Proving that neuroinflammation exists in the brain of actively depressed patients has been difficult. Obtaining brain tissue or cerebral spinal fluid from actively depressed patients is intrinsically challenging. But recent advances in neuroimaging has finally allowed us to investigate this more convincingly. Positron emission tomography (PET) may now be applied

to measure translocator protein (TSPO) binding in vivo. TPSO is a protein that is located on the outer mitochondrial membranes in microglia. TPSO expression is increased when microglia are activated during neuroinflammation. Therefore, higher measures of TSPO implies greater neuroinflammation. Patients in an active major depression episode, relative to matched controls, showed higher TSPO density in all brain regions measured (including the prefrontal cortex, anterior cingulate and insula)[141]. This finding was replicated in a later study[142]. In the last study, researchers found that patients with longer duration of depression had higher levels of TSPO relative to patients with a shorter duration of depression and to controls[143]. These findings support our observation that the recurrent depression group is associated with more inflammation that the single depression episode group.

There seems to be a plausible biological relationship between infection and depression through an immunoinflammatory link. What about injury? In this study, hospitalisation for injury had an association with recurrent depression in the unadjusted model. Results were adjusted for chronic disability and the association was lost. This implied that it might have been the loss of function after injury that was associated with

recurrent depression, not the acute injury itself. Both infection and injury are common precipitants of an inflammatory response, but from what has been discussed, infections seem more capable of perpetuating a prolonged inflammatory course and hence resulted in a stronger association with chronic recurrent depression.

Regular fish oil supplementation showed a protective effect against recurrent major depression, a form of depression that appears to have a stronger inflammatory aetiology. The anti-inflammatory mechanisms of action of fish oil (omega-3 fatty acid) were explained in the introduction. Studies have attempted to quantify omega-3 fatty acids in depressed individuals. Depressed subjects, compared with healthy controls, had lower serum concentrations of omega-3 fatty acids and higher omega-6 fatty acids to omega-3 fatty acids ratio[144,145], which seems to suggest that low dietary fish oil may contribute towards depression. In our UKB study, the dose, duration and frequency of fish oil supplementation was not captured, and the temporality of its use in relation to depression could not be established. Even if those information were available, the efficacy of fish oil supplementation will still need to be determined in a randomised clinical trial. Two recent meta-analyses on the adjuvant use

of omega-3 fatty acids in clinical trials found it had only small-moderate treatment effects in MDD[146,147]. Where our UKB results may help would be to guide the selection of participants. Since our results indicate that fish oil supplementation only protected against recurrent major depression, future trials may evaluate whether including only patients with recurrent episode major depression might result in bigger treatment effects.

This chapter used an epidemiological approach to study the relationship between inflammation and depression. The associations derived prompt further thought into the role of inflammation and the course of illness, and how manipulating our body's inflammatory response might change the course of illness. The next chapter will complement this chapter by investigating whether a novel and rapid acting antidepressant agent is able to alter the morphology and function of inflammation regulating microglial cells, and whether the antidepressant mechanism of action involve receptors that are located on immune cells.

E) Summary

Findings in this chapter showed that recurrent major depression was more strongly associated with inflammation-causing conditions than single episode depression. There was a significant dose-response relationship between the number of inflammatory events and the odds of recurrent major depression. Of the infective events, viral infection showed the most significant association with recurrent depression. Regular fish oil supplementation may be protective against the recurrent depression.

F) References

1. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386:743–800.

2. World Health Organization. Depression Fact Sheet. Available from: http://www.who.int/mediacentre/factsheets/fs369/en/ [Accessed 2nd January 2018].

3. World Health Organisation. The ICD-10 Classification of mental and behavioural disorders; clinical descriptions and diagnostic guidelines. Genova: World Health Organisation; 1992.

4. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th Edition (Text Revised). Washington, DC: American Psychiatric Association; 2000.

5. Boland RJ, Keller MB. The course of depression. In: Davis KL, Charney D, Coyle J., Nemeroff C, editors. Neuropsychopharmacology, the 5th Generation of

Progress New York: American College of Neuropsychopharmacology; 2002:1009–1015.

6. Spijker J, de Graaf R, Bijl RV, Beekman AT, Ormel J, Nolen WA. Duration of major depressive episodes in the general population: results from The Netherlands Mental Health Survey and Incidence Study (NEMESIS). British Journal of Psychiatry. 2002 Sep;181:208–13.

7. Eaton WW, Shao H, Nestadt G, Lee HB, Bienvenu OJ, Zandi P. Population-based study of first onset and chronicity in major depressive disorder. Arch Gen Psychiatry 2008;65:513–20.

8. Mattisson C, Bogren M, Horstmann V, Munk-Jörgensen P, Nettelbladt P. The long-term course of depressive disorders in the Lundby Study. Psychol Med. 2007 Jun;37(6):883-91.

9. Mueller TI, Leon AC, Keller MB, Solomon DA, Endicott J, Coryell W, Warshaw M, Maser JD. Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. Am J Psychiatry. 1999 Jul;156(7):1000-6.

10. Maj M, Veltro F, Pirozzi R, Lobrace S, Magliano L. Pattern of recurrence of illness after recovery from an episode of major depression: a prospective study. Am J Psychiatry. 1992 Jun;149(6):795-800.

11. American Psychiatric Association (2000). Diagnostic and statistical manual of mental disorders (4th ed., text rev.). Washington, DC: Author.

12. Solomon DA, Keller MB, Leon AC, Mueller TI, Lavori PW, Shea MT, Coryell W, Warshaw M, Turvey C, Maser JD, Endicott J. Multiple recurrences of major depressive disorder. Am J Psychiatry. 2000 Feb;157(2):229-33.

13. Merikangas KR, Wicki W, Angst J. Heterogeneity of depression. Classification of depressive subtypes by longitudinal course. Br J Psychiatry. 1994 Mar;164(3):342-8

14. Wilhelm K, Parker G, Dewhurst-Savellis J, Asghari A. Psychological predictors of single and recurrent major depressive episodes. Journal of Affective Disorders 1999;54:139–147.

15. Wittchen HU, Nelson CB, Lachner G. Prevalence of mental disorders and psychosocial impairments in adolescents and young adults. Psychological Medicine 1998;28:109–126.

16. Klerman GL, Weissman MM. The course, morbidity, and costs of depression. Archives of General Psychiatry 1992;49:831–834.

17. Schmid-Schonbein, GW. Analysis of inflammation. Annual Review of Biomedical Engineering. 2006;8:93–131.

18. Hart BL. Biological basis of the behavior of sick animals. Neuroscience & Biobehavioral Reviews. 1998;12:23–137.

19. Dantzer R. Cytokine-induced sickness behaviour: where do we stand? Brain, Behavior, and Immunity. 2001;15,7–24

20. Shattuck EC, Muehlenbein MP. Human sickness behavior: Ultimate and proximate explanations. American Journal of Physical Anthropology. 2015 May;157(1):1–18.

21. Jones DS, Podolsky SH, Greene JA. The burden of disease and the changing task of medicine. New England Journal of Medicine. 2012 Jun 21;366(25):2333–8.

22. World Health Organization (2017): The Top 10 Causes of Death Fact Sheet. Available from: http://www.who.int/mediacentre/ factsheets/fs310/en/ [Accessed 2nd January 2018].

23. Centres for Disease Control and Prevention. Ten Leading Causes of Death and Injury - 2015. Available from: https://www.cdc.gov/injury/wisqars/Leading Causes.html [Accessed 2nd January 2018].

24. McCaig LF, Burt CW: National Hospital Ambulatory Medical Care Survey:1999 Emergency Department Summary. Advance Data from Vital and Health Statistics 320. Hyattsville, Md, National Centre for Health Statistics, 2001.

25. Watson WL, Ozanne-Smith J: Injury surveillance in Victoria, Australia: developing comprehensive injury incidence estimates. Accid Anal Prev 2000; 32:277–286.

26. Bluthe RM, Michaud B, Kelley KW, Dantzer R. Vagotomy blocks behavioural effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes. Neuroreport. 1996;7:2823–2827.

27. Bluthe RM, Laye S, Michaud B, Combe C, Dantzer R, Parnet P. Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. European Journal of Neuroscience. 2000;12:4447–4456.

28. Kent S, Bluthe RM, Dantzer R, Hardwick AJ, Kelley KW, Rothwell NJ, Vannice JL. Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. Proceedings of the National Academy of Sciences of the United States of America. 1992;89:9117–9120.

29. Skelly DT, Hennessy E, Dansereau, MA, Cunningham C. A systematic analysis of the peripheral and CNS effects of systemic LPS, IL-1Beta, TNF-alpha and IL-6 challenges in C57BL/6 mice. PLoS ONE. 2013 Jul 1;8(7):e69123.

30. Stefferl A, Hopkins SJ, Rothwell NJ, Luheshi GN. The role of TNF-alpha in fever: opposing actions of human and murine TNF-alpha and interactions with IL-beta in the rat. British Journal of Pharmacology. 1996; 118:1919–1924.

31. Wieczorek M, Swiergiel AH, Pournajafi-Nazarloo H, Dunn AJ. Physiological and behavioral responses to interleukin-1beta and LPS invagotomised mice. Physiology & Behavior. 2005;85:500–511.

32. Palin K, Bluthe RM, McCusker RH, Moos F, Dantzer R, Kelley KW. TNF alpha-induced sickness behavior in mice with functional 55 kD TNF receptors is blocked by central IGF-I. Journal of Neuroimmunology. 2007;187,55–60.

33. Palin K, McCusker RH, Strle K, Moos F, Dantzer R, Kelley KW. Tumour necrosis factor-alpha-induced sickness behaviour is impaired by central administration of an inhibitor of c-jun N-terminal kinase. Psychopharmacology. 2008;197:629–635.

34. Palin K, Bluthe RM, McCusker RH, Levade T, Moos F, Dantzer R, Kelley KW. The type 1 TNF receptor and its associated adapter protein, FAN, are required for TNFalpha-induced sickness behaviour. Psychopharmacology. 2009;201:549–556.

35. Dantzer R. Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am. 2009;29(2):247-64.

36. Bluthe RM, Michaud B, Poli V, Dantzer R. 2000b. Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. Physiology and Behaviour. 2000b;70:367–373.

37. Harden LM, du Plessis I, Poole S, Laburn HP. Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behaviour. Physiology and Behaviour. 2006;89:146–155.

38. Harden LM, du Plessis I, Poole S, Laburn HP. Interleukin (IL)-6 and IL-1 beta act synergistically within the brain to induce sickness behaviour and fever in rats. Brain, Behaviour, and Immunity. 2008;22:838–849.

39. Howren MB, Lamkin DM, Suls J. Associations of depression with C reactive protein, IL-1, and IL-6: a meta-analysis. Psychosomatic Medicine. 2009;71:171-186.

40. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL. A meta-analysis of cytokines in major depression. Biological Psychiatry. 2010;67:446–457.

41. Liu Y, Ho RC, Mak A: Interleukin (IL)-6, tumour necrosis factor alpha (TNFα) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major

depressive disorder: a meta-analysis and meta-regression. Journal of Affective Disorders. 2012;139:230–239.

42. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, Stubbs B, Solmi M, Veronese N, Herrmann N, Raison CL, Miller BJ, Lanctôt KL, Carvalho AF. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatrica Scandinavica. 2017;135(5):373–387.

43. Köhler CA, Freitas TH, Stubbs B, Maes M, Solmi M, Veronese N, de Andrade NQ, Morris G, Fernandes BS, Brunoni AR, Herrmann N, Raison CL, Miller BJ, Lanctôt KL, Carvalho AF. Peripheral Alterations in Cytokine and Chemokine Levels After Antidepressant Drug Treatment for Major Depressive Disorder: Systematic Review and Meta-Analysis. Mol Neurobiol. 2018;55(5):4195-4206.

44. Taylor JL, Grossberg SE. The effects of interferon-alpha on the production and action of other cytokines. Seminars in Oncology. 1998;25(1 Suppl 1):23–9.

45. Cassidy EM, Manning D, Byrne S, Bolger E, Murray F, Sharifi N, Wallace E, Keogan M, O'Keane V. Acute effects of low-dose interferon-alpha on serum cortisol and plasma interleukin-6. Journal of Psychopharmacology. 2002;16(3):230–4.

46. Capuron L, Raison CL, Musselman DL, Lawson DH, Nemeroff CB, Miller AH. Association of exaggerated HPA axis response to the initial injection of interferon-alpha with development of depression during interferon-alpha therapy. American Journal of Psychiatry. 2003;160:1342–1345.

47. Bonaccorso S, Puzella A, Marino V, Pasquini M, Biondi M, Artini M, Almerighi C, Levrero M, Egyed B, Bosmans E, Meltzer HY, Maes M. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Research. 2001;105(1-2):45–55.

48. Wichers MC, Kenis G, Koek GH, Robaeys G, Nicolson NA, Maes M. Interferon-alpha-induced depressive symptoms are related to changes in the cytokine network but not to cortisol. Journal of Psychosomatic Research. 2007;62(2):207–14.

49. Raison CL, Borisov AS, Woolwine BJ, Massung B, Vogt G, Miller AH. Interferon-alpha effects on diurnal hypothalamic-pituitary-adrenal axis activity: relationship with proinflammatory cytokines and behaviour. Molecular Psychiatry. 2010;15(5):535–47.

50. Capuron L, Gumnick JF, Musselman DL, Lawson DH, Reemsnyder A, Nemeroff CB, Miller AH. Neurobehavioural effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology. 2002; 26(5),643–52.

51. Constant A, Castera L, Dantzer R, Couzigou P, de Ledinghen V, Demotes-Mainard J, Henry C. Mood alterations during interferon alfa therapy in patients with chronic hepatitis C: evidence for an overlap between manic/hypomanic and depressive symptoms. Journal of Clinical Psychiatry. 2005;66:1050–1057.

52. Dieperink E, Ho SB, Tetrick L, Thuras P, Dua K, Willenbring ML. Suicidal ideation during interferon-α2b and ribavirin treatment of patients with chronic hepatitis C. General Hospital Psychiatry. 2004;26:237–240.

53. Wichers MC, Koek GH, Robaeys G, Praamstra AJ, Maes M. Early increase in vegetative symptoms predicts IFN-alpha-induced cognitive-depressive changes. Psychological Medicine. 2005;35:433–41.

54. Udina M, Hidalgo D, Navinés R, Forns X, Solà R, Farré M, Capuron L, Vieta E, Martín-Santos R. Prophylactic antidepressant treatment of interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. Journal of Clinical Psychiatry. 2014;75(10):e1113–21.

55. Malaguarnera M, Laurino A, Di Fazio I, Pistone G, Castorina M, Guccione N, Rampello L. Neuropsychiatric effects and type of IFN-alpha in chronic hepatitis C. Journal of Interferon & Cytokine Research. 2001;21(5):273–8

56. Udina M, Castellví P, Moreno-España J, Navinés R, Valdés M, Forns X, Langohr K, Solà R, Vieta E, Martín-Santos R. Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. Journal of Clinical Psychiatry. 2012;73(8):1128–38.

57. Machado MO, Oriolo G, Bortolato B, Köhler CA, Maes M, Solmi M, Grande I, Martín-Santos R, Vieta E, Carvalho AF. Biological mechanisms of depression following treatment with interferon for chronic hepatitis C: A critical systematic review. Journal of Affective Disorders. 2017;209:235–45.

58. Friebe A, Horn M, Schmidt F, Janssen G, Schmid-Wendtner MH, Volkenandt M, Hauschild A, Goldsmith CH, Schaefer M. Dose-dependent development of depressive symptoms during adjuvant interferon-{alpha} treatment of patients with malignant melanoma. Psychosomatics. 2010;51:466–73.

59. Chiu WC, Su YP, Su KP, Chen PC. Recurrence of depressive disorders after interferon-induced depression. Translational Psychiatry. 2017;7(2):e1026.

60. Valkanova V, Ebmeier KP, Allan CL. CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. Journal of Affective Disorders. 2013;150(3):736–44.

61. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. Brain, Behavior and Immunity. 2015;49:206–15.

62. Ole Köhler-Forsberg, Liselotte Petersen, Christiane Gasse, Preben B. Mortensen, Soren Dalsgaard, Robert H. Yolken, Ole Mors, Michael E. Benros. A Nationwide Study in Denmark of the Association Between Treated Infections and the Subsequent Risk of Treated Mental Disorders in Children and Adolescents. JAMA Psychiatry, 2019 Mar 1;76(3):271-279.

63. Khandaker GM, Stochl J, Zammit S, Goodyer I, Lewis G, Jones PB. Childhood inflammatory markers and intelligence as predictors of subsequent persistent depressive symptoms: a longitudinal cohort study. Psychol Med. 2018 Jul;48(9):1514-1522.

64. Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB. Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. JAMA Psychiatry. 2014 Oct;71(10):1121-8.

65. Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. Mol Psychiatry. 2016 May;21(5):642-9.

66. Baumeister D, Lightman SL, Pariante CM. The interface of stress and the HPA axis in behavioural phenotypes of mental illness. Curr Top Behav Neurosci 2014; 18: 13–24.

67. Zunszain PA, Anacker C, Cattaneo A, Carvalho LA, Pariante CM. Glucocorticoids, cytokines and brain abnormalities in depression. Prog Neuro-Psychopharmacology Biol Psychiatry 2011; 35: 722–729.

68. Pace TWW, Hu F, Miller AH. Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav Immun 2007; 21: 9–19.

69. Wang X, Zhang L, Lei Y, Liu X, Zhou X, Liu Y, Wang M, Yang L, Zhang L, Fan S, Xie P. Meta-analysis of infectious agents and depression. Sci Rep. 2014;4:4530

70. Benros ME, Waltoft BL, Nordentoft M, Ostergaard SD, Eaton WW, Krogh J, Mortensen PB. Autoimmune diseases and severe infections as risk factors for mood disorders: a nationwide study. JAMA Psychiatry. 2013;70(8):812-20.

71. Jeng JS, Li CT, Chen MH, Lin WC, Bai YM, Su TP, et al. Repeated low-grade infections predict antidepressant-resistant depression: a nationwide population-based cohort study. Journal of Clinical Psychiatry. 2018;79(1):17m11540.

72. Patten SB, Williams JV, Lavorato DH, Eliasziw M. Major depression and injury risk. Can J Psychiatry. 2010 May;55(5):313-8.

73. Skogstad L, Tøien K, Hem E, Ranhoff AH, Sandvik L, Ekeberg Ø. Psychological distress after physical injury: a one-year follow-up study of conscious hospitalised patients. Injury. 2014 Jan;45(1):289-98.

74. Cashman JN. The mechanisms of action of NSAIDs in analgesia. Drugs. 1996;52 Suppl 5:13-23.

75. Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. Blood. 2007 Mar 15;109(6):2285-92.

76. Elkind MS. Inflammation, atherosclerosis, and stroke. Neurologist. 2006 May;12(3):140-8.

77. Moriya J. Critical roles of inflammation in atherosclerosis. J Cardiol. 2019 Jan;73(1):22-27.

78. Boissonneault GA. Dietary fat, immunity, and inflammatory disease. In: Chow CK, editor. Fatty Acids in Foods and Their Health Implications. New York: Marcel Dekker: 2000. p. 777–807.

79. Logan AC. Neurobehavioural aspects of omega-3 fatty acids: possible mechanisms and therapeutic value in major depression. Alternative Medicine Review. 2003;8:410–425.

80. Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysohoou C, Skoumas Y, Stefanadis C. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. Journal of the American College of Cardiology. 2005;46(1):120–124.

81. Niu K, Hozawa A, Kuriyama S, Ohmori-Matsuda K, Shimazu T, Nakaya N et al. Dietary long-chain n-3 fatty acids of marine origin and serum C-reactive protein concentrations are associated in a population with a diet rich in marine products. American Journal of Clinical Nutrition. 2006;84(1):223–229.

82. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. Circulation. 2003;108(2): 155–160.

83. He K, Liu K, Daviglus ML, Jenny NS, Mayer-Davis E, Jiang R et al. Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). American Journal of Cardiology. 2009;103(9):1238–1243.

84. Rangel-Huerta OD, Aguilera CM, Mesa MD, Gil A. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomakers: a

systematic review of randomised clinical trials. British Journal of Nutrition. 2012 Jun;107 Suppl 2:S159–70.

85. Rationale, design and development of a large-scale prospective resource [internet]. UK Biobank. 2007. http://www.ukbiobank.ac.uk/resources/

86. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12: 1–10.

87. Wigmore EM, Clarke TK, Howard DM, Adams MJ, Hall LS, Zeng Y, Gibson J, Davies G, Fernandez-Pujals AM, Thomson PA, Hayward C, Smith BH, Hocking LJ, Padmanabhan S, Deary IJ, Porteous DJ, Nicodemus KK, McIntosh AM. Do regional brain volumes and major depressive disorder share genetic architecture? A study of Generation Scotland (n=19 762), UK Biobank (n=24 048) and the English Longitudinal Study of Ageing (n=5766). Translational Psychiatry. 2017;7(8):e1205.

88. Ward J, Strawbridge RJ, Bailey MES, Graham N, Ferguson A, Lyall DM, Cullen B, Pidgeon LM, Cavanagh J, Mackay DF, Pell JP, O'Donovan M, Escott-Price V, Smith DJ. Genome-wide analysis in UK Biobank identifies four loci associated with mood instability and genetic correlation with major depressive disorder, anxiety disorder and schizophrenia. Translational Psychiatry. 2017;7(11):1264.

89. IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

90. Health Survey for England-2014. Health and Social Care Information Centre, Leeds, 2015. http://healthsurvey.hscic.gov.uk/ support-guidance /public-health/health-survey-for-england-2014/mental-health-problems.aspx

91. Hasin DS, Sarvet AL, Meyers JL, Saha TD, Ruan WJ, Stohl M, Grant BF. Epidemiology of Adult DSM-5 Major Depressive Disorder and Its Specifiers in the United States. JAMA Psychiatry. 2018 Apr 1;75(4):336-346. Rothman KJ. Measuring interactions in Epidemiology: An Introduction. New York: Oxford University Press; 2002. p168–180.

92. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, Collins R, Allen NE. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants with Those of the General Population. Am J Epidemiol. 2017 Nov 1;186(9):1026-1034.

93. Rothman K., Gallacher J., Hatch E. Why representativeness should be avoided. Int J Epidemiol. 2013;42:(4):1012–1014.

94. Ward AC. The role of causal criteria in causal inferences: Bradford Hill's "aspects of association". Epidemiol Perspect Innov. 2009 Jun 17;6:2.

95. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal anti-inflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci. 2013;16(5):821-47.

96. Villani AM, Crotty M, Cleland LG, James MJ, Fraser RJ, Cobiac L, Miller MD. Fish oil administration in older adults: is there potential for adverse events? A systematic review of the literature. BMC Geriatr. 2013 May 1;13:41.

97. Kroenke, K., Spitzer, R.L., Williams, J.B., 2003. The patient health questionnaire-2: validity of a two-item depression screener. Med. Care 41, 1284–1292.

98. Kessler, R. C., Andrews, G., Mroczek, D., Ustun, B., & Wittchen, H. U. The World Health Organization composite international diagnostic interview short-form (CIDI-SF).International Journal of Methods in Psychiatric Research. 1998;(4),171–185.

99. Haro JM, Arbabzadeh-Bouchez S, Brugha TS, de Girolamo G, Guyer ME, Jin R, Lepine JP, Mazzi F, Reneses B, Vilagut G, Sampson NA, Kessler RC. Concordance of the Composite International Diagnostic Interview Version 3.0 (CIDI 3.0) with standardized clinical assessments in the WHO World Mental Health surveys. Int J Methods Psychiatr Res. 2006;15(4):167-80.

100. Irwin MR., Levin MJ., Carrillo C et al. Major depressive disorder and immunity to varicella-zoster virus in the elderly. Brain Behav Immun. 2011;25:759– 66.

101. Troidle L., Watnick S., Wuerth DB., Gorban-Brennan N., Kliger AS., Finkelstein FO. Depression and its association with peritonitis in long-term peritoneal dialysis patients. Am J Kidney Dis. 2003;42:350–54.

102. Andersson NW, Goodwin RD, Okkels N, Gustafsson LN, Taha F, Cole SW, Munk-Jørgensen P. Depression and the risk of severe infections: prospective analyses on a nationwide representative sample. Int J Epidemiol. 2016 Feb;45(1):131-9.

103. Irwin MR., Cole SW. Reciprocal regulation of the neural and innate immune systems. Nat Rev Immunol 2011;11:625–32.

104. Glaser R., Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol 2005;5:243–51.

105. Kiecolt-Glaser JK., Glaser R. Depression and immune function: central pathways to morbidity and mortality. J Psychosom Res 2002;53:873–76.

106. Nordstrom DL, Zwerling C, Stromquist AM, et al. Epidemiology of unintentional adult injury in a rural population. J Trauma. 2001;51:758 –766.2.

107. Peele PB, Tollerud DJ. Depression and occupational injury: results of a pilot investigation. J Occup Environ Med. 2005;47:424 – 427.3.

108. Sprince NL, Zwerling C, Lynch CF, et al. Risk factors for falls among Iowa farmers: a case–control study nested in the Agricultural Health Study. Am J Ind Med. 2003;44(3):265–272.4.

109. Sprince NL, Park H, Zwerling C, et al. Risk factors for animal-related injury among Iowa large-livestock farmers: a case-control study nested in the Agricultural Health Study. J Rural Health. 2003;19(2):165–173.

110. Patten SB, Williams JV, Lavorato DH, Eliasziw M. Major depression and injury risk. Can J Psychiatry. 2010;55(5):313-8.

111. Smith KJ, Au B, Ollis L, Schmitz N. The association between C-reactive protein, interleukin-6 and depression among older adults in the community: a systematic review and meta-analysis. Experimental Gerontology. 2018;102:109–132.

112. Valkanova V, Ebmeier KP, Allan CL. CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. Journal of Affective Disorders. 2013;150(3):736-44.

113. Neal Nathanson N, Francisco González-Scarano F. Patterns of Infection: Unwanted guests – Quick Visits and Extended Stays. In: Katze M, Korth MJ, Law L, Nathanson N, editors. Viral Pathogenesis. Third Edition. Elsevier; 2016. p.81–94.

114. Konsman JB, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: Mechanisms and implications. Trends in Neurosciences. 2002;25:154–159.

115. Clement HW, Buschmann J, Rex S, Grote C, Opper D, Gemsa W, Wesemann W. Effects of interferon-gamma, interleukin-1 beta, and tumor necrosis

factor-alpha on the serotonin metabolism in the nucleus raphe dorsalis of the rat. Journal of Neural Transmission. 1997;104:981–91.

116. Hayley S, Wall P, Anisman H. Sensitization to the neuroendocrine, central monoamine and behavioural effects of murine tumor necrosis factor-a: peripheral and central mechanisms. European Journal of Neuroscience. 2002;15:1061–76.

117. Tsao CW, Lin YS, Cheng JT, Lin CF, Wu HT, Wu SR, Tsai WH. Interferon-alpha-induced serotonin uptake in Jurkat T cells via mitogen-activated protein kinase and transcriptional regulation of the serotonin transporter. Journal of Psychopharmacology. 2008;22(7):753–60.

118. Song C. The interaction between cytokines and neurotransmitters in depression and stress: possible mechanism of antidepressant treatments. Hum Psychopharmacology. 2000; 15:199–211.

119. Petitto JM, McCarthy DB, Rinker CM, Huang Z, Getty T. Modulation of behavioural and neurochemical measures of forebrain dopamine function in mice by species-specific interleukin-2. Journal of Neuroimmunology. 1997;73:183–90.

120. Connor TJ, Song C, Leonard BE, Merali Z, Anisman H. An assessment of the effects of central interleukin-1b, -2, -6, and tumor necrosis factor-a administration on some behavioural, neurochemical, endocrine and immune parameters in the rat. Neuroscience. 1998;84(3):923–33.

121. Lacosta S, Merali Z, Anisman H. Influence of interleukin-1b on exploratory behaviours, plasma ACTH, corticosterone, and central biogenic amines in mice. Psychopharmacology (Berl). 1998;137:351–61.

122. M ller N, Schwarz MJ. The immune-mediated alteration of serotonin and glutamate: Towards an integrated view of depression. Molecular Psychiatry. 2007;12: 988–1000.

123. McNally L, Bhagwagar Z, Hannestad J. Inflammation, glutamate, and glia in depression: a literature review. CNS Spectrums. 2008;13:501–10.

124. Ida T, Hara M, Nakamura Y, Kozaki S, Tsunoda S, Ihara H. Cytokine-induced enhancement of calcium-dependent glutamate release from astrocytes mediated by nitric oxide. Neuroscience Letters. 2008;432:232–6.

125. Tilleux S, Hermans E. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. Journal of Neuroscience Research. 2007;85:2059–70.

126. Stone TW. Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection. Trends in Pharmaceutical Sciences. 2000;21:149–54.

127. Cortese GP, Barrientos RM, Maier SF, Patterson SL. Aging and a peripheral immune challenge interact to reduce mature brain-derived neurotrophic factor and activation of TrkB, PLCgamma1, and ERK in hippocampal synaptoneurosomes. Journal of Neuroscience. 2011;31: 4274–4279.

128. Felger JC, Lotrich FE. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience. 2011; 246:199–229.

129. Lisak RP, Benjamins J, Bealmear B, et al. Differential effects of Th1, monocyte/macrophage and Th2 cytokine mixtures on early gene expression for glial and neural-related molecules in central nervous system mixed glial cell cultures: neurotrophins, growth factors and structural proteins. Journal of Neuroinflammation. 2007;4:30.

130. Lotrich FE, Albusaysi S, Ferrell RE. Brain-derived neurotrophic factor serum levels and genotype: association with depression during interferon-alpha treatment. Neuropsychopharmacology. 2013;38:989–95.

131. Sachar E. Cortisol production in depressive illness: a clinical and biochemical clarification. Archives of General Psychiatry. 1971;23:289–298.

132. Carroll BJ. A specific laboratory test for the diagnosis of melancholia: standardization, validation, and clinical utility. Journal of Clinical Endocrinology and Metabolism. 1981;51:433–437.

133. Silverman MN, Pearce BD, Biron CA, Miller AH. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunology. 2005;18:41–78.

134. McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. Endocrine Reviews. 1999;20:435–459.

135. Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychological Review. 1998;105:83–107.

136. Bogie JF, Stinissen P, Hendriks JJ. Macrophage subsets and microglia in multiple sclerosis. Acta Neuropathologica. 2014 Aug;128(2):191–213.

137. Banks WA. Cytokines, CVOs, and the blood–brain barrier. In: R Ader, DL Felten, N Cohen, editors. Psychoneuroimmunology. New York: Academic Press; 2001. p.483–97.

138. Plotkin SR, Banks WA, Maness LM, Kastin AJ. Differential transport of rat and human interleukin-1a across the blood–brain barrier and blood–testis barrier in rats. Brain Research. 2000;881:57–61.

139. Cunningham Jr. ET, DeSouza EB. Interleukin-1 receptors in the brain and endocrine tissue. Immunology Today.1993;14:171–6.

140. Tancredi V, DArcangelo G, Grassi F, Tarroni P, Palmier G, Santoni A et al. Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neuroscience Letters. 1992;146:176–8.

141. Setiaan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G, Suridjan I, Kennedy JL, Rekkas PV, Houle S, Meyer JH. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. JAMA Psychiatry. 2015 Mar;72(3):268–75.

142. Holmes SE, Hinz R, Conen S, Gregory CJ, Matthews JC, Anton-Rodriguez JM, Gerhard A, Talbot PS. Elevated Translocator Protein in Anterior Cingulate in Major Depression and a Role for Inflammation in Suicidal Thinking: A Positron Emission Tomography Study. Biological Psychiatry. 2018;83(1):61–69.

143. Setiawan E, Attwells S, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Xu C, Sharma S, Kish S, Houle S, Meyer JH. Association of translocator protein total distribution volume with duration of untreated major depressive disorder: a cross-sectional study. Lancet Psychiatry. 2018;5(4):339–347.

144. Tiemeier H, van Tuijl HR, Hofman A, Kiliaan AJ, Breteler MM. Plasma fatty acid composition and depression are associated in the elderly: the Rotterdam study. American Journal of Clinical Nutrition. 2003;78:40–46.

145. Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H. Fatty acid composition in major depression: decreased omega-3 fractions in cholesteryl esters

and increased C20:4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. Journal of Affective Disorders. 1996;38:35–46.

146. Appleton KM, Sallis HM, Perry R, Ness AR, Churchill R. ω-3 Fatty acids for major depressive disorder in adults: an abridged Cochrane review. BMJ Open. 2016;6(3):e010172.

147. Mocking RJ, Harmsen I, Assies J, Koeter MW, Ruhé HG, Schene AH. Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. Transl Psychiatry. 2016;6:e756.

Chapter 2

Ketamine's antidepressant effect and the roles of microglial cells and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors

A) Introduction

Major depressive disorder (MDD) is a debilitating mental illness that affects millions of individuals worldwide, and has harmful effects on our general health and the economy[1,2]. However, studies show that current antidepressant medications only provide remission for less than one-third of MDD patients, even after three to four months of treatment[3,4]. Remission in patients often required more than six months of treatment and multiple antidepressant trials[4]. Standard antidepressants also have the inherent issue of delayed therapeutic onset and this continues to be a major bane for patients. The situation becomes more critical in depressed patients who have suicidal ideations, as treatment delays increase the risk of suicide[5,6].

Current antidepressants mainly target the monoamine neurotransmitter system. The key therapeutic effect is believed to be the increase of monoamines (serotonin, norepinephrine, dopamine) in brain synapses. The delayed therapeutic onset suggests that longer-term effects downstream of monoamine signaling are of greater importance[7], though it remains unclear which mechanisms are responsible for treatment efficacy. Clinicians are still using these relatively inefficacious medications for depression because there has been a lack of major breakthroughs in drug development.

Ketamine is emerging as a prototype for a new class of rapid-acting antidepressants. The fact that ketamine could induce an antidepressant response within hours of administration[8,9] took psychiatrists by surprise. Ketamine is an old drug that anaesthesiologists use for anaesthesia. Its use in psychiatry is spurring new research into the glutamatergic neurotransmission system, which is a departure from the long-held monoamine-based system. The following subsection details the pharmacological properties of ketamine.

Ketamine – a brief history

Ketamine was first synthesised in 1962 at Parke-Davis laboratories, Michigan. It was developed for use as a fast-acting general anaesthetic. Following United States FDA approval in 1972, doctors began to use it in the battlefield during the Vietnam War. Unfortunately, socio-recreational use of ketamine increased in the late 1970s through to the 1980s and 1990s. Ketamine users widely experienced its dissociative and hallucinatory effects. The mounting illicit use of ketamine in the United States led to its Schedule III classification under the Controlled Substance Act in August 1999. In the United Kingdom, it was labeled a Class C drug under the Misuse of Drugs Act in January 2006. Ketamine is nevertheless valued as an efficacious anaesthetic with minimal cardiovascular and respiratory side effects. It is still commonly used for anaesthesia in hospitals around the world.

Pharmacokinetics of ketamine

Ketamine (C13H16ClNO) is an arylcyclohexylamine drug with two active enantiomers: S(+) and R(-) ketamine. Being both lipid soluble and partially water soluble, it can be absorbed through intravenous, intramuscular, intranasal, oral and topical routes. Ketamine rapidly crosses the blood-brain

barrier because it has low molecular weight (237.7 Da), low ionisation at physiologic pH (pKa 7.5), and high lipid solubility. Peak plasma concentration and maximal effect can be reached within one minute of intravenous infusion. Ketamine has a half-life of two to three hours. It undergoes hepatic metabolism to form norketamine and dehydronorketamine through the cytochrome P450 enzyme system. Norketamine is the main metabolite which has approximately one-third the potency of ketamine. Metabolites of ketamine are conjugated and mostly eliminated via renal excretion (90%) and fecal excretion (5%), with about 4% being excreted unchanged in urine.

Pharmacodynamics of ketamine

Ketamine is a non-competitive antagonist of the N-methyl-d-aspartate (NMDA) receptor. It has a binding site within the receptor's ion channel. Activation of the NMDA receptor causes Ca^{2+} and Na+ influx and the neuron depolarises. Intracellular Ca^{2+} also activates various calcium-dependent signaling cascades. The NMDA receptor is a complex modulator of glutamate neurotransmission. NMDA blockade is thought to increase glutamate neurotransmission through the preferential inhibition of γ-amino butyric acid (GABA) interneurons, which in turn disinhibits excitatory neurons, leading to

increased glutamatergic firing[10,11]. NMDA antagonism could also reduce the activity of calcium-dependent nitric oxide synthase, leading to lower levels of nitric oxide (NO) in the neuron. As NO acts as a retrograde messenger to presynaptic neurons, reduced NO signaling could ultimately lead to decreased presynaptic glutamate release[12,13]. NMDA is an ionotropic glutamate receptor that is widely distributed in the brain. Together with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors, they mediate virtually all the fast-glutamatergic excitatory signals. Glutamate is the most abundant excitatory neurotransmitter in the human brain. It is an essential neurotransmitter for normal cognitive function. It is also a mediator of synaptic plasticity and neuronal cell death[14]. Glutamate is synthesised in the cytoplasm and stored in synaptic vesicles. After release into the synaptic cleft, it is taken up by astrocytes and is oxidatively degraded or converted to glutamine by glutamine synthetase. Glutamine is in turn released from the astrocytes, accumulated by neurons, and converted back to glutamate by glutaminase. Extracellular glutamate concentrations are usually maintained at low levels to prevent neuronal excitotoxicity. The NMDA receptor mediates sensory input from the spinal level up to the subcortical and cortical levels. Ketamine, an NMDA antagonist, blocks or interferes with sensory input to higher centres of the central nervous system (CNS). It also affects emotional

responses to stimuli and the processes of learning and memory[15]. Clinical effects of ketamine at intravenous doses greater than 2mg/kg are anaesthetic and analgesic in nature[16]. At subanaesthetic doses, psychomimetic effects can be predominant and are reportedly similar to symptoms of schizophrenia[17,18]. It is at even lower doses of around 0.5mg/kg that antidepressant effects are experienced, and these are the doses that have been reported in clinical trials for depression[8,9]. More randomised controlled trials have been conducted since the two pivotal studies[8,9], Several meta-analyses have confirmed the rapidity and short-term efficacy of intravenous low-dose ketamine in the treatment of MDD[19-21].

The first part of this chapter will focus on whether ketamine has any immunomodulatory effect on microglial cells at its antidepressant dose. About 10% of all brain cells are microglia. These macrophage-like cells are crucial in regulating both protective and toxic processes in the brain. Non-activated microglia constantly monitor the cerebral environment for threats and when there is a disturbance in brain homeostasis, microglia become activated and changes in morphology and function[22]. Microglia may be activated into two distinct or "classical" types[22], though other subtypes have been described[23]. In

M1 activation, microglial cells may become hyper-ramified or amoeboid. They may release pro-inflammatory cytokines, glutamate and free radicals in this cytotoxic mode against pathogens. In M2 activation, microglia cells release anti-inflammatory cytokines and neurotrophic factors, and function mainly to heal and repair tissue. Although the M1/M2 phenotype has been classically described, not all experts agree that there's such a rigid dichotomy. Some of the reasons[24-26] that have been put forth include: 1) more the two activation states have been observed; 2) different cell lines may respond differently to the same stimulus; 3) the background environment can affect polarization; 4) induced phenotypes in vitro may not be representative of in vivo phenotypes due to complexity of stimuli; 5) microglia can control their own polarization through paracrine and autocrine processes. The jury is still out on whether a new classification in future will be universally accepted, given the present diversity of opinions. For this study, the focus will not be on classifying microglia, but to describe the observed changes in morphology and functional expression.

In depression, acute stress and cortisol release could induce pro-inflammatory and proliferative responses in microglia[27], leading to neuroinflammation[28,29].

Microglia are also sensitive to adverse stimuli like infections and injuries[30,31], and in response to these noxious stimuli they become hyperactive and release even more pro-inflammatory cytokines[32,33]. An overexpression of pro-inflammatory cytokines could increase the production of reactive oxygen species in the brain, leading to oxidative damage and neuronal death[34]. Prolonged activation of microglia could also suppress hippocampal neurogenesis and neuroplasticity[35,36]. Under inflammatory conditions, microglia could induce apoptosis of hippocampal neuroblast through IL-6 or TNF-α[35,36]. It could also inhibit GluR1 (AMPAR) phosphorylation, which is required for synaptic plasticity and spatial memory[37]. As the brain ages, amyloid and tauopathy sets in and through complement-mediated mechanisms, microglia could cause synaptic engulfment and synaptic loss[38].

In depressed patients, postmortem studies of microglia have been inconclusive. Some studies observed activated microglia in depressed patients, while other studies found no difference between those depressed and not depressed[39-42]. In vivo positron emission tomography (PET) imaging of microglia has yielded more consistent results. In brief, PET imaging uses radioactive ligands that bind to the translocator protein (TSPO). TSPOs are

located primarily on the outer mitochondrial membranes of microglia. Its expression is relatively specific to microglia in the brain and when there is a neuroinflammatory insult, its expression becomes rapidly upregulated. The first PET-TSPO study[43] failed to identify any difference between depressed patients and controls, but following that, three additional studies[44,45] observed higher TSPO signals in MDD patients relative to controls. This provided in vivo evidence of neuroinflammation and highlighted microglia's role in depression. Although PET-TSPO imaging has been a major advancement, it is not without its limitations. Current PET-TSPO imaging is able to distinguish active from inactive microglial but is still unable to show whether the activated microglia are pro-inflammatory or anti-inflammatory[46].

Pre-clinical studies are still useful for elucidating the pathological states of microglia and in identifying microglia's response to drugs. Antidepressants like tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs) are drugs that target the monoamine (serotonin, norepinephrine, dopamine) neurotransmitter system. It was previously thought that they did not have any anti-inflammatory mechanism of action, but there is now pre-clinical evidence that SSRIs and

other antidepressants can inhibit pro-inflammatory cytokine (IL-1β and TNF-α) release from lipopolysaccharide (LPS)-activated microglial cells[47-51]. Antidepressants can also attenuate microglial activation and inhibit its production of free radical nitric oxides[52,53]. However in vitro studies have no behavioural correlates and the use of drug concentrations may not be physiologically representative, therefore in vivo studies are needed. Chronic stress can induce both depressive-like symptoms and microglia activation in rodents. Rats chronically restrained for up to 21 days exhibit depressive behaviour and microglial change [e.g. increased cell numbers, hyper-ramification, expression of activation markers][54-58]. Chronic unpredictable stress in rats could also activate microglia and increase pro-inflammatory cytokine production (e.g. IL-1β)[58].

This study aims are to explore whether ketamine's antidepressant effect has an immunomodulatory mechanism of action, and to investigate whether ketamine's antidepressant effect is solely due to NMDA receptor (NMDAR) antagonism. Multiple lines of evidence are leading scientists to question NMDAR's role in treating depression. Molecules that are also NMDAR antagonists, such as memantine, AZD6765, CP-101 606, Ro25–6981 and even

MK-801 which binds to the same receptor site as ketamine, have failed to replicate ketamine's robust antidepressant effects[59-62]. Surprisingly, experiments with other glutamate receptors, such as AMPA receptor (AMPAR), have led to the discovery that AMPAR may be crucial for ketamine to exert its antidepressant effect. 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzoquinoxaline-2,3-dione (NBQX) is both an AMPAR and kainate receptor antagonist. In animal studies, when NBQX is administered minutes to hours after ketamine injection, ketamine's antidepressant effect becomes negated[62,63]. It is unclear whether the nullification of ketamine's effect was due to AMPAR or kainite receptor blockade. An interesting point to note is that AMPAR-type receptors are also present on microglia and these receptors regulate pro-inflammatory release from microglia. The work in this chapter will aim to elucidate AMPAR's role in depression treatment more clearly by using a selective AMPAR antagonist and AMPAR potentiator.

Research questions

1) Can ketamine alter the morphology of microglia in depression?
2) Can ketamine alter the expression of inflammatory markers in depression?

3) Are there other glutamate receptors involved in ketamine's antidepressant effect?

This chapter aims to determine: 1) whether a single antidepressant dose of ketamine (versus vehicle) at the end of a chronic stress period is able to attenuate microglial morphology; 2) whether a single antidepressant dose of ketamine (versus vehicle) at the end of the chronic stress period is able to change mRNA and protein markers of inflammation in microglia (including cytokines, chemokines and neurotrophic factors); 3) whether ketamine's antidepressant effect will be influenced by selective AMPAR antagonism and potentiation.

The first hypothesis is that three weeks chronic stress will induce changes in microglia and depressive-like symptoms in rats. Ketamine when given at the end of three weeks will change the morphology of activated microglia to a less activated state and produce antidepressant behavioural effects. The second hypothesis is that three weeks of chronic stress will increase the expression of pro-inflammatory markers in rats. Ketamine when given at the end of three weeks will reduce the expression of pro-inflammatory markers. The third

hypothesis is that a selective AMPAR blocker will negate ketamine's antidepressant effect while an AMPA potentiator with enhance its effect.

B) Materials and Methods

Study Overview

For the first two study aims, 24 rats were randomly assigned into one of four groups:

1) Stress + Vehicle

2) Stress + Ketamine

3) No Stress + Vehicle

4) No Stress + Ketamine

For three weeks, eight rats underwent chronic mild stress while the other eight were not stressed. At the end of three weeks, a single administration of intraperitoneal (i/p) 10mg/kg ketamine was given to rat groups 2) and 4) while rat groups 1) and 3) received the i/p vehicle (placebo). Ketamine 10mg/kg was chosen as the optimal antidepressant dose from prior dose testing (supplementary Fig.S1) which was consistent with the antidepressant dose

used in other studies[63,64]. All rats were put through the forced swim test (FST) 24 hours post-injection. Rats were then sacrificed, and brain tissues were harvested for further experiments.

For the third study aim, 6-8 rats were assigned into each drug comparison group. Perampanel, a selective AMPAR antagonist, was administered i/p 10-15 minutes before ketamine 10mg/kg was given and FST was conducted 24 hours later. Doses at 2.5mg/kg, 5mg/kg and 10mg/kg were investigated. Piracetam, an AMPAR potentiator, was administered i/p 10-15 minutes before ketamine 10mg/kg was given and FST was conducted 24 hours later. Doses at 75mg/kg, 150mg/kg and 300mg/kg were investigated. The dosages of perampanel and piracetam in this study have been previously used to investigate CNS effects in rodents[65-67].

Animals

Six-week-old male Murine Pathogen Free™ NTac: Sprague-Dawley rats were purchased from InVivos (Singapore). The animals were housed in pairs within polycarbonate cages upon arrival and were allowed to acclimatise for one

week before any experimental manipulation. Food and water were available *ad libitum*. All animals' related procedures were approved by the Institutional Animal Care and Use Committee (IACUC), National University of Singapore, and were carried out in compliance to animal handling guidelines developed by the National Advisory Committee for Laboratory Animal Research, Singapore.

Chronic Unpredictable Mild Stress (CMS)

The CMS procedure conducted was adapted and modified from the CMS model of depression by Willner et al[68]. The CMS schedule (supplementary Table S1) comprises of a variety of unpredictable mild stressors including white noise at 85db for one hour, isolation housing overnight, elevated stress for 30 minutes, food or water deprivation (16 hours), rotation on a shaker for one hour, damp and dirty bedding, 45 degree cage tilt for one hour, confinement to space for one hour, light/dark lighting reversals and stroboscopic lighting (300 flashes/minute) for one hour. A semi-variable schedule of two stressors per day was repeated for three consecutive weeks. The non-stressed control animals were housed in normal conditions. After three weeks of CMS, all animals underwent behavioural testing.

Forced swim test

The overall experimental design was counterbalanced (rats from each treatment groups was represented in each session). Camera and dividers were well-positioned to obtain the best possible resolution of the rats. Water tanks were filled with room temperature tap water (23 to 25°C) up to the determined level. Water temperature was checked with the thermometer. Prior to starting the test, a white noise generator was started. The level of white noise was sufficient to mask out external noises. Rats were brought into the room and given at least one hour to acclimatise. Video recording commenced before rats were placed into the water tanks. Rats were held by their tails, and gently and slowly placed in the water. When the rat was in the water, its tail was slowly released. Once all rats were in their respective tanks, countdown on the stopwatch began. The test length was six minutes. The experimenter was at a reasonable distance from the animals to prevent any noticeable/distracting movements. After six minutes, the recording stopped. Prior to stopping, a note containing the ID/subject number of the rat was shown to the camera. The rat will be removed from the water by its tail in the same order that they were put in. The rat will then be gently dried with the drying paper and heated lamp and placed back into its home cage.

Tissue processing

Within seven days of drug treatment, 13-week-old rats were deeply anaesthetised by an intraperitoneal injection with Pentobarbitone (0.5 ml/kg). Animals were transcardially perfused with phosphate-buffer saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were harvested and post-fixed for two days at 4 °C. After post-fixation, brains were saturated in 30% (w/v) sucrose in PBS and the right hemisphere was subjected to cryosection at 30μm thickness with a cryostat (Leica, Germany). Sections were stored in PBS until fluorescent immunohistochemistry staining was performed.

Fluorescent immunohistochemistry

Immunohistochemistry staining was performed using the standard free-floating method. Three coronal sections (one in series of five) per animal from the hippocampus and prefrontal cortex were used. Briefly, free-floating sections were washed twice with PBS and permeabilised with PBS containing 0.1% Tx-100 (PBS-Tx). Following this, they were pre-incubated for two hours in blocking solution (5% Donkey serum) at room temperature. Thereafter, the sections were incubated at 4°C overnight with rabbit anti-IBA1 (Wako, Osaka,

Jp, 1:500 in blocking solution). On the next morning, sections were washed and incubated in the Donkey Anti-Rabbit Alexa Fluor® 488 secondary antibody (Invitrogen, Oregon, USA, 1:500 in blocking solution) at room temperature for two hours. Subsequently, sections were washed and incubated for 10 minutes with DAPI stain (Invitrogen, Oregon, USA, 1:50000 in PBS-TX). Next, sections were washed, dehydrated before mounting with homemade 2.5% PVA/DABCO mountant.

Fluorescence images showing IBA1 immunoreactivity and DAPI stain were captured using Zeiss LSM710 confocal laser scanning microscope for microglia cell counting and for quantifying morphological changes, at 20x and 40x magnification respectively. The anatomical location of the region of interest was identified using a rat brain atlas. The hippocampus and prefrontal cortex were chosen because these brain regions have been implicated in depression and prior studies have reported chronic mild stress effects on these regions[69]. One image per section covering the dentate gyrus of the hippocampus while three images per section covering the anterior cingulate cortex (AcG), infralimbic (IL), prelimbic (PL) medial prefrontal cortex were imaged pseudo randomly (n=3 or 4). Z stack images of 20 slices were taken to detect

microglia throughout the entire thickness of each section. For comparison, the parameters (contrast, brightness and pinhole) for each confocal scan were kept the same. Images were rendered into a two-dimensional diagram with Imaris software, before the numbers of microglia were counted manually with Image J software. To analyse the morphological changes in microglia, z-stack images were used for three-dimensional reconstruction of the microglia. Surface rendering was performed on each microglia before applying filament tracer to trace their processes. The following parameters were used to analyse the morphological changes: volume, surface area, total process length, branch point, branch depth and number of terminal points. All parameters are mean values of a single microglial cell. Only cells with entire processes shown with DAPI stained nuclei were selected for analysis.

Real-time polymerase chain reaction (RT-PCR)

Tissue: Rat brain tissues rats were stored in RNA latter solution at -80 ºC until analysis. Real Time (RT) PCR was done to detect the mRNA expression level of various chemo and cytokines in the hippocampus and PFC. As a first step, the hippocampus and PFC stored in RNA latter solution were extracted using the RNA extraction kit from Qiagen (RNeasy Plus Mini Kit, Qiagen).

Tissue was homogenised using lysis buffer provided in the kit. RNA was extracted according to the protocol of the manufacturer. RNA purity and concentration were determined using NanoDrop One (Thermo Scientific). Four hundred nanograms of RNA was converted to cDNA using RevertAid First Strand cDNA Synthesis Kit, (Thermo Scientific) according to the protocol of the manufacturer. All RT-PCR Primers were obtained from the publications and validated using NCBI primer blast. The list of primers and validation details are attached with the SOP for RT-PCR. PowerUp™ SYBR™ Green Master Mix Applied Biosystems was used for PCR reactions. Applied Biosystems Vii a 7 real time PCR system was used for the reactions.

Protein Milliplex Assay

Rat brain tissues were frozen with liquid nitrogen and stored at -80 °C until analysis. Ice cold phosphate buffered saline with protease inhibitors was used to homogenise around 30 milligrams of brain hippocampus tissue. Clear supernatant was used for the assay to estimate the protein concentrations of cytokines in an ELISA based assay (MILLIPLEX MAP Rat Cytokine/Chemokine Magnetic Bead Panel, Milliplex, RECYTMAG-65K-10) Assay protocol was followed from the manufacturer instructions. Milliplex MAGPIX® System was used for the quantification assay.

Statistical Analysis

Analysis of variance (ANOVA) was used to test for group differences. Main and interaction effects were reported when two-way ANOVA was used to analyse the effects of two independent variables on one dependent variable. Post-hoc analysis (Tukey) was performed when one-way ANOVA was used. SPSS software version 24 was used. Based on our preliminary test results using ketamine and the forced swim test, a minimum of 4 animals per group was needed to detect an effect size of 1.9, with 80 % power and 0.05 alpha.

C) Results

Ketamine had different effects on microglia, which depended on the state of microglia before ketamine was given. In the prefrontal cortex, ketamine reduced the level of ramification in microglia when they were hyper-ramified under stress (Fig. 1). In the hippocampus, ketamine increased the level of ramification in both resting and hypo-ramified microglia (Fig. 2). Detailed analyses were performed to quantify these visual observations (Fig. 3 & Fig. 4).

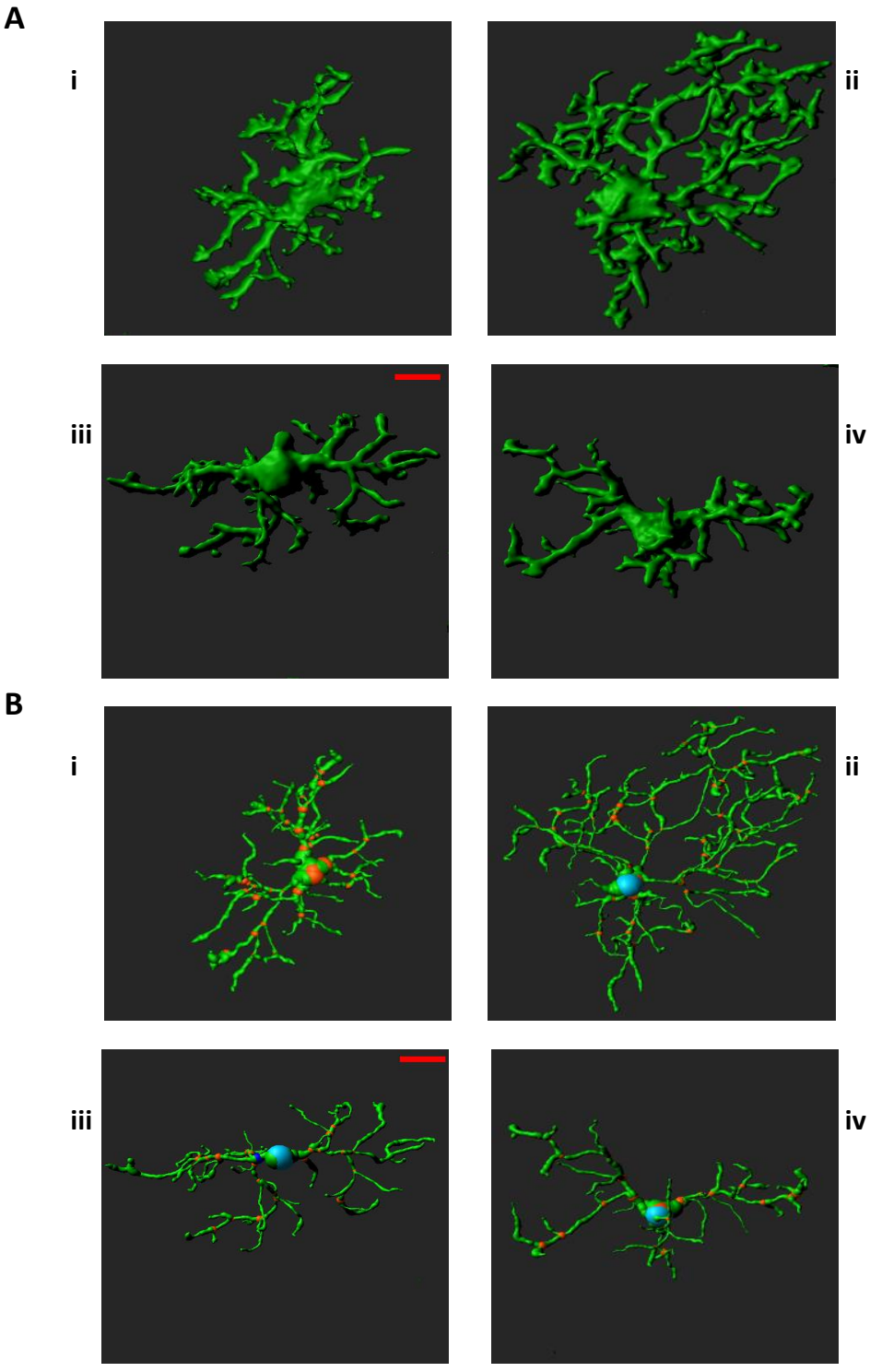

Figure 1. Morphology of microglia in the prefrontal cortex. Representative images showing processes tracing of IBA-1 labelled microglia from **(i)** Control **(ii)** CMS **(iii)** Control + Ket10 **(iv)** CMS + Ket10 groups by **(A)** surface rendering and using **(B)** filament tracer in Imaris (red for branch points, blue for point of origin). Scale bar = 10um.

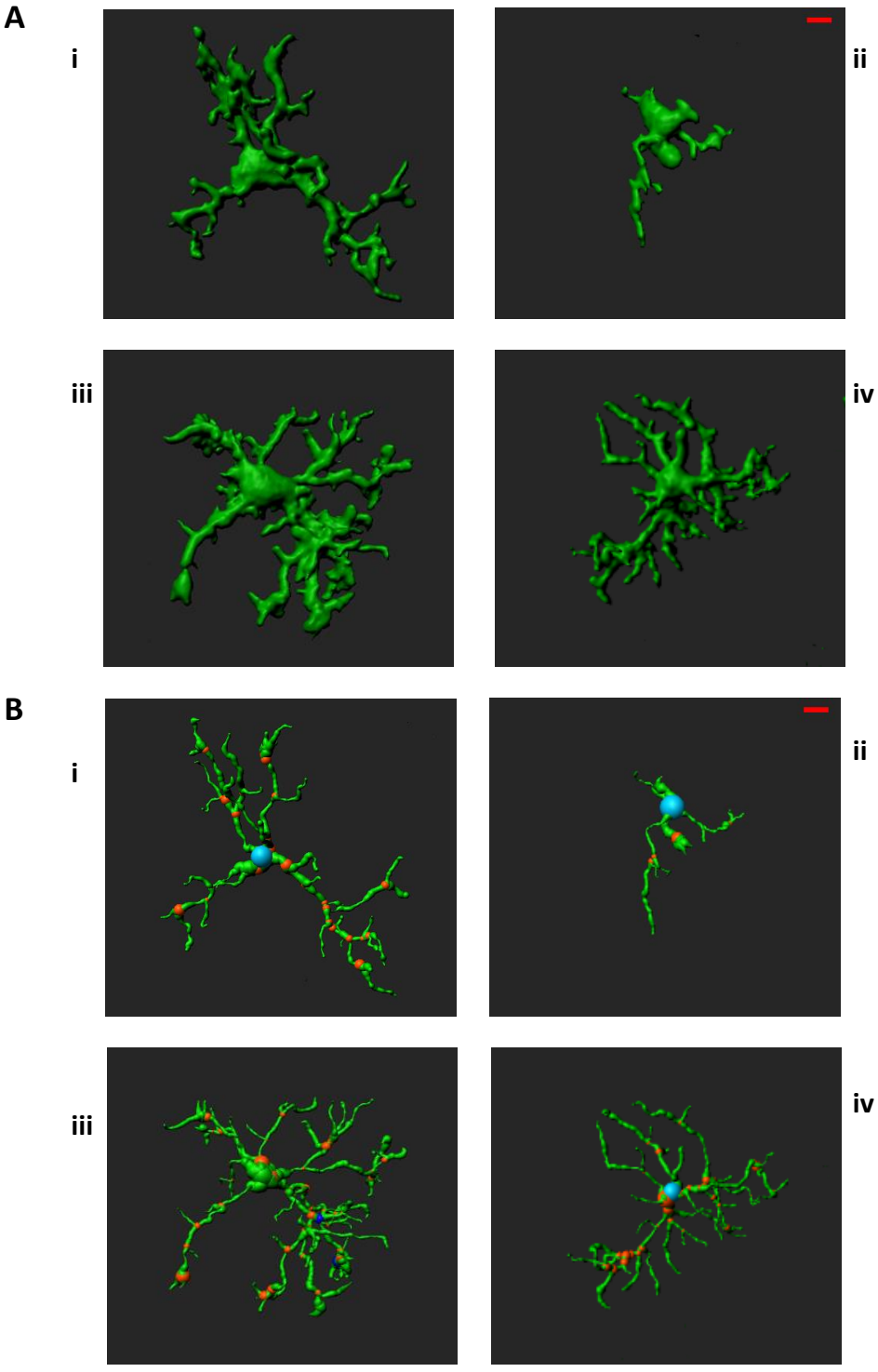

Figure 2. Morphology of microglia in the hippocampus. Representative images showing processes tracing of IBA-1 labelled microglia from **(i)** Control **(ii)** CMS **(iii)** Control + Ket10 **(iv)** CMS + Ket10 groups by **(A)** surface rendering and using **(B)** filament tracer in Imaris (red for branch points, blue for point of origin). Scale bar = 10um.

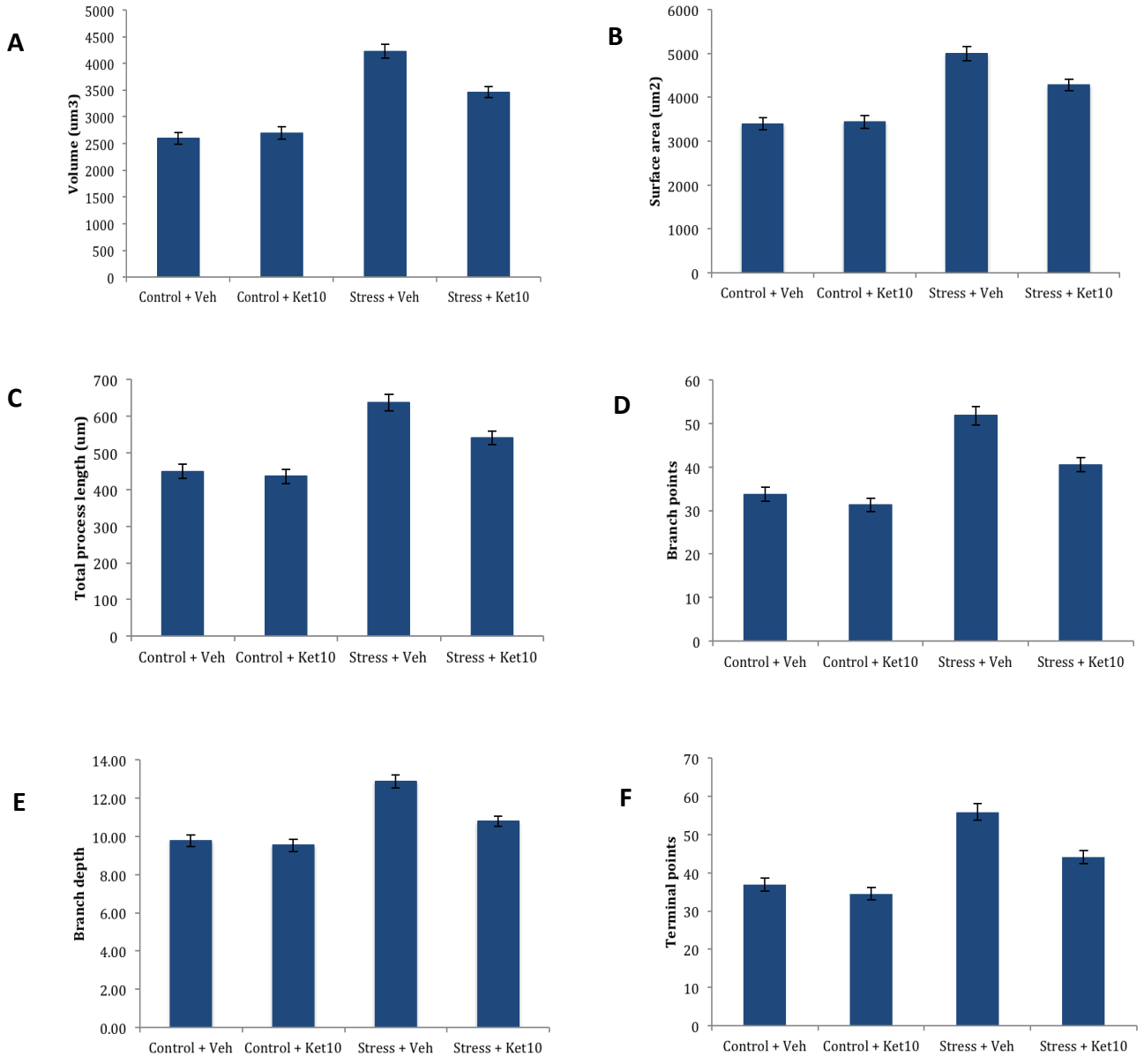

Figure 3. Bar graphs depicting the detailed morphological characteristics of microglia in the prefrontal cortex. (A) Volume, (B) Surface area, (C) Total process length, (D) Branch points, (E) Branch depth, (F) Terminal points. Mean values with standard error bars shown. CMS, Chronic mild stress; Ket, Ketamine; Veh, Vehicle.

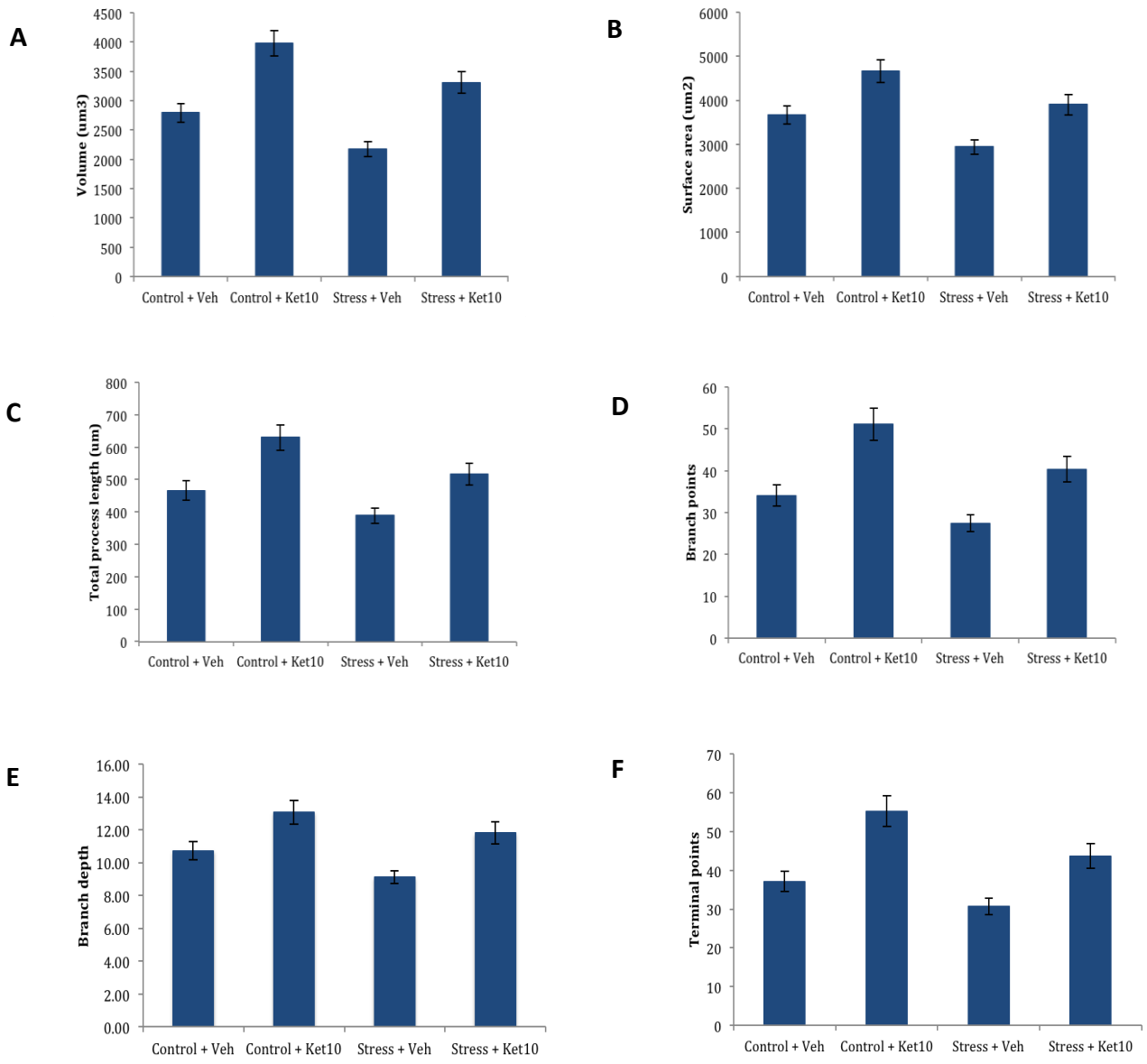

Figure 4. Bar graphs depicting the detailed morphological characteristics of microglia in the hippocampus. (A) Volume, (B) Surface area, (C) Total process length, (D) Branch points, (E) Branch depth, (F) Terminal points. Mean values with standard error bars shown. CMS, Chronic mild stress; Ket, Ketamine; Veh, Vehicle.

In the prefrontal cortex, there was significant interaction between CMS and ketamine (Table 1). Interaction effects altered microglial volume, surface area, total process length, branch points, branch depth and terminal points. CMS increased ramification of microglia in the prefrontal cortex (Fig. 3, Table 1). Ketamine decreased microglial volume [F (1,743)=24.33, p<0.001], surface area [F (1,743)=13.74, p<0.001], total process length [F (1,746)=12.71, p<0.001], branch points [F (1,746)=22.77, p<0.001], branch depth [F (1,745)=27.22, p<0.001] and terminal points [F (1,746)=22.91, p<0.001] in CMS rats, but had no effect on these measures in non-CMS rats.

In the hippocampus, there were significant main effects for both CMS and ketamine (Table 2). Interaction effects were not present. CMS induced decreased ramification of microglia in the hippocampus (Fig. 4). Stressed rats had decreased microglial volume [F (1,573)=9.06, p<0.01], surface area [F (1,573)=7.49, p<0.01], total process length [F (1,574)=7.29, p<0.01], branch points [F (1,574)=7.29, p<0.01], branch depth [F (1,227)=5.96, p<0.05] and terminal points [F (1,574)=7.04, p<0.01] relative to non-stressed rats. Rats given ketamine had increased microglial volume [F (1,573)=82.38, p<0.001],

surface area [F (1,573)=41.31, p<0.001], total process length [F (1,574)=35.08, p<0.001], branch points [F (1,574)=42.86, p<0.001], branch depth [F (1,227)=18.46, p<0.001] and terminal points [F (1,574)=42.16, p<0.001] compared to rats not given ketamine.

CMS and ketamine had no significant effect on the number of microglia in both the prefrontal cortex and hippocampus.

Table 1. Morphological characteristics of microglia in the prefrontal cortex.

Category	**Control + Vehicle**	**Control + Ketamine**	**CMS + Vehicle**	**CMS + Ketamine**	Main effect CMS	Main effect Ketamine	Interaction
Volume (μm^3)	2598.6 (109.9)	2697.9 (116.0)	4224.5 (134.4)	3455.8 (105.4)	<0.001	<0.01	<0.001
Surface area (μm^2)	3395.6 (136.7)	3445.3 (148.6)	4994.1 (163.0)	4277.2 (131.5)	<0.001	<0.05	<0.05
Total process length (µm)	449.4 (20.0)	435.6 (19.6)	636.8 (22.8)	540.6 (18.5)	<0.001	<0.01	<0.05
Branch points	33.7 (1.7)	31.3 (1.6)	51.8 (2.1)	40.5 (1.6)	<0.001	<0.001	<0.05
Branch depth	9.8 (0.3)	9.5 (0.3)	12.9 (0.3)	10.8 (0.3)	<0.001	<0.001	<0.01
Terminal points	36.9 (1.7)	34.5 (1.6)	55.9 (2.2)	44.1 (1.7)	<0.001	<0.001	<0.05

Table 2. Morphological characteristics of microglia in the hippocampus.

Category	**Control + Vehicle**	**Control + Ketamine**	**CMS + Vehicle**	**CMS + Ketamine**	Main effect CMS	Main effect Ketamine	Interaction
Volume (μm^3)	2795.2 (160.8)	3978.4 (217.0)	2175.5 (129.7)	3311.5 (188.6)	<0.01	<0.001	0.706
Surface area (μm^2)	3664.7 (203.2)	4660.6 (253.5)	2942.2 (166.5)	3902.0 (237.3)	<0.01	<0.001	0.414
Total process length (um)	466.2 (30.2)	629.9 (38.8)	388.5 (23.2)	516.6 (34.4)	<0.01	<0.001	0.203
Branch points	34.1 (2.5)	51.1 (3.8)	27.5 (2.0)	40.3 (3.1)	<0.01	<0.001	0.155
Branch depth	10.7 (0.6)	13.1 (0.7)	9.1 (0.4)	11.8 (0.7)	<0.05	<0.001	0.774
Terminal points	37.1 (2.6)	55.2 (4.0)	30.6(2.1)	43.7 (3.2)	<0.01	<0.001	0.126

RT-PCR was performed to identify differential mRNA expressions of cytokines [tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL- 1β), IL-4, IL-6, IL-10, granulocyte macrophage-colony stimulating factor (GM-CSF), transforming growth factor beta (TGF-β)], chemokines [fractalkine/fractalkine receptor, monocyte chemotactic protein (MCP), macrophage inflammatory protein-1alpha (MIP-1α)], neurotropic factors [brain derived neurotropic factor (BDNF)], and microglia activation markers [mannose receptor C-type1 (MRC1), ionized calcium-binding adaptor molecule 1 (IBA-1), cluster of differentiation 68 (CD68)].

Of the cytokines, TNF-α, IL-1β, IL-6 and GM-CSF are pro-inflammatory, and IL-4, IL-10 and TGF-β are anti-inflammatory. MCP and MIP-1α are pro-inflammatory chemokines. BDNF is a neuronal growth factor. Fractalkine receptors (CX3CR1) are exclusively expressed by microglia and fractalkine (CX3CL1) is a signalling molecule produced by neurons; this forms a signalling pathway that has immunomodulatory effects on microglia[70]. MRC-1 is a marker of microglial immunoregulatory activation[70]. IBA-1 and CD68 are cytoplasmic and lysosomal markers of microglial activation respectively[71].

In the prefrontal cortex (Fig. 5), ketamine had significant main effects on TGF-β, MRC-1 and CD68, such that rats given ketamine had lower expressions of TGF-β [F (1,21)=7.92, p<0.05], MRC-1 [F (1,21)=6.30, p<0.05] and CD68 [F (1,21)=5.62, p<0.05] than rats that were given vehicle. CMS had no significant main effect on any of the biomarkers (Table 3). For fractalkine, there was a significant interaction effect between ketamine and CMS [F (1,21)=5.78, p<0.05]. Ketamine significantly lowered fractalkine expressions in non-CMS rats (p<0.01) but had no effect on fractalkine in CMS rats.

In the hippocampus, the main effect of ketamine on IL-1β was significant, such that IL-1β levels were lower in rats given ketamine [F (1,10)=7.88, p<0.05] than in rats given vehicle. (Fig. 6). CMS had no significant main effect on IL-1β expression and there was no interaction between CMS and ketamine. For the other PCR measures, ketamine and CMS had no main effects and there were no interaction effects.

Neither CMS nor ketamine had main effects on protein markers which included fractalkine, MRC-1, MIP-1α, IL-1β, IL-6, TNF-α, IL-4 and IL-10.

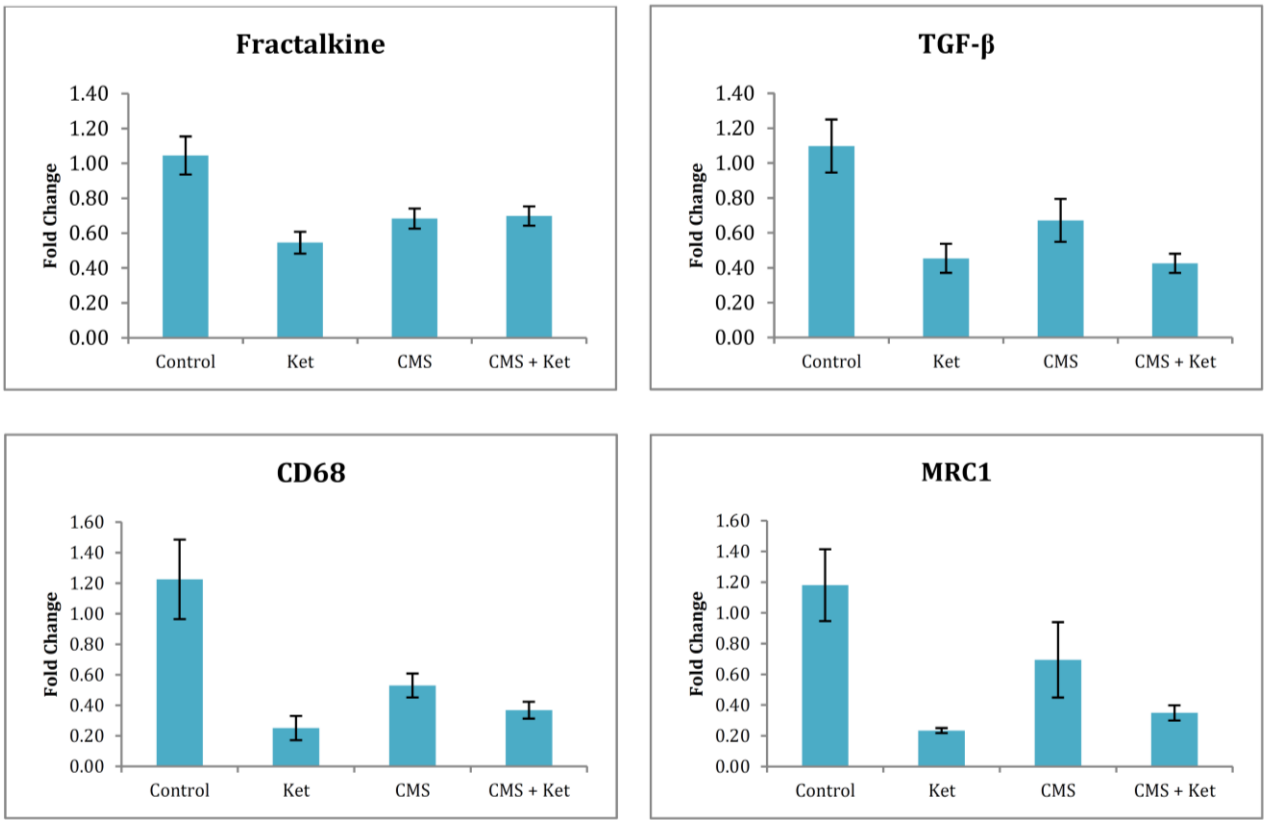

Figure 5. Real-time PCR quantification of biomarkers in the prefrontal cortex.
Ket, Ketamine; CMS, Chronic mild stress; TGF-β, transforming growth factor beta; CD68, cluster of differentiation 68; MRC1, mannose receptor C-type1.

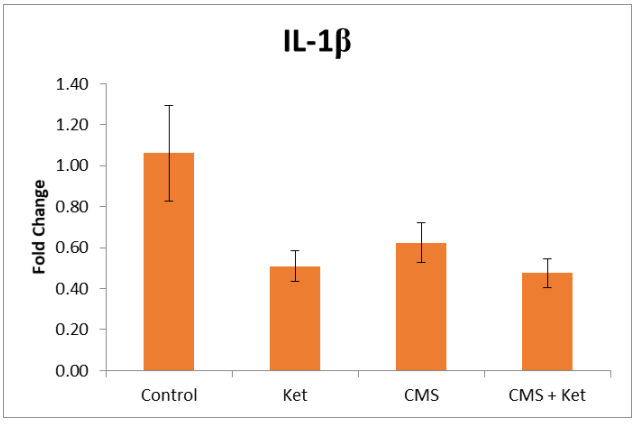

Figure 6. Real-time PCR quantification of biomarkers in the hippocampus.
Ket, Ketamine; CMS, Chronic mild stress; IL- 1β, interleukin-1 beta.

Table 3. Real-time PCR quantification of biomarkers in the prefrontal cortex.

Category	**Control + Vehicle**	**Control + Ketamine**	**Stress + Vehicle**	**Stress + Ketamine**	Main effect Stress	Main effect Ketamine	Interaction
Fractalkine	1.05 (0.11)	0.55 (0.06)	0.68 (0.06)	0.70 (0.06)	0.342	**<0.05**	**<0.05**
Fractalkine receptor	1.04 (0.09)	0.80 (0.15)	0.77 (0.13)	0.61 (0.11)	0.095	0.150	0.752
TNF-α	1.11 (0.18)	0.59 (0.09)	0.85 (0.14)	0.80 (0.10)	0.879	0.143	0.219
IL-6	3.60 (2.36)	2.85 (1.32)	1.62 (0.37)	0.84 (0.22)	0.171	0.588	0.992
TGF-β	1.10 (0.15)	0.45 (0.08)	0.67 (0.12)	0.43 (0.06)	0.165	**<0.05**	0.223
IL-1β	1.26 (0.31)	0.66 (0.19)	1.17 (0.19)	1.14 (015)	0.551	0.323	0.375
BDNF	1.07 (0.13)	0.63 (0.09)	0.82 (0.09)	0.78 (0.12)	0.748	0.095	0.154
IBA-1	1.14 (0.18)	0.70 (0.14)	0.80 (0.19)	0.40 (0.06)	0.127	0.053	0.945
CD68	1.22 (0.26)	0.25 (0.08)	0.53 (0.08)	0.37 (0.06)	0.241	**<0.05**	0.105
MRC-1	1.18 (0.23)	0.23 (0.02)	0.69 (0.25)	0.35 (0.05)	0.478	**<0.05**	0.256

The CMS procedure induced depressive-like behaviour (Fig. 7) as exemplified by increased immobility time relative to controls during the forced swim test (FST). Ketamine 10mg/kg, when administered 24 hours before FST, conferred an antidepressant effect by decreasing immobility time. There was a significant interaction effect [F (1,20)=5.02, p<0.05] between CMS and ketamine. Rats which underwent CMS had significantly increased immobility time (p<0.01) relative to rats without CMS, but CMS had no effect on immobility time when ketamine was given before FST (Fig. 7).

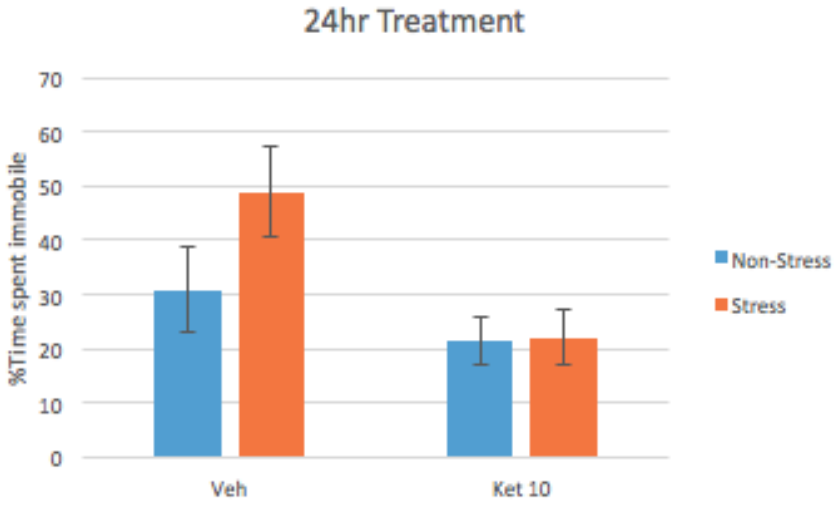

Figure 7. Ketamine effects on FST in stressed and non-stressed rats.
Veh, Vehicle; Ket, Ketamine.

In the AMPAR analysis, PML began to block ketamine's effect on FST at 5mg/kg and by PML 10mg/kg its blocking effect had maximised. In the experiment, there was a significant difference [F (4,31)=6.46, p<0.01] in immobility time between groups (Fig. 8). Post-hoc analysis showed that group (vehicle + ketamine 10mg/kg) and group (PML 2.5mg/kg + ketamine 10mg/kg) both had significantly lower immobility times (p<0.01) than control (vehicle + vehicle). This indicated that PML 2.5mg/kg was insufficient to block ketamine's effect. Both group (PML 5mg/kg + ketamine 10mg/kg) and group (PML 10mg/kg + ketamine 10mg/kg) did not have statistically different immobility times compared with control (vehicle + vehicle), which indicated that at higher PML doses, ketamine's antidepressant effect was fully blocked. There was no difference in immobility times between group (PML 5mg/kg + ketamine 10mg/kg) and group (PML 10mg/kg + ketamine 10mg/kg).

PCT doses from 75mg/kg to 300mg/kg produced no significant enhancement of ketamine's (10mg/kg) antidepressant effect on FST. It was possible that a ceiling effect had been reached. However, when ketamine was given at a lower dose of 5mg/kg, PCT augmentation was able to enhance ketamine's effect. This was achieved in the absence increased locomotor activity. In the

experiment, there was a significant difference [F (4,43)=19.46, p<0.001] in immobility time between groups (Fig. 9). Post-hoc analysis showed that group (vehicle + ketamine 5mg/kg) had significantly lower (p<0.05) immobility time than control (vehicle + vehicle), and all other groups (Fig. 9) also had significantly lower (p<0.001) immobility time than control (vehicle + vehicle). Compared with group (vehicle + ketamine 5mg/kg), the addition of PCT to ketamine in groups (PCT 150mg/kg + ketamine 5mg/kg) and (PCT 300mg/kg + ketamine 5mg/kg) both lowered immobility time further (p<0.01). The immobility times of (PCT 150mg/kg + ketamine 5mg/kg) and (PCT 300mg/kg + ketamine 5mg/kg) did not significantly differ from (vehicle + ketamine 10mg/kg). These results indicated that PCT augmentation at 150mg/kg and 300mg/kg enhanced ketamine's 5mg/kg effect to the equivalent of ketamine 10mg/kg.

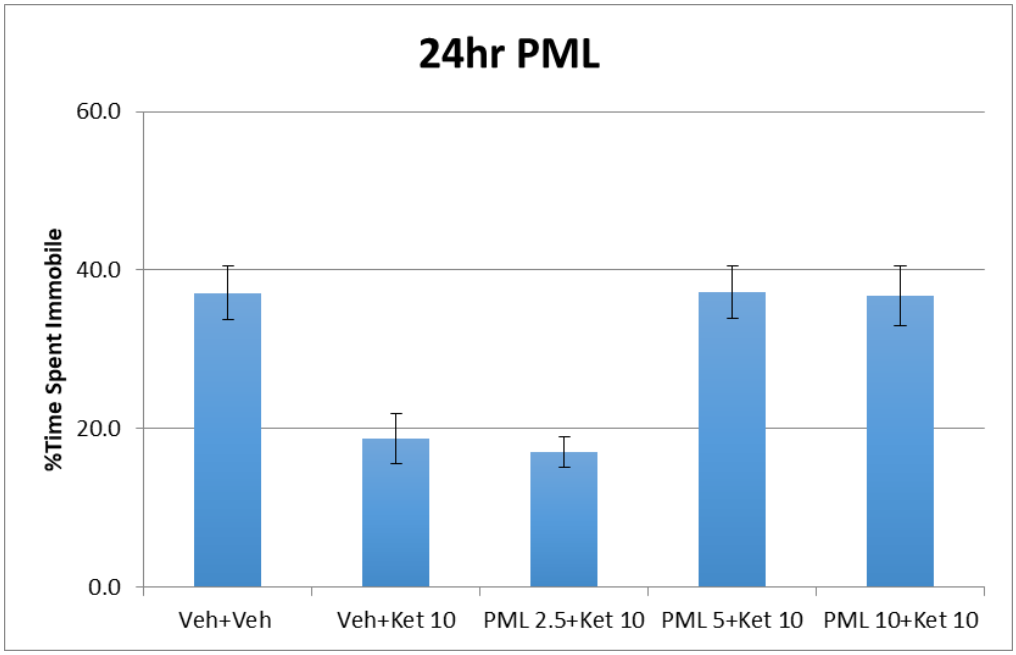

Figure 8. Ketamine's effect on FST with PML pre-treatment.
Veh, Vehicle; Ket, Ketamine; PML, Perampanel.

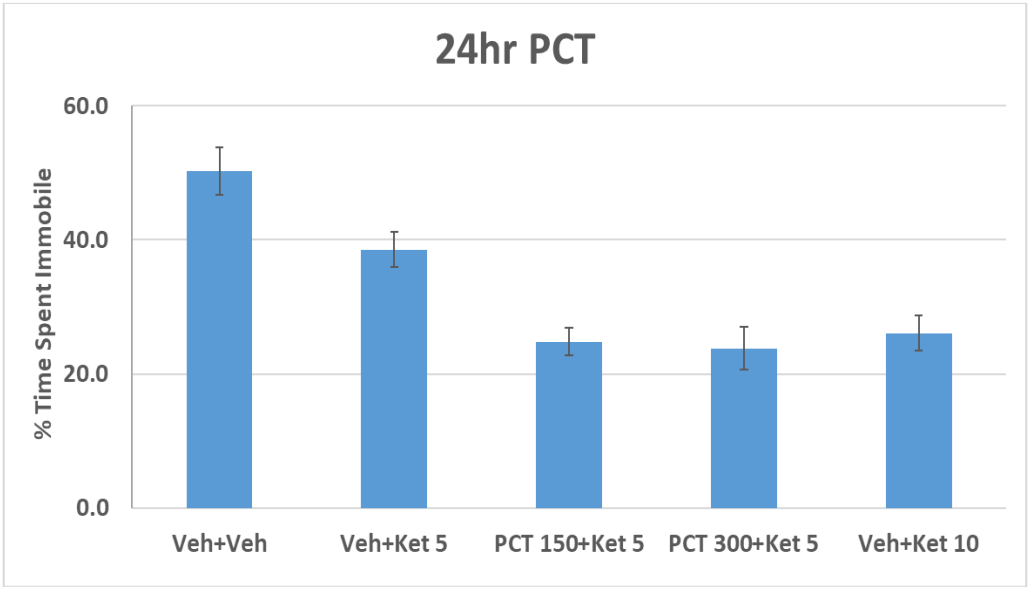

Figure 9. Ketamine's effect on FST with PCT pre-treatment.
Veh, Vehicle; Ket, Ketamine; PCT, Piracetam.

D) Discussion

Morphological changes to microglia were observed when there was exposure to chronic stress. With CMS, microglia became hyper-ramified in the prefrontal cortex and de-ramified in the hippocampus. Different changes in response to chronic stress in these two brain regions have previously been described[54,72]. Hyper-ramification in the prefrontal cortex may represent microglial activation[54] and de-ramification in the hippocampus could signify microglial dystrophy[72]. It is increasingly being recognised that the form and function of microglia is not homogenous throughout brain[73,74], and microglia's response to adverse stimuli could be different due to region-specific signals and interactions in the surrounding microenvironment[75,76]. Surprisingly, ketamine reversed both forms of stress-induced change – hyper-ramified microglia became less ramified and de-ramified microglia became more ramified. To our knowledge, these effects of ketamine have not been previously reported and they support our hypothesis that ketamine could induce morphological change in microglia.

Few studies have examined the effects of antidepressants on microglial morphology. Imipramine, a tricyclic antidepressant (TCA), was shown to

prevent chronic stress-induced microglial dystrophy after 5 weeks of daily oral imipramine[77]. Venlafaxine, a serotonin and norepinephrine reuptake inhibitor (SNRI) antidepressant, ameliorated chronic stress-induced hyper-ramification of microglia after 30 days of daily intraperitoneal venlafaxine[78]. A point to note is that these studies required chronic administration of a standard antidepressant to change microglial morphology and to achieve behavioural response. Ketamine was able to elicit similar effects, but in a much shorter time, and with just a single dose of the drug. The rate of change in microglial morphology appears commensurate with the rate of antidepressant response. These observations suggest that there is a biological link between microglial morphology and depressive symptoms, but is microglial change a cause or an effect of the change in depressive symptomatology? Minocycline, an inhibitor of microglial activity, prevented the onset of depressive-like symptoms when prophylactically administered to rodents before LPS or interferon-α challenge[79,80], and reversed depressive symptoms when administered after the onset of learned helplessness depression[81]. These additional lines of evidence appear to suggest that it is microglial function/dysfunction that influences depressive symptoms.

While morphological changes in microglia could be directly observed, the interpretation of its function and relation to associated biomarkers is less straightforward. In the prefrontal cortex, stress-induced hyper-ramification of microglia was not associated with higher mRNA expressions of activation or inflammation, in contrast to the injury/inflammation model where expressions of inflammation would be expected[82-84]. Recent investigations that used chronic stress to induce morphological and functional changes in microglia have reported that hyper-ramification could occur without elevated mRNA expressions of activation or inflammation[54,56,78]. The hyper-ramified form of microglia is still not well understood. It may represent a primed, surveillant form of microglia that is between resting and activated states – meaning it could become reactive and phagocytic when there is further pathological stimulus, or it could revert back to the resting state when there is no longer any CNS distress[83]. Ketamine's ability to reduce ramification in these hyper-ramified cells under stress conditions may be related to its action on NMDARs. Glutamate acts on NMDARs to trigger adenosine triphosphate (ATP) release from neuronal dendrites, which stimulates outgrowth of microglial processes[85-87]. When stressed, corticosterone is released and acts on glucocorticoid receptors in neurons. With increased glucocorticoid stimulation, prefrontal cortex neurons selectively upregulate post-synaptic

surface expressions of NMDAR, leading to an increase in NMDAR activation and ATP release[88]. Ketamine, an NMDAR antagonist, could have blocked excessive postsynaptic NMDAR activation, which effectively cut down the ATP signal to microglia for ramification. This blocking effect of ketamine may have been evident only when glutamate transmission was high, but not when glutamatergic transmission was at its basal physiological rate, as ketamine did not reduce microglial ramification in non-stress conditions. Ketamine's binding to NMDAR requires the removal of a voltage dependent Mg2+ channel block, therefore the greater the depolarization and firing rate of neurons, the greater the number of NMDAR channels ketamine could bind to[89].

In the hippocampus, the stress-induced de-ramification/dystrophy of microglia observed was in accordance with previous studies[72,77]. Those previous studies additionally reported loss in hippocampal microglial numbers, which was not observed in our study, possibly because the animals in those studies were exposed to longer chronic stress paradigms. Hippocampal microglia may have responded to stress and corticosterone more vigorously than prefrontal cortex microglia because glucocorticoid receptors are more abundantly expressed in hippocampal microglia[90]. The combination of prolonged

corticosterone stimulation from chronic stress and a high glucocorticoid to mineralocorticoid receptor ratio might have led to overactivation dystrophy in hippocampal microglia[91]. Ketamine's ability to elicit trophic ramification of microglia in the hippocampus appeared independent of stress, because both stressed and non-stressed microglia showed robust response to ketamine in that regard. In the hippocampus, low dose ketamine preferentially blocks NMDARs on inhibitory GABAergic interneurons, which reduces tonic inhibition on excitatory pyramidal neurons[92]. This resultant net increase in basal glutamatergic transmission activates postsynaptic NMDARs, which in turn triggers ATP release and stimulates ATP-mediated microglial ramification[85,86]. Compared to stressed conditions in the prefrontal cortex, there was probably less concomitant NMDAR blockade with ketamine, because chronic stress-induced upregulation of NMDAR activity is not a feature of hippocampal neurons[93]. In non-stressed conditions, microglia in the prefrontal cortex did not show increased ramification in response to ketamine. This may be because ketamine reduced fractalkine in the prefrontal cortex but not the hippocampus. Fractalkine from neurons sends a tonic signal to microglia to regulate activation; in pathological conditions this signal comes off and microglia assumes a neurotoxic state[94]. Under physiological conditions, fractalkine signaling regulates process branching and motility in microglia to

continuously survey their environment[94]. Microglial morphology responds rapidly to fractalkine signals. Ex vivo microglia increase in process branches and terminal points when exposed to exogenous fractalkine, but readily reverse those changes when fractalkine is removed[95]. Thus ketamine, in lowering fractalkine, may have prevented the ramification process in the prefrontal cortex. The mechanisms through which ketamine acts on neurons to effect change in fractalkine is unclear and will need further investigation.

Findings from mRNA and protein analyses in general indicated that the morphological changes observed in microglia were not associated with a pro-inflammatory background and classical markers of microglial activation were also not elevated. Instead, the only consistent pattern observed was that ketamine lowered mRNA expressions of microglial immunological activity, irrespective of chronic stress. Overall, ketamine reduced expressions of pro-inflammatory (IL-1β) and anti-inflammatory (TGF-β) cytokines, and markers of microglial activity (MRC-1, CD68). These findings do suggest that ketamine has immunomodulatory effects, and these effects may have been polarised if there was a strong inflammatory stimulus (e.g. LPS)[96]. Nonetheless, ketamine's effect in this study suggests it lowered microglial activation. There is evidence

that this effect is through direct antagonism of NMDAR on microglia. One of the early studies reported that NMDAR antagonism (with MK801), under hypoxic conditions, downregulated IL-1β, TNF-α, inducible nitric oxide synthase (iNOS) and nitric oxide (NO) expressions in microglia[97]. A following study later confirmed that stimulation of microglial NMDAR with NMDA in vitro released both pro-inflammatory (IL-1β, TNF-α, GM-CSF, MCP) and anti-inflammatory (IL-4, IL-10, IL13) cytokines/chemokines, and pre-treatment with MK801 reduced the release of these molecules[98].

With regards to CMS, it had no significant main effect on mRNA expressions, though trend decreases in some markers were observed. It is possible that there may have been a difference if the duration of CMS was longer, but it is also known that CMS can yield variable results on inflammatory markers, with some studies reporting elevated cytokines[64,99], some reporting deceased levels[100] and yet others reporting no effect of chronic stress on inflammatory cytokines levels[101,102]. These inconsistencies may be due to varying chronic stress procedures, the ambient environment, animal handling, innate resilience, and batch and strain differences. Nonetheless, this study allowed us to observe states of microglia that are rarely reported with CMS. Hyper-ramified

microglia are surveillant and pre-activated forms that could turn either pro- or anti-inflammatory[83]. Dystrophic/de-ramified microglia are pre-apoptotic and could be dysfunctional or immunoparalysed[77]. The non-polarised mRNA expressions observed in this study may have well represented these morphological forms.

The next important finding in this study is the confirmation that AMPAR function is essential for ketamine to exert its antidepressant effect. Previous studies[103,104] reported that NBQX was able to block ketamine's antidepressant effect, but it was unclear whether it was because it antagonised AMPA receptors or kainate receptors because NBQX is an antagonist to both. A selective AMPA receptor antagonist was in this study to ensure it was AMPAR antagonism and not kainite antagonism that blocked ketamine's antidepressant effect. An AMPAR potentiator was also used in this study to determine whether AMPAR potentiation was synergistic to ketamine's antidepressant effect. The hypothesised mechanism behind this is that ketamine will first trigger pre-synaptic neurons to release glutamate, glutamate then activates post-synaptic AMPAR[105,106] while post-synaptic NMDAR remains blocked; this way all available extracellular glutamate will get shunted to stimulate AMPAR.

The study findings support the hypothesis that AMPAR activation is crucial to ketamine's antidepressant effect. PML dose dependently blocked ketamine's antidepressant effect on the FST, while PCT dose dependently enhanced ketamine's antidepressant effect on the FST. The significance of these findings is twofold: 1) Novel therapeutic drugs that target AMPAR could be developed. 2) Existing use of ketamine could be augmented with AMPAR agents for treatment efficacy without the risk of using higher ketamine doses. There are compelling reasons to pursue therapeutic development in these directions. Despite the huge potential and promise of ketamine, it is important to note that even at subanaesthetic doses, ketamine use is associated with psychotomimetic, dissociative and cognitive disturbances[107]. There are also cardiovascular risks of tachycardia and hypertension[108]. Prolonged use bears the additional risk of drug abuse[109], neurotoxicity[110,111] and urological toxicity[112,113]. It is hoped that a better understanding of ketamine's mechanism of action will drive further development of novel antidepressants that are rapid-acting but without the adverse features that currently limit ketamine's wider clinical use.

Beyond the therapeutic efficacy of alleviating depressive symptoms, it is also imperative to address the neuroinflammation that is associated with depression, because prolonged inflammation could contribute towards depression recurrence and chronicity[114]. This study found that AMPARs are crucial to ketamine's antidepressant effect. Future research into AMPAR-based antidepressants should also explore whether they possess immunomodulatory properties. Several AMPA-type (GluR1–GluR4) receptors that have been identified in microglia[115,116]. Preliminary findings suggest that AMPAR allows microglia to receive glutamate signals directly, and when exposed to pathological conditions, they trigger the release of TNF-α and IL-1β[117]. Another study showed that treatment with an AMPAR antagonist reduced microglial activation post-infection[118]. It remains to be studied whether ketamine's immunomodulatory effect on microglia is also medicated by AMPAR. Research into AMPAR-based therapeutics is gaining increasing attention. The goal will be to ultimately develop an antidepressant that could not only relieve symptoms rapidly but could also prevent future recurrences effectively.

Limitations

Although CMS is a widely used and validated rodent model of depression, the behavioural phenotype induced could still be variable for reasons noted above. Experiments could be repeated using different models of depression (e.g. social defeat and learned helplessness paradigms) to increase validity, but that will come at additional cost which may not be feasible for all investigators.

Examining the biological process from cellular stimuli to cellular response is essential for the understanding of treatment and disease, but it is a multilayered process that is fraught with variability. In the context of this study, animals were presented with a similar stimulus, but not all microglia would have responded to the stimuli. For those that did, the response may have been at different rates, and may vary according to other accompanying signals in the microenvironment. mRNA expressions can correlate poorly with protein expressions due to variations in transcription rates, translation modulations, post-transcription regulatory processes, delays in protein synthesis and transport, and autophagy of proteins[119]. Correlations between mRNA expressions and secreted cytokines vary from r=0.9 to no correlation

at all[120]. Different approaches have been developed to improve mRNA-protein coupling. Some have used automated single cell microscopy, mRNA and protein analysis systems that allow simultaneously analysis of different cytokines over progressive time points[121]. Others have opted for high throughput RNA-sequencing and high-resolution mass spectroscopy with nanoflow cytometry to improve fidelity of data. Despite these advances, discordance of data still exists. The methods used in this study are still widely used and reported, but one should be mindful of the limitations when interpreting the results.

In the AMPAR segment, although receptor blockade and potentiation correlated with behavioural response, the degree of blockade/potentiation and cross-reactivity with other glutamate ionotropic receptors was not determined. To assess this, whole-cell voltage clamp methods could be used to examine individual AMPA, NMDA and kainite excitatory post-synaptic currents (EPSCs), by sequentially using their respective receptor antagonists and analysing their EPSC ratios.

E) Summary

This study found that ketamine had immunomodulatory and morphological effects on microglia, which are core immune cells that regulate the neuro-immune system in the brain. The results suggest that ketamine may have the potential to rapidly moderate neuroinflammation, in addition to being a rapid-acting antidepressant. This chapter also examined AMPARs' role in ketamine's mechanism of action and conclude that they are essential for ketamine to produce an antidepressant effect. Future studies should investigate whether ketamine's immunomodulatory function is also mediated by AMPAR.

E. Supplementary data

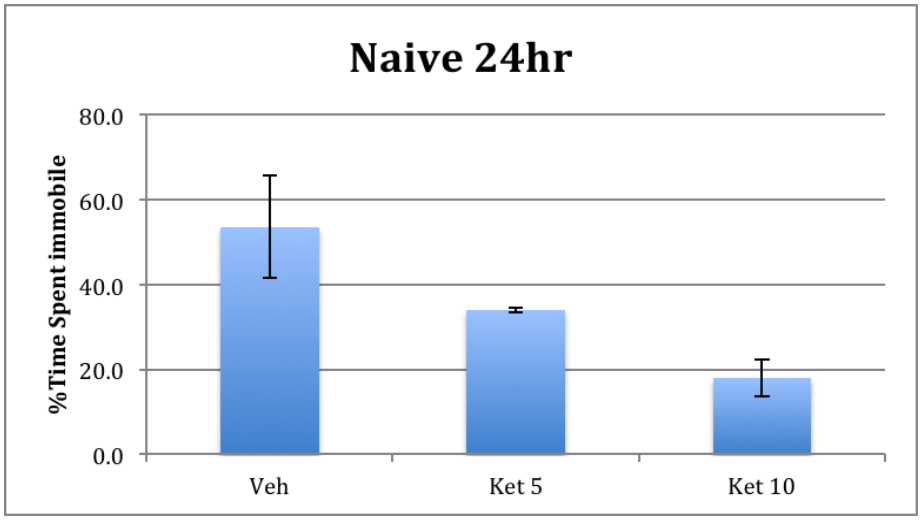

Figure S1. Effects of ketamine on FST (5mg/kg, 10mg/kg, Veh=Vehicle)

Table S1. Chronic mild stress schedule

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
1. Lights on (overnight)	x									x							x			x
2. 30-degree cage tilt (3h)	x					x					x							x		
3. Food deprivation (16h)		x				x						x								
4. Confined space (1h)		x					x						x						x	
5. Damp bedding overnight			x				x				x			x						
6. Lights off (10am to 1pm)			x					x				x			x					x
7. Water deprivation (16h)				x				x					x			x		x		
8. Rotation on shaker (1h)				x					x					x			x			
9. Isolation housing overnight					x				x						x				x	
10. Elevated stress					x					x						x				

F) References

1. Baune BT, Adrian I, Jacobi F. Medical disorders affect health outcome and general functioning depending on comorbid major depression in the general population. Journal of Psychosomatic Research. 2007;62(2):109−118.

2. Kessler RC, Akiskal HS, Ames M, Birnbaum H, Greenberg P, Hirschfeld RM et al. Prevalence and effects of mood disorders on work performance in a nationally representative sample of U.S. workers. American Journal of Psychiatry. 2006;163(9):1561−1568.

3. Thase, ME, Haight BR, Richard N, Rockett CB, Mitton M, Modell JG et al. Remission rates following antidepressant therapy with bupropion or selective serotonin reuptake inhibitors: a meta-analysis of original data from 7 randomized controlled trials. Journal of Clinical Psychiatry. 2005; 66(8):974−981.

4. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR∗D: implications for clinical practice. American Journal of Psychiatry. 2006;163(1):28−40.

5. Szanto K, Mulsant BH, Houck P, Dew MA, Reynolds CF 3rd. Occurrence and course of suicidality during short-term treatment of late-life depression. Archives of General Psychiatry. 2003;60:610–617.

6. Jick H, Kaye JA, Jick SS. Antidepressants and the risk of suicidal behaviors. Journal of the American Medical Association. 2004;292:338–343.

7. Payne JL, Quiroz JA, Zarate CA Jr, Manji HK. Timing is everything: does the robust upregulation of noradrenergically regulated plasticity genes underlie the rapid antidepressant effects of sleep deprivation? Biological Psychiatry. 2002;52(10):921−926.

8. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS et al. Antidepressant effects of ketamine in depressed patients. Biological Psychiatry. 2000;47(4):351−354.

9. Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Archives of General Psychiatry. 2006;63(8):856−864.

10. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. Journal of Neuroscience. 1997;17:2921–2927.

11. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. Journal of Neuroscience. 2007;27:11496–11500.

12. Keilhoff G, Becker A, Grecksch G, Wolf G, Bernstein HG. Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and cFOS similar to those found in human schizophrenia. Neuroscience. 2004;126:591–598.

13. O'Dell, TJ, Hawkins RD, Kandel ER, Arancio O. 1991. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. Proceedings of the National Academy of Sciences USA. 1991; 88:11285–11289.

14. Danbolt NC. Glutamate uptake. Progress in Neurobiology. 2001;65(1):1–105.

15. Bergman SA. Ketamine: review of its pharmacology and its use in pediatric anesthesia. Anesthesia Progress. 1999;46:10–20.

16. Grahame-Smith DG, Aronson JK. Oxford textbook of clinical pharmacology and drug therapy. 3rd Edition. Oxford: Oxford University Press; 2002.

17. Adler CM, Malhotra AK, Elman I, Goldberg T, Egan M, Pickar D, Breier A. Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. American Journal of Psychiatry. 1999;156:1646–1649.

18. Umbricht D, Schmid L, Koller R, Vollenweider FX, Hell D, Javitt DC. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. Archives of General Psychiatry. 2000;57:1139–1147.

19. Lee EE, Della Selva MP, Liu A, Himelhoch S. Ketamine as a novel treatment for major depressive disorder and bipolar depression: a systematic review and quantitative meta-analysis. General Hospital Psychiatry. 2015 Mar-Apr;37(2):178–184.

20. McGirr A, Berlim MT, Bond DJ, Fleck MP, Yatham LN, Lam RW. A systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials of ketamine in the rapid treatment of major depressive episodes. Psychological Medicine. 2015 Mar;45(4):693–704.

21. Fond G, Loundou A, Rabu C, Macgregor A, Lançon C, Brittner M et al. Ketamine administration in depressive disorders: a systematic review and meta-analysis. Psychopharmacology (Berl). 2014 Sep;231(18):3663–76.

22. Boche D, Perry VH, Nicoll JA. Review: activation patterns of microglia and their identification in the human brain. Neuropathology and Applied Neurobiology. 2013;39:3–18.

23. Chhor V, Le Charpentier T, Lebon S, Oré MV, Celador IL, Josserand J et al. Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia in vitro. Brain, Behavior and Immunity. 2013;32:70–85.

24. Davis MJ, Tsang TM, Qiu Y, Dayrit JK, Freij JB, Huffnagle GB, Olszewski MA. Macrophage M1/M2 polarization dynamically adapts to changes in cytokine microenvironments in Cryptococcus neoformans infection. MBio. 2013 Jun 18;4(3):e00264-13.

25. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 2014 Mar 3;6:13.

26. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. Front Immunol. 2019 24;10:1084.

27. Nair A, Bonneau RH. Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. Journal of Neuroimmunology. 2006;171(1-2):72–85.

28. Hanisch UK. Microglia as a source and target of cytokines. Glia 2002:40;140–155.

29. Smith JA., Das A., Ray SK., Banik NL. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull. 2012:87;10–20.

30. Goshen I., Kreisel T., Ben-Menachem-Zidon O., Licht T., Weidenfeld J., Ben-Hur T., et al. Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. Mol. Psychiatry 2008;13:717–728.

31. Ramirez K., Fornaguera-Trías J., Sheridan J. F. Stress-induced microglia activation and monocyte trafficking to the brain underlie the development of anxiety and depression. Curr Top Behav. Neurosci. 2016;31:155–172.

32. Dunn AJ. Effects of cytokines and infections on brain neurochemistry. Clin Neurosci Res. 2006:6;52–68.

33. Fenn A.M., Gensel JC., Huang Y., Popovich PG., Lifshitz J., Godbout JP. Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. Biol Psychiatry. 2014:76;575–584.

34. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science. 1993;262(5134):689–95.

35. Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science. 2003 Dec 5;302(5651):1760-5.

36. Cacci E, Claasen JH, Kokaia Z. Microglia-derived tumor necrosis factor-alpha exaggerates death of newborn hippocampal progenitor cells in vitro. J Neurosci Res. 2005 Jun 15;80(6):789-97.

37. Liu M, Li J, Dai P, Zhao F, Zheng G, Jing J, Wang J, Luo W, Chen J. Microglia activation regulates GluR1 phosphorylation in chronic unpredictable stress-induced cognitive dysfunction. Stress. 2015 Jan;18(1):96-106.

38. Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. J Cell Biol. 2018 Feb 5;217(2):459-472.

39. Bayer TA, Buslei R, Havas L, Falkai P. Evidence for activation of microglia in patients with psychiatric illnesses. Neuroscience Letters. 1999;271:126–128.

40. Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C et al. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. Journal of Psychiatric Research. 2008;42:151–157.

41. Torres-Platas SG, Cruceanu C, Chen GG, Turecki G, Mechawar N. Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. Brain, Behavior and Immunity. 2014;42:50–59.

42. Schnieder TP, Trencevska I Rosoklija G, Stankov A, Mann JJ, Smiley J, Dwork AJ. Microglia of prefrontal white matter in suicide. Journal of Neuropathology & Experimental Neurology. 2014;73:880–890.

43. Hannestad J, DellaGioia N, Gallezot JD, Lim K, Nabulsi N, Esterlis I. The neuroinflammation marker translocator protein is not elevated in individuals with mild-to-moderate depression: a [11C]PBR28 PET study. Brain, Behavior and Immunity. 2013;33:131–138.

44. Setiaan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. JAMA Psychiatry. 2015 Mar;72(3):268–75.

45. Holmes SE, Hinz R, Conen S, Gregory CJ, Matthews JC, Anton-Rodriguez JM, Gerhard A, Talbot PS. Elevated Translocator Protein in Anterior Cingulate in Major Depression and a Role for Inflammation in Suicidal Thinking: A Positron Emission Tomography Study. Biological Psychiatry. 2018 Jan 1;83(1):61–69.

46. Setiawan E, Attwells S, Wilson AA, Mizrahi R, Rusjan PM, Miler L et al. Association of translocator protein total distribution volume with duration of untreated major depressive disorder: a cross-sectional study. Lancet Psychiatry. 2018 Apr;5(4):339–347.

47. Hashioka S, Klegeris A, Monji A, Kato T, Sawada M, McGreer PL, Kanba S. Antidepressants inhibit interferon-gamma-induced microglial production of IL-6 and nitric oxide. Experimental Neurology. 2007;206(1):33–42.

48. Horikawa H, Kato TA, Mizoguchi Y, Monji A, Seki Y, Ohkuri T et al. Inhibitory effects of SSRIs on IFN-gamma induced microglial activation through the regulation of intracellular

calcium. Progress in Neuro-psychopharmacology & Biological Psychiatry. 2010;34(7):1306–1316.

49. Tynan RJ, Weidenhofer J, Hinwood M, Cairns MJ, Day TA, Walker FR. A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. Brain, Behavior and Immunity. 2012;26(3):469–47.

50. Hwang J, Zheng LT, Ock J, Lee MG, Kim SH, Lee HW et al. Inhibition of glial inflammatory activation and neurotoxicity by tricyclic antidepressants. Neuropharmacology. 2008;55(5):826–834.

51. Bielecka AM, Paul-Samojedny M, Obuchowicz E. Moclobemide exerts anti-inflammatory effect in lipopolysaccharide-activated primary mixed glial cell culture. Naunyn Schmiedeberg's Archives of Pharmacology. 2010;382 (5–6):409–417.

52. Lim CM, Kim SW, Park JY, Kim C, Yoon SH, Lee JK. Fluoxetine affords robust neuroprotection in the postischemic brain via its anti-inflammatory effect. J Neurosci Res. 2009 Mar;87(4):1037-45.

53. Hashioka S, Klegeris A, Monji A, Kato T, Sawada M, McGeer PL, Kanba S. Antidepressants inhibit interferon-gamma-induced microglial production of IL-6 and nitric oxide. Exp Neurol. 2007 Jul;206(1):33-42.

54. Hinwood M, Tynan RJ, Charnley JL, Beynon SB, Day TA, Walker FR. Chronic stress induced remodeling of the prefrontal cortex: structural re-organization of microglia and the inhibitory effect of minocycline. Cerebral Cortex. 2012;23:1784–1797.

55. Tynan RK, Naicker S, Hinwood M, Nalivaiko E, Buller KM, Pow DV et al. Chronic stress alters the density and morphology of microglia in a subset of stress-responsive brain regions. Brain, Behavior and Immunity. 2010; 24:1058–1068.

56. Hinwood M, Morandini J, Day TA, Walker FR. Evidence that microglia mediate the neurobiological effects of chronic psychological stress on the medial prefrontal cortex. Cerebral Cortex. 2012;22: 1442–1454.

57. Kongsui R, Beynon SB, Johnson SJ, Mayhew J, Kuter P, Nilsson M, Walker R. Chronic stress induces prolonged suppression of the P2X7 receptor within multiple regions of the hippocampus: a cumulative threshold spectra analysis. Brain, Behavior and Immunity. 2014;42:69–80.

58. Pan Y, Chen XY, Zhang QY, Kong LD. Microglial NLRP3 inflammasome activation mediates IL-1beta-related inflammation in prefrontal cortex of depressive rats. Brain, Behavior and Immunity. 2014;41:90–100.

59. Newport DJ, Carpenter LL, McDonald WM, Potash JB, Tohen M, Nemeroff CB, APA Council of Research Task Force on Novel Biomarkers and Treatments. Ketamine and Other NMDA Antagonists: Early Clinical Trials and Possible Mechanisms in Depression. American Journal of Psychiatry. 2015 Oct;172(10):950–66.

60. Maeng S, Zarate CA, Jr, Du J, et al. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biological Psychiatry. 2008;63:349–52.

61. Jiménez-Sanchez L, Campa L, Auberson YP, Adell A. The role of GluN2A and GluN2B subunits on the effects of NMDA receptor antagonists in modelling schizophrenia and treating refractory depression. Neuropsychopharmacology. 2014;39:2673–80.

62. Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature. 2016 May 26;533(7604):481–6.

63. Koike H, Chaki S. Requirement of AMPA receptor stimulation for the sustained antidepressant activity of ketamine and LY341495 during the forced swim test in rats. Behavioural Brain Research. 2014;271:111–5.

64. Yang C, Hong T, Shen J, Ding J, Dai XW, Zhou ZQ, et al. Ketamine exerts antidepressant effects and reduces IL-1β and IL-6 levels in rat prefrontal cortex and hippocampus. Exp Ther Med. 2013;5:1093–6.

65. Hanada T, Hashizume Y, Tokuhara N, et al. Perampanel: a novel, orally active, noncompetitive AMPA-receptor antagonist that reduces seizure activity in rodent models of epilepsy. Epilepsia. 2011;52(7):1331-40.

66. Rägo LK, Allikmets LH, Zarkovsky AM. Effects of piracetam on the central dopaminergic transmission. Naunyn Schmiedebergs Arch Pharmacol. 1981;318(1):36-7.

67. Ennaceur A, Cavoy A, Costa JC, et al. A new one-trial test for neurobiological studies of memory in rats. II: Effects of piracetam and pramiracetam. Behav Brain Res. 1989;33(2):197-207.

68. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berlin). 1987;93(3):358–364.

69. Calcia MA, Bonsall DR, Bloomfield PS, Selvaraj S, Barichello T, Howes OD. Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. Psychopharmacology (Berl). 2016 May;233(9):1637-50.

70. Chhor V, Le Charpentier T, Lebon S, Oré MV, Celador IL, Josserand J, Degos V, Jacotot E, Hagberg H, Sävman K, Mallard C, Gressens P, Fleiss B. Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia in vitro. Brain Behav Immun. 2013 Aug;32:70-85.

71. Hendrickx DAE, van Eden CG, Schuurman KG, Hamann J, Huitinga I. Staining of HLA-DR, Iba1 and CD68 in human microglia reveals partially overlapping expression depending on cellular morphology and pathology. J Neuroimmunol. 2017 Aug 15;309:12-22.

72. Tong L, Gong Y, Wang P, Hu W, Wang J, Chen Z, Zhang W, Huang C. Microglia Loss Contributes to the Development of Major Depression Induced by Different Types of Chronic Stresses. Neurochem Res. 2017 Oct;42(10):2698-2711.

73. Böttcher C, Schlickeiser S, Sneeboer MAM, Kunkel D, Knop A, Paza E, Fidzinski P, Kraus L, Snijders GJL, Kahn RS, Schulz AR, Mei HE; NBB-Psy, Hol EM, Siegmund B, Glauben R, Spruth EJ, de Witte LD, Priller J. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. Nat Neurosci. 2019 Jan;22(1):78-90.

74. Li Q, Cheng Z, Zhou L, Darmanis S, Neff NF, Okamoto J, Gulati G, Bennett ML, Sun LO, Clarke LE, Marschallinger J, Yu G, Quake SR, Wyss-Coray T, Barres BA. Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. Neuron. 2019 Jan 16;101(2):207-223.e10.

75. Silverman HA, Dancho M, Regnier-Golanov A, Nasim M, Ochani M, Olofsson PS, Ahmed M, Miller EJ, Chavan SS, Golanov E, Metz CN, Tracey KJ, Pavlov VA. Brain region-specific alterations in the gene expression of cytokines, immune cell markers and cholinergic system components during peripheral endotoxin-induced inflammation. Mol Med. 2015 Mar 11;20:601-11.

76. Pepe G, De Maglie M, Minoli L, Villa A, Maggi A, Vegeto E. Selective proliferative response of microglia to alternative polarization signals. J Neuroinflammation. 2017 Dec 4;14(1):236.

77. Kreisel T, Frank MG, Licht T, Reshef R, Ben-Menachem-Zidon O, Baratta MV, Maier SF, Yirmiya R. Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. Mol Psychiatry. 2014 Jun;19(6):699-709.

78. Hellwig S, Brioschi S, Dieni S, Frings L, Masuch A, Blank T, Biber K. Altered microglia morphology and higher resilience to stress-induced depression-like behavior in CX3CR1-deficient mice. Brain Behav Immun. 2016 Jul;55:126-137.

79. J.C. O'Connor, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol Psychiatry. 2009;14:511-522.

80. L.S. Zheng, et al. Minocycline treatment ameliorates interferon-alpha- induced neurogenic defects and depression-like behaviors in mice. Front Cell Neurosci. 2015;9:5.

81. S. Arakawa, et al. Minocycline produced antidepressant-like effects on the learned helplessness rats with alterations in levels of monoamine in the amygdala and no changes in BDNF levels in the hippocampus at baseline. Pharmacol Biochem Behav. 2012;100:601-606.

82. Davis EJ, Foster TD, Thomas WE. Cellular forms and functions of brain microglia. Brain Res Bull. 1994;34(1):73–8.

83. Streit WJ, Walter SA, Pennell NA. Reactive microgliosis. Prog Neurobiol. 1999 Apr;57(6):563-81.

84. Kettenmann H, Hanisch U-K, Noda M, Verkhratsky A. Physiology of Microglia. Physiol Rev. 2011;91(2):461–553.

85. Dissing-Olesen L, LeDue JM, Rungta RL, Hefendehl JK, Choi HB, MacVicar BA. Activation of neuronal NMDA receptors triggers transient ATP-mediated microglial process outgrowth. J Neurosci. 2014;34,10511–10527.

86. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci. 2005 Jun;8(6):752-8.

87. Ohsawa K, Irino Y, Sanagi T, Nakamura Y, Suzuki E, Inoue K, Kohsaka S. P2Y12 receptor-mediated integrin-beta1 activation regulates microglial process extension induced by ATP. Glia. 2010 May;58(7):790-801.

88. Yuen EY, Liu W, Karatsoreos IN, Feng J, McEwen BS, Yan Z. Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory. Proc Natl Acad Sci U S A. 2009 Aug 18;106(33):14075-9.

89. Glasgow NG, Siegler Retchless B, Johnson JW. Molecular bases of NMDA receptor subtype-dependent properties. J Physiol. 2015 Jan 1;593(1):83-95.

90. Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS, Bulloch K. Steroid hormone receptor expression and function in microglia. Glia. 2008 Apr 15;56(6):659-74.

91. Liu B, Wang K, Gao HM, Mandavilli B, Wang JY, Hong JS. Molecular consequences of activated microglia in the brain: overactivation induces apoptosis. J Neurochem. 2001 Apr;77(1):182-9.

92. Widman AJ, McMahon LL. Disinhibition of CA1 pyramidal cells by low-dose ketamine and other antagonists with rapid antidepressant efficacy. Proc Natl Acad Sci U S A. 2018 Mar 27;115(13):E3007-E3016.

93. Calabrese F, Guidotti G, Molteni R, Racagni G, Mancini M, Riva MA. Stress-induced changes of hippocampal NMDA receptors: modulation by duloxetine treatment. PLoS One. 2012;7(5):e37916.

94. Arnoux I, Audinat E. Fractalkine Signaling and Microglia Functions in the Developing Brain. Neural Plast. 2015;2015:689404.

95. Liang KJ, Lee JE, Wang YD, Ma W, Fontainhas AM, Fariss RN, Wong WT. Regulation of dynamic behavior of retinal microglia by CX3CR1 signaling. Invest Ophthalmol Vis Sci. 2009 Sep;50(9):4444-51.

96. Chang Y, Lee JJ, Hsieh CY, Hsiao G, Chou DS, Sheu JR. (2009). Inhibitory effects of ketamine on lipopolysaccharide-induced microglial activation. Mediators of Inflammation. 2009;2009:705379.

97. Murugan M, Sivakumar V, Lu J, Ling EA, Kaur C. Expression of N-methyl D-aspartate receptor subunits in amoeboid microglia mediates production of nitric oxide via NF-κB signaling pathway and oligodendrocyte cell death in hypoxic postnatal rats. Glia. 2011 Apr;59(4):521-39.

98. Kaindl AM, Degos V, Peineau S, Gouadon E, Chhor V, Loron G, et al. Activation of microglial N-methyl-D-aspartate receptors triggers inflammation and neuronal cell death in the developing and mature brain. Ann Neurol. 2012 Oct;72(4):536-49.

99. Wang N, Yu HY, Shen XF, Gao ZQ, Yang C, Yang JJ, Zhang GF. The rapid antidepressant effect of ketamine in rats is associated with down-regulation of pro-inflammatory cytokines in the hippocampus. Ups J Med Sci. 2015;120(4):241-8.

100. Bartolomucci A, Palanza P, Parmigiani S, Pederzani T, Merlot E, Neveu PJ, Dantzer R. Chronic psychosocial stress down-regulates central cytokines mRNA. Brain Res Bull. 2003 Dec 30;62(3):173-8.

101. Girotti M, Donegan JJ, Morilak DA. Chronic intermittent cold stress sensitizes neuro-immune reactivity in the rat brain. Psychoneuroendocrinology. 2011 Sep;36(8):1164-74.

102. Plata-Salamán CR, Ilyin SE, Turrin NP, Gayle D, Flynn MC, Bedard T, Merali Z, Anisman H. Neither acute nor chronic exposure to a naturalistic (predator) stressor influences the interleukin-1beta system, tumor necrosis factor-alpha, transforming growth factor-beta1, and neuropeptide mRNAs in specific brain regions. Brain Res Bull. 2000 Jan 15;51(2):187-93.

103. Li N, Lee B, Liu RJ, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329:959–64.

104. Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature. 2016;533(7604):481–6.

105. Chowdhury GM, Zhang J, Thomas M, et al. Transiently increased glutamate cycling in rat PFC is associated with rapid onset of antidepressant-like effects. Mol Psychiatry. 2016;22:120–6.

106. Moghaddam B, Adams B, Verma A, et al. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci. 1997;17:2921–7.

107. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr, Charney DS. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry. 1994 Mar;51(3):199-214.

108. Christ G, Mundigler G, Merhaut C, Zehetgruber M, Kratochwill C, Heinz G, Siostrzonek P. Adverse cardiovascular effects of ketamine infusion in patients with catecholamine-dependent heart failure. Anaesth Intensive Care. 1997 Jun;25(3):255-9.

109. Jansen KL, Darracot-Cankovic R. The nonmedical use of ketamine, part two: A review of problem use and dependence. J Psychoactive Drugs. 2001;33:151–8.

110. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999;283:70–4.

111. Zou X, Patterson TA, Divine RL, Sadovova N, Zhang X, Hanig JP, et al. Prolonged exposure to ketamine increases neurodegeneration in the developing monkey brain. Int J Dev Neurosci. 2009;27:727–31.

112. Chu PS, Ma WK, Wong SC, Chu RW, Cheng CH, Wong S, et al. The destruction of the lower urinary tract by ketamine abuse: A new syndrome? BJU Int. 2008;102:1616–22.

113. Shahani R, Streutker C, Dickson B, Stewart RJ. Ketamine-associated ulcerative cystitis: A new clinical entity. Urology. 2007;69:810–2.

114. Liu CH, Zhang GZ, Li B, Li M, Woelfer M, Walter M, Wang L. Role of inflammation in depression relapse. J Neuroinflammation. 2019 Apr 17;16(1):90.

115. Noda M, Nakanishi H, Nabekura J, et al. AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. J Neurosci 2000;20:251–8.

116. Hagino Y, Kariura Y, Manago Y, et al. Heterogeneity and potentiation of AMPA type of glutamate receptors in rat cultured microglia. Glia 2004;47:68–77.

117. Sivakumar, V.; Ling, E.A.; Lu, J.; Kaur, C. Role of glutamate and its receptors and insulin-like growth factors in hypoxia induced periventricular white matter injury. Glia 2010, 58, 507–523.

118. Brison E, Jacomy H, Desforges M, Talbot PJ. Glutamate excitotoxicity is involved in the induction of paralysis in mice after infection by a human coronavirus with a single point mutation in its spike protein. J Virol. 2011 Dec;85(23):12464-73.

119. Liu Y, Beyer A, Aebersold R. On the Dependency of Cellular Protein Levels on mRNA Abundance. Cell. 2016 Apr 21;165(3):535-50.

120. Shebl FM, Pinto LA, García-Piñeres A, Lempicki R, Williams M, Harro C, Hildesheim A. Comparison of mRNA and protein measures of cytokines following vaccination with human papillomavirus-16 L1 virus-like particles. Cancer Epidemiol Biomarkers Prev. 2010 Apr;19(4):978-81.

121. Kalb DM, Adikari SH, Hong-Geller E, Werner JH. Single-cell correlations of mRNA and protein content in a human monocytic cell line after LPS stimulation. PLoS One. 2019 Apr 19;14(4):e0215602.

Chapter III

Conclusion and future directions

Immunoinflammatory function plays a major role in depression. This thesis endeavored to illustrate that challenges to the immunoinflammatory system could affect the severity of depression, and that the treatment of depression may involve immunomodulation.

The first chapter highlighted the relationship between inflammation and depression. Inflammatory events were associated with recurrent depression, which is a more severe form depression, but had no association with single episode depression. The odds of recurrent depression got higher as the number of inflammatory events increased. In addition, the consumption of an anti-inflammatory supplement (fish oil) showed a protective effect against recurrent depression but had no association with single episode depression. These findings suggest that cumulative inflammatory stress over time might lead to a more protracted course of depression, and efforts to reduce the inflammatory load might prevent illness recurrence. Based on these initial

findings, future studies could examine this concept further with more data points, by incorporating more inflammatory diseases, psychosocial stressors/trauma, drug treatments, dietary and lifestyle choices that influence the level of inflammation, and details on depression onset, severity and duration in relation to the above factors. These results could have important clinical implications on disease modification.

As described earlier in the chapter, recurrent depression is associated with the highest morbidity and mortality, but there is a striking dearth of modifiable risk factors for recurrent depression. In a recent comprehensive meta-review, prognostic risk factors for recurrence with the strongest evidence included childhood maltreatment, history of prior depressive episodes, and residual symptoms of depression[1]. Although these factors influence the risk of recurrence, they are not 'modifiable' risk factors in the traditional sense. Successful identification and modification of risk factors like hypertension, hyperlipidemia, obesity, smoking and physical inactivity have been applied to medical conditions like heart disease and stroke. Similar preventive measures have not been strongly advocated for clinical depression because the identification of risks and the effects of risk modification on long-term

outcomes are not well established yet. Our study supports the notion that inflammation could be a modifiable risk factor for recurrent depression. This could be in the form of dietary change or supplementation. As our results suggest, fish oil consumption might protect against recurrent depression. The mechanisms for this have been described earlier. Physical exercise could also be recommended as it has been shown to both improve depression and lower levels of inflammation[2-4]. By modifying the inflammation risk in recurrent depression, the risk of comorbid diseases with chronic inflammation, such as coronary heart disease and diabetes, might also be mitigated[5].

In terms of therapeutics, there is currently no anti-inflammatory drug that is approved for treating clinical depression. Existing antidepressant drugs that have approved clinical indication for depression mainly target the monoaminergic system. The most recent antidepressant, esketamine, was approved by the US FDA in February 2019. Unlike previous antidepressants, it is the first in its class to target the glutamatergic system, and the only one that is rapid-acting. In the second chapter, ketamine was studied to determine whether it had immunomodulatory effects in the brain, and to glean insight into whether its effects on neuroimmune cells were also rapid-acting.

Microglia was chosen as the cell to study as it is a core immune cell in the brain and the first line of defense against injury or pathogens. Ketamine altered the morphology of microglia significantly within 24 hours of drug administration. Its effects on morphology seemed immunoregulatory and homeostatic. Correspondingly, ketamine lowered mRNA expressions of microglial activation, but showed no distinct polarization of its inflammatory markers.

In recent years, increasing attention has been paid to the study of microglial function in depressed patients with suicidal thoughts and in patients who had completed suicide. Suicide is the most concerning outcome of depression. One postmortem study found an increased primed to resting microglia ratio and higher mRNA expressions of MCP in depressed victims of suicide compared to controls who died without psychiatric, neurological or inflammatory illnesses[6]. Another postmortem study reported lower microglial reaction in non-suicide depressed patients versus depressed patients who committed suicide[7]. In living subjects, PET imaging of microglial activity (marked by TSPO) revealed significantly higher TSPO in depressed patients with suicidal thoughts than in depressed patients without[8]. Intriguingly,

ketamine, which we found to rapidly modulate microglia, has robust effects against suicidal ideations. A recent meta-analysis of clinical trials showed that intravenous ketamine could specifically reduce suicidal ideations within 1 day of drug administration (Cohen's d=0.85, 95% CI=0.53–1.17)[9]. It is postulated that microglia's pathomechanism is mediated by the tryptophan-kynurenine pathway (TKP). Activated microglia produce several neurotoxic metabolites in this pathway [e.g. 3-hydroxykynurenine (3-HK), quinolinic acid (QA)][10]. 3-HK is an oxidative stressor while QA is both an excitotoxic NMDAR agonist and oxidative stressor. When activated, microglia also release cytokines that induce indoleamine 2,3-dioxygenase activity in the TKP, which depletes tryptophan and reduces serotonin in the brain[11]. By lowering microglial activation and blocking NMDAR, ketamine could acutely reduce neurotoxins and restore serotonin levels, which might be important mechanisms in diminishing suicidality.

There is evidence to suggest that anti-inflammatory agents may influence depressive symptoms. In a meta-analysis of randomized placebo-controlled trials, the pooled estimate for cytokine inhibitors (etanercept, adalimumab, ustekinumab, infliximab) and NSAIDs (celecoxib, ibuprofen, naproxen)

indicated that these agents could reduce depressive symptoms (SMD=−0.34, 95% CI; −0.57 to −0.11, $I^2 = 90\%$) in the short-term[12]. It should be noted that almost all the trials did not measure levels of inflammation; many trials were tested in patient groups that had symptomatic comorbidities like osteoarthritis or psoriasis and most patients were not diagnosed with major depressive disorder. These patients might have felt better because their comorbid inflammation was treated. For a clearer understanding of whether anti-inflammatory drugs could be used to treat major depression, future trials should test them on patients who are diagnosed with major depression and without confounding comorbidities. Even if antidepressant effects were established, there are still concerns over the long-term use of anti-inflammatory drugs. NSAIDs are associated with gastrointestinal and cardiovascular adverse effects, and cytokine inhibitors might increase the risk of infections. Newer trials have investigated the adjunct use of minocycline, a microglia inhibitor, to treat major depressive disorder. Results have been mixed; one study showed a large treatment effect (standardised effect size = 1.21)[13] while another showed no significant difference from placebo[14]. This calls to attention the need for better identification of patients to suit certain treatments, because neither our patients nor our treatments are homogenous.

Information from prior chapters highlight the potential of precision medicine. In the first chapter, it was shown that patients with recurrent depression have a higher inflammatory load and a risk score based on biodata could be used to delineate the level of inflammation. This could be further refined by using a more inclusive model as noted above. Blood biomarkers of inflammation could additionally be used to identify patients for anti-inflammatory treatment. For example, a study showed that depressed patients with low levels of C-reactive protein (CRP) failed to have any antidepressant response to infliximab, while patients with high levels of CRP responded[15]. Another study noted that depressed patients with high levels of IL-1ra, IL-6, CRP and leptin had significantly better antidepressant response to omega-3 fatty acid (eicosapentaenoic acid) than those with low levels[16]. A panel of inflammatory blood markers, in the form of a high sensitivity test kit, may be developed in future to characterise a patient's inflammatory profile. As new anti-inflammatory therapeutics are being developed, existing anti-depressant drugs could also be examined for anti-inflammatory properties. A research group demonstrated that the anti-inflammatory effects of SSRIs and SNRIs could be quantified by measuring the level of pro-inflammatory cytokine production in LPS-stimulated microglia, and discovered that antidepressants had different

anti-inflammatory capabilities, even for antidepressants that belonged to the same class[17]. By matching patients' level of inflammation to the anti-inflammatory strength of antidepressants, the potential of personalised medicine may soon be truly realised and used to improve treatment outcomes.

References

1. Buckman JEJ, Underwood A, Clarke K, Saunders R, Hollon SD, Fearon P, Pilling S. Risk factors for relapse and recurrence of depression in adults and how they operate: A four-phase systematic review and meta-synthesis. Clin Psychol Rev. 2018;64:13–38.

2. Schuch FB, Vancampfort D, Richards J, Rosenbaum S, Ward PB, Stubbs B. Exercise as a treatment for depression: A meta-analysis adjusting for publication bias. J Psychiatr Res. 2016;77:42-51.

3. Hayashino Y, Jackson JL, Hirata T, Fukumori N, Nakamura F, Fukuhara S, et al. . Effects of exercise on C-reactive protein, inflammatory cytokine and adipokine in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. Metabolism 2014:63:431–40.

4. Swardfager W, Herrmann N, Cornish S, Mazereeuw G, Marzolini S, Sham L, et al. . Exercise intervention and inflammatory markers in coronary artery disease: a meta-analysis. Am Heart J. 2012:163:666–76.

5. Haffner SM. The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. Am J Cardiol. 2006;97(2A):3A-11A.

6. Torres-Platas SG, Cruceanu C, Chen GG, Turecki G, Mechawar N. Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. Brain Behav Immun. 2014;42:50-9.

7. Ralf Brisch, Johann Steiner, Christian Mawrin, Marta Krzyżanowska, Zbigniew Jankowski, Tomasz Gos. Microglia in the dorsal raphe nucleus plays a potential role in both suicide facilitation and prevention in affective disorders. Eur Arch Psychiatry Clin Neurosci. 2017;267(5):403–415.

8. Holmes SE, Hinz R, Conen S, Gregory CJ, Matthews JC, Anton-Rodriguez JM, Gerhard A, Talbot PS. Elevated Translocator Protein in Anterior Cingulate in Major Depression and a Role for Inflammation in Suicidal Thinking: A Positron Emission Tomography Study. Biol Psychiatry. 2018;83(1):61-69.

9. Wilkinson ST, Ballard ED, Bloch MH, Mathew SJ, Murrough JW, Feder A, Sos P, Wang G, Zarate CA Jr, Sanacora G. The Effect of a Single Dose of Intravenous Ketamine on Suicidal Ideation: A Systematic Review and Individual Participant Data Meta-Analysis. Am J Psychiatry. 2018;175(2):150-158.

10. Garrison AM, Parrott JM, Tuñon A, Delgado J, Redus L, O'Connor JC. Kynurenine pathway metabolic balance influences microglia activity: Targeting kynurenine monooxygenase to dampen neuroinflammation. Psychoneuroendocrinology. 2018;94:1-10.

11. Myint AM, Kim YK, Verkerk R, Scharpé S, Steinbusch H, Leonard B. Kynurenine pathway in major depression: evidence of impaired neuroprotection. J Affect Disord. 2007;98(1-2):143-51.

12. Köhler O, Benros ME, Nordentoft M, Farkouh ME, Iyengar RL, Mors O, Krogh J. Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. JAMA Psychiatry. 2014;71(12):1381-91.

13. Husain MI, Chaudhry IB, Husain N, Khoso AB, Rahman RR, Hamirani MM, Hodsoll J, Qurashi I, Deakin JF, Young AH. Minocycline as an adjunct for treatment-resistant depressive symptoms: A pilot randomised placebo-controlled trial. J Psychopharmacol. 2017;31(9):1166-1175.

14. Dean OM, Kanchanatawan B, Ashton M, Mohebbi M, Ng CH, Maes M, Berk L, Sughondhabirom A, Tangwongchai S, Singh AB, McKenzie H, Smith DJ, Malhi GS, Dowling N, Berk M. Adjunctive minocycline treatment for major depressive disorder: A proof of concept trial. Aust N Z J Psychiatry. 2017:51(8):829-840.

15. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, Haroon E, Miller AH. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. JAMA Psychiatry. 2013;70(1):31-41.

16. Rapaport MH, Nierenberg AA, Schettler PJ, Kinkead B, Cardoos A, Walker R, Mischoulon D. Inflammation as a predictive biomarker for response to omega-3 fatty acids in major depressive disorder: a proof-of-concept study. Mol Psychiatry. 2016;21(1):71-9.

17. Tynan RJ, Weidenhofer J, Hinwood M, Cairns MJ, Day TA, Walker FR. A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. Brain Behav Immun. 2012;26(3):469-79.