Investigating the Effects of Inflammation on Emotional Processing

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The greatest mistake in the treatment of diseases is that there are physicians for the body and physicians for the soul, although the two cannot be separated.

Plato (427–347 BC)

Abstract

Elevated levels of pro-inflammatory cytokines are implicated in the pathogenesis of major depression. Human and animal studies have shown that pro-inflammatory cytokines can induce a behavioural repertoire of symptoms collectively referred to as 'sickness behaviours', which include cognitive and mood symptoms, social withdrawal and sleep disturbance. When likened to clinical depression, these symptoms appear to be strikingly similar. Moreover, subsets of depressed patients have raised inflammatory markers, 30-50% of patients receiving cytokine treatment in the form of interferon-α (IFN-α) therapy develop depressive symptoms, and significantly higher rates of depression are associated with chronic inflammatory conditions, such as rheumatoid arthritis (RA). Converging evidence has led to the hypothesis that chronic, low-grade inflammation could lead to more persistent alterations in neuropsychological function that might be instrumental in the pathophysiology of depression. However, the mechanisms for this potential modulation of mood and cognitive function are unclear. The current thesis therefore aimed to enhance understanding of the neuropsychological underpinnings of the link between inflammation and depression.

Negative emotional processing biases are well-recognised in the aetiology of depression; however potential inflammation-induced alterations in emotional processing remain unexplored. Thus, a series of studies were conducted using human models of immune system activation to examine neuropsychological function. The first study demonstrated that IFN-α treatment in patients with hepatitis C produced negative biases in emotional categorisation, attentional vigilance and a specific effect of enhanced recognition of disgust. The subsequent study found a specific effect of false discrimination of disgusted faces in a healthy volunteer model of inflammatory challenge with typhoid vaccination, however further effects on emotional processing were limited. Typhoid vaccination was also shown to disrupt sleep continuity in ways that may be relevant to depression in the third study. Negative biases were not evident, however, in patients with RA. The final study found that neuropsychological effects of the atypical antidepressant tianeptine were similar to effects following IFN-α, which may be of interest considering tianeptine's purported serotonergic re-uptake enhancing properties and the effects of cytokines on serotonin metabolism. This thesis provides intriguing, yet preliminary, evidence that inflammatory pathways may modulate emotional processing – a mechanism which, if supported, may have future implications for improved identification and treatment of subgroups of depressed patients.

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Table of Contents

Chapter 3

Investigating the Effects of Typhoid Vaccine on Emotional Processing and Cognition

Chapter 5

Investigating Emotional Processing in a Model of Chronic Inflammation in Patients with Rheumatoid Arthritis

Chapter 6

Tianeptine in an Experimental Medicine Model of Antidepressant Action

Chapter 7

General Discussion

Long Abstract

Elevated levels of pro-inflammatory cytokines are implicated in the pathogenesis of major depression. Both human and animal studies have shown that pro-inflammatory cytokines can induce a behavioural repertoire of symptoms collectively referred to as 'sickness behaviours', which include cognitive and mood symptoms, such as depression, anhedonia and memory impairment, in addition to social withdrawal, fatigue and sleep disturbance. When likened to clinical depression, these symptoms appear to be strikingly similar. Additionally, subsets of depressed patients have raised levels of pro-inflammatory cytokines, up to half of patients receiving cytokine treatment in the form of interferon-alpha (IFN-α) therapy develop depressive symptoms, and higher rates of depression are associated with chronic inflammatory conditions, such as rheumatoid arthritis (RA). Such observations have contributed to the hypothesis that chronic, or unregulated, low-grade inflammation may cause more persistent alterations in neuropsychological function that might be instrumental in the pathophysiology of major depression. The precise mechanisms for this potential modulation of mood and cognitive function, however, remain unclear.

This thesis aimed to extend understanding of the neuropsychological underpinnings of the link between inflammation and depression. More specifically, the current body of work primarily investigated whether pro-inflammatory cytokines can modulate emotional processing. Negative affective biases in information processing are well-recognised in the aetiology and maintenance of depression, and are thus highly relevant to clinical mood disorders, however potential inflammation-induced alterations in emotional processing remain undefined. The studies presented here used behavioural techniques to explore the effects of inflammation on emotional processing, as well as non-emotional cognition and sleep, using human models of immune system activation.

Chapter 1 provides an overview of evidence that supports the relevance of negative affective biases in depression and the role of inflammation in depression pathophysiology. In addition, mechanisms of deleterious effects of pro-inflammatory cytokines on neurotransmitter function and neural circuitry are discussed, which may be relevant to emotional information processing.

Chapter 2 presents a study in which the effects of chronic IFN-α treatment on emotional processing in patients with hepatitis C virus were investigated. It was found that IFN-α, which activates the endogenous pro-inflammatory cytokine network, produced negative biases in emotional processing. IFN-α had a highly specific effect of enhancing recognition of disgusted faces and speeded reaction times to negative emotional stimuli in a facial expression recognition task. Negative biases were also demonstrated by increased accuracy in negative self-referent categorisation of emotional trait words, and a trend towards enhanced negative emotional memory. Negative attentional biases in the dot probe task were also found following IFN-α. This study provides novel evidence that inflammatory mechanisms may be capable of modulating emotional processing, which might be important in the development of depression in some patients receiving this treatment, and subsets of depressed patients with elevated inflammation more widely.

In order to examine the effects of inflammation on emotional processing further, and without potential confounds of physical illness symptoms, Chapter 3 utilised a healthy volunteer model of inflammatory challenge with typhoid vaccination. A specific effect of false discrimination of disgust was found, whereby the typhoid vaccine group misclassified more faces as disgusted in the facial expression recognition task compared to the placebo group. Negative biases in emotional categorisation, emotional memory or attentional vigilance, however, were not found. A battery of non-emotional cognitive tasks was also included in

this study; typhoid vaccine had no effects on sustained attention, no marked effects on working memory, mild effects on improving learning in an auditory verbal learning task, as well as slightly improved task performance in a probabilistic reward learning task, which is an opposite effect to a recently published study.

Chapter 4 investigated the effects of inflammation on sleep polysomnography using the typhoid vaccination model. In a cross-over design study, typhoid vaccination, but not placebo, was shown to disrupt several sleep continuity parameters, including decreased sleep efficiency and increased wake after sleep onset. This study thereby supports previous evidence that suggests innate immune cytokine activation can disrupt sleep continuity in ways that may be pertinent to depression.

The following study presented in Chapter 5 examined the effects of chronic inflammation on emotional processing in patients with RA. Negative biases expected in facial expression recognition were not observed – on the contrary, impairments in emotional face recognition were found in patients with high inflammation. Negative biases were also not apparent in emotional categorisation, emotional memory or attentional vigilance tasks. Instead, data were arguably more suggestive of cognitive impairment.

Chapter 6 investigated the effects of the atypical antidepressant tianeptine on emotional processing, which may be of interest when consideration is given to its purported acute serotonergic re-uptake enhancing properties and the effects of pro-inflammatory cytokines on serotonin metabolism. Tianeptine reduced positive emotional memory and enhanced avoidance of happy faces in a similar manner to effects seen with IFN-α in Chapter 2.

Finally, Chapter 7 presents a summary of the results and a discussion of the potential implications of this research on our understanding of the possible neuropsychological link between inflammation and depression, including a hypothesis presented in this thesis that

enhanced disgust processing may represent a distinct effect of inflammation on emotional processing. This chapter also addresses important methodological considerations that should be taken into account when interpreting results presented in this thesis, including limitations of using healthy volunteer models of inflammation-associated depression and studying patients with chronic physical illness. Suggestions for further research are also discussed.

Abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
AVLT	Auditory verbal learning task
BDI	Beck depression inventory
BMI	Body mass index
CNS	Central nervous system
CSF	Cerebrospinal fluid
CRP	C-reactive protein
DSM-IV	Diagnostic and statistical manual of mental disorders
ETB	Emotional test battery
EPQ	Eysenck personality questionnaire
FERT	Facial expression recognition task
HAM-D	Hamilton depression rating scale
HCV `	Hepatitis C virus
HPA	Hypothalamic-pituitary-adrenal
IDO	indoleamine 2,3-dioxygenase
IFN-α	Interferon-α
IL-1	Interleukin-1
IL-6	Interleukin-6
KYN	Kynurenine
mL	Millilitre
ms	Millisecond
NART	National adult reading test
PANAS	Positive and negative affective schedules
QUIN	Quinolinic acid
RA	Rheumatoid arthritis
SD	Standard deviation
SEM	Standard error of the mean
STAI	State trait anxiety inventory
SSRE	Selective serotonin re-uptake enhancer
SSRI	Selective serotonin re-uptake inhibitor
TNF-α	Tumour necrosis factor-α
VAS	Visual analogue scale

Chapter 1

Research Background

1.1 General introduction

Major depression is a commonly occurring, highly debilitating, and frequently recurrent disorder that afflicts up to 20% of individuals at some point in their lives (Kessler *et al.*, 2010). Estimates from the World Health Organisation suggest that it is a major contributor to the global burden of disease, representing the leading cause of disability worldwide (WHO, 2016), and it is associated with substantial reductions in quality of life, in addition to significant medical morbidity and mortality (Kessler and Bromet, 2013). Depression has a complex and multifactorial aetiology; as such it is characterised by a broad range of symptoms and marked heterogeneity between patients. The central symptomatology of clinical depression consists of depressed mood, pessimistic thoughts, diminished interest or enjoyment in activities (anhedonia) and fatigue or loss of energy. In addition, individuals with depression commonly experience poor concentration, memory impairment, reduced confidence and self-esteem, as well as feelings of guilt, unworthiness and self-shame. Disturbed sleep and appetite also frequently occur (Cowen, 2013). Although effective treatments are available, approximately one third of patients with depression fail to respond to conventional antidepressant therapies (Rush *et al.*, 2006). Thus, there is a pressing clinical need to enhance understanding of the pathophysiology of depression in order to identify additional pathways that could be targeted by novel therapeutics.

An increasing body of evidence indicates that inflammatory pathways may play an important role in the pathogenesis of depression. Both clinical and animal studies have shown that pro-inflammatory cytokines can rapidly induce a behavioural repertoire of symptoms collectively referred to as ‘sickness behaviour’, which represent coordinated reorganisation

of physiological, psychological and motivational states that are believed to promote conservation of energy expenditure to shift energy resources towards combating infection (Konsman *et al.*, 2002, Maes *et al.*, 2012). Symptoms associated with this behavioural repertoire display marked similarity to depressive symptomatology (Dantzer *et al.*, 2008b, Raison *et al.*, 2006), including mood and cognitive symptoms, such as depression, anhedonia and memory impairment, in addition to social withdrawal and neurovegetative symptoms, notably fatigue, psychomotor retardation and sleep disturbance (Capuron *et al.*, 1999, Dantzer *et al.*, 2008b, Motivala *et al.*, 2005, Reichenberg *et al.*, 2001). These common symptoms of sickness are a normal physiological response mediated by pro-inflammatory cytokines such as interleukin (IL)-1β, interleukin (IL)-6 and tumour necrosis factor-α (TNF-α) (Dantzer *et al.*, 2008b), which are released by activated immune cells during the host response to a pathogen, tissue injury and also psychological stress (Capuron and Miller, 2011). However, they also provide demonstrable evidence of the ability of pro-inflammatory cytokines to act centrally in the brain to cause behavioural and perceptual changes in state. Indeed, multiple lines of evidence linking inflammation and depression (discussed below) have contributed to the hypothesis that chronic, or unregulated, low-grade inflammation may cause more persistent alterations in neuropsychological function that might be instrumental in the pathogenesis of neuropsychiatric disorders, including major depression (Anisman, 2009, Dantzer *et al.*, 2008a, Miller *et al.*, 2009), though the precise mechanisms for this potential modulation of mood and cognitive function remain unclear.

The work presented within this thesis primarily aimed to investigate the effects of inflammation and pro-inflammatory cytokines on emotional processing, since negative affective biases are thought to play a central role in depression pathophysiology. The present introductory chapter therefore provides an overview of the modulation of emotional processing in depression, before presenting data that point to the immune system as a

potentially key modulator of behaviours that may contribute to the development of depression. Evidence of elevated inflammatory profiles in depressed patients are discussed, in addition to depression in medical illness, which is relevant to the study presented towards the end of this thesis that examined emotional processing in patients with a chronic inflammatory condition, rheumatoid arthritis (RA). Studies presented in this thesis also utilised human models of immune depression, including interferon (IFN)-α therapy and typhoid vaccination; thus evidence from previous studies using these models will be introduced to provide a background to data presented in the ensuing chapters. Furthermore, existing understanding of immune pathways thought to facilitate the communication between the periphery and the brain, and an overview of the pathophysiological effects of inflammatory cytokines on neurotransmitter metabolism and neurocircuitry will be introduced, as these mechanisms are thought to be involved in inflammation-associated depression and may be relevant to the work presented herein.

1.2 Emotional processing biases in depression

Negative affective biases in emotional information processing are well-recognised in the aetiology and maintenance of depression (Beck *et al.*, 1979, Elliott *et al.*, 2011). The cognitive model of depression was first developed by Beck (1967), providing a framework that characterised factors contributing to the onset and maintenance of depression. Central to this model is the notion that risk of developing depression is associated with the presence of negative self-referential schemas (e.g. 'I will never be a success') that can be activated by subsequent stressors (Clark and Beck, 2010). Activation of latent schemas by environmental triggers confers vulnerability to depression, as information processing is skewed towards negative self-referential thoughts about the self, world and future, and attentional resources are directed towards negative affective stimuli, while positive information is filtered out (Beck, 2008, Disner *et al.*, 2011). Ultimately, negatively biased information processing

(including biased attention, processing and memory) can lead to distorted negative interpretation, evaluation and appraisal of reality, and to additional symptoms of depression such as sadness, hopelessness, anhedonia and social withdrawal (Beck, 2008, Roiser *et al.*, 2012).

Negative biases in information processing have been extensively researched and have been linked to both risk of developing depression and risk of relapse (Bouhuys *et al.*, 1999, Hale, 1998, Harmer, 2013). Depressed patients have been shown to be more likely to interpret, focus on and remember negative compared to positive emotional cues in self-referent neuropsychological tasks (Harmer *et al.*, 2011a, Harmer *et al.*, 2009a, Mathews and MacLeod, 2005). For example, depressed patients have been found to be more likely to interpret ambiguous or neutral facial stimuli as being sad (Bouhuys *et al.*, 1999, Douglas and Porter, 2010, Gollan *et al.*, 2008, Leppanen *et al.*, 2004) and typically display impaired recognition of happy facial expressions (Bourke *et al.*, 2010, Gur *et al.*, 1992, Harmer *et al.*, 2009b). Negative biases in emotional memory have also been observed in depressed patients, which can manifest as increased incidental recall of negative material or reduced recall of positive material (Bradley *et al.*, 1995, Harmer *et al.*, 2009b, Leppanen, 2006, Roiser *et al.*, 2012). Furthermore, depressed patients exhibit negative biases in attentional tasks (Gotlib and Joormann, 2010, Roiser *et al.*, 2012), for example the attentional dot probe task, that are usually demonstrated by a tendency to attend to negative, over neutral, emotional stimuli, or to orient attention away from positive emotional cues (Fritzsche *et al.*, 2010, Gotlib *et al.*, 2004, Leppanen, 2006, McCabe and Gotlib, 1995, Roiser *et al.*, 2012).

Neuroimaging studies suggest that negative affective processing biases in major depression have an identifiable neuronal basis that is characterised by impairments in cortico-limbic circuitry (reviewed by Disner *et al.*, 2011, Elliott *et al.*, 2011). The amygdala, anterior insula, orbitofrontal cortex and ventral striatum are all implicated in emotion recognition

and generation of emotional reactions to the presentation of affective stimuli, and the anterior cingulate cortex (ACC) and prefrontal cortex are implicated in the regulation of emotional reactions to affective cues (Adolphs, 2002, Leppanen, 2006, Phillips *et al.*, 2003a, b). For example, implicit face processing paradigms in depression are associated with heightened responses in the amygdala, insula and ventral striatum to negative emotional expressions (Fu *et al.*, 2004, Surguladze *et al.*, 2005), and conversely decreased activity in the thalamus, amygdala, hippocampus and putamen in response to happy facial expressions (Fu *et al.*, 2007, Harmer *et al.*, 2009a, Lawrence *et al.*, 2004). Furthermore, it has been demonstrated that depressed patients display decreased connectivity between the ACC and limbic regions compared to healthy controls (Anand *et al.*, 2005).

Early, non-conscious amelioration of negative affective biases has been proposed as a final common pathway for the effects of a range of clinically effective antidepressant agents in the cognitive neuropsychological model of antidepressant action (Pringle *et al.*, 2011, Roiser *et al.*, 2012). This model advocates that early modification of emotional processing biases promotes a more positive social environment in which individuals with depression can re-learn emotional associations; in time this can lead to alleviation of depressed mood (Pringle *et al.*, 2011). Indeed, a large body of evidence shows that early effects of antidepressant treatments on emotional processing have been seen in both depressed patients and healthy volunteers, independently of subjective changes in mood (reviewed by Pringle and Harmer, 2015, Warren *et al.*, 2015). For example, reversal of negative biases in depressed patients has been seen following a single dose of reboxetine, whereby positive affective responses in facial expression recognition, emotional categorisation and emotional memory were increased, before subjective changes were seen (Harmer *et al.*, 2009b). Healthy volunteer models have also been used to examine direct actions of treatments on emotional processing, allowing exploration of effects without potential confounds of shifts in clinical symptoms.

For instance, seven days of citalopram reduced facial expression recognition of anger, disgust and fear, and decreased negative emotional memory compared to placebo in healthy subjects (Harmer *et al.*, 2004). Similar effects have been seen with a range of antidepressant treatments, including reboxetine (Harmer *et al.*, 2003a), duloxetine (Harmer *et al.*, 2008), mirtazapine (Arnone *et al.*, 2009) and agomelatine (Harmer *et al.*, 2011b). Effects found in experimental medicine models in healthy subjects would be expected to alleviate or reverse negative affective biases found in depression, thereby diminishing the influence of negative biases that are proposed to be a key mechanism in maintaining depression. Furthermore, recognition of happy facial expressions with two weeks of antidepressant treatment has been shown to correlate with beneficial therapeutic effects four weeks later in depressed patients, which suggests that early modification of emotional processing may underlie later treatment response (Tranter *et al.*, 2009).

Taken together, evidence suggests negative biases in emotional processing may be a significant process in the development and maintenance of depression (Harmer, 2013, Leppanen, 2006), and it is a key hypothesis that targeting affective biases may represent a common pathway for a range of pharmacologically distinct drugs to treat depression (Harmer, 2013, Harmer *et al.*, 2011a, Roiser *et al.*, 2012). The 'Cytokine effects on neurocircuitry' section below introduces intriguing evidence that inflammatory cytokines are capable of modulating neural activity in brain regions discussed above, however potential effects of inflammatory cytokines on emotional processing biases remain unknown.

1.3 Inflammatory cytokines and depression

1.3.1 Cytokines in major depression

There is rich literature suggesting that increased circulating inflammatory markers, including pro-inflammatory cytokines and acute phase proteins, are evident in the blood and cerebrospinal fluid (CSF) of patients with major depression, in the absence of additional medical illness (Raison *et al.*, 2006, Zorrilla *et al.*, 2001). Indeed, recent meta-analyses provide support for the modest, yet significant, elevation of inflammatory markers in subgroups of patients with idiopathic depression (Dowlati *et al.*, 2010, Howren *et al.*, 2009, Liu *et al.*, 2012a, Valkanova *et al.*, 2013). Associations with IL-6 and C-reactive protein (CRP) have been most frequently reported in major depression (Alesci *et al.*, 2005, Danner *et al.*, 2003, Ford and Erlinger, 2004, Lanquillon *et al.*, 2000, Miller *et al.*, 2002, Raison *et al.*, 2006, Tiemeier *et al.*, 2003), though increases in IL-1β and TNF-α have also been suggested to be reliably elevated markers of inflammation in depressed patients compared to non-depressed individuals (Hestad *et al.*, 2003, Lanquillon *et al.*, 2000, Levine *et al.*, 1999, Thomas *et al.*, 2005, Tuglu *et al.*, 2003). Inflammatory markers have also been associated with severity of depressive symptoms (Alesci *et al.*, 2005, Lindqvist *et al.*, 2009, Martinez *et al.*, 2012, Thomas *et al.*, 2005), and with individual depression-related symptoms, such as cognitive dysfunction and fatigue (Bower *et al.*, 2009, Capuron and Miller, 2011, Musselman *et al.*, 2001). However, it should be noted that few studies have measured central inflammatory markers and the extent to which peripheral markers mirror central inflammatory cytokine levels (Byrne *et al.*, 2016). Nevertheless, IL-6 in CSF has been found to be higher in suicide attempters (Lindqvist *et al.*, 2009) and functional allelic variants of genes for TNF-α, IL-1β and CRP might be associated with greater risk of depression (Bufalino *et al.*, 2013).

Elevated inflammatory markers have also been implicated in antidepressant treatment resistance, leading to hypotheses that cytokines may somehow circumvent the mechanisms of conventional antidepressant treatments (Miller *et al.*, 2009, Raison *et al.*, 2013). Indeed, depressed patients exhibiting antidepressant non-response have been shown to display elevated levels of pro-inflammatory cytokines, such as IL-6 and TNF-α (Fitzgerald *et al.*, 2006, Lanquillon *et al.*, 2000), as well as single nucleotide polymorphisms (SNPs) in the IL-6 and IL-1β genes that may be linked to reduced antidepressant responsiveness (Bufalino *et al.*, 2013, Uher *et al.*, 2010).

Furthermore, childhood trauma, which has been shown to be predictive of a greater risk of developing depression (Miller and Raison, 2015) and represents a potential factor in reduced treatment efficacy (Capuron and Miller, 2011), has been implicated in elevated inflammation (Baumeister *et al.*, 2016, Danese *et al.*, 2008, Danese *et al.*, 2007). For example, elevated IL-6 levels in adolescents with a history of childhood adversity were found to precede development of depression (Miller and Cole, 2012). Early life stress has also been implicated in greater inflammatory responses to stress tests in depressed patients (Pace *et al.*, 2006) and healthy adult subjects with a history of childhood maltreatment (Carpenter *et al.*, 2010).

Exaggerated stress-induced increases in TNF-α and IL-6, followed by elevated CRP and cortisol, have been documented in depressed patients (Weinstein *et al.*, 2010). Moreover, depressed subjects exhibit impaired ability for glucocorticoids to inhibit cytokine production in response to an acute psychological stressor, suggesting depression may be associated with increased resistance to molecules that under normal circumstances halt the inflammatory cascade (Miller *et al.*, 2005). Converging data therefore point to a potentially important relationship between stress, inflammation and depression, which has been proposed to be mediated via neuroendocrine pathways such as stress-induced hypothalamic-pituitary-

adrenal (HPA) axis alterations, or inflammasome activation that may drive stress-induced inflammatory responses pertinent to depression pathophysiology (this is beyond the scope of the present work, however it is reviewed by Felger and Lotrich, 2013, Miller and Raison, 2015).

Sleep disturbance is thought to be an additional risk factor for the development of depression, and sleep alterations are frequently experienced by depressed patients (Nutt *et al.*, 2008). Intriguingly, inflammatory pathways have also been implicated in sleep (Bryant *et al.*, 2004, Motivala *et al.*, 2005, Opp, 2005, Suarez, 2008) and sleep deprivation studies have shown that inflammatory markers are increased following sleep disruption (Irwin, 2015, Meier-Ewert *et al.*, 2004). However, data are limited, particularly objective data such as sleep polysomnography, thus further work is required to clarify the potential role of inflammation in sleep modulation (see Chapter 4).

Since not all patients with depression have raised inflammatory markers, it has been suggested that patients with elevated inflammation may represent a subtype of depression, in which immune pathways are biologically relevant to the development of disease. This hypothesis has been supported by studies demonstrating that elevated levels of IL-6 and CRP can predict subsequent development of depression and cognitive symptoms of depression (Gimeno *et al.*, 2009, Pasco *et al.*, 2010), although there are some conflicting data (Copeland *et al.*, 2012, Dantzer, 2012). Furthermore, anti-inflammatory treatment via TNF-α blockade has been shown to improve depressive symptoms in treatment resistant depressed patients with elevated CRP, but not in patients without inflammation (Raison *et al.*, 2013), and evidence suggests anti-inflammatory agents, particularly celecoxib, may have beneficial antidepressant properties in some patients (Kohler *et al.*, 2014).

It is noteworthy that although there is compelling evidence to suggest an important role of inflammation in depression pathophysiology, a significant proportion of depressed patients do not exhibit raised inflammatory markers (Krishnadas and Harrison, 2016). Thus, enhanced understanding of potential immune mechanisms that may contribute to depression in subgroups of patients is certainly required. In addition, inflammatory marker elevation in depressed patients has not been found to be overly pronounced, however in the wider literature even minor increases in inflammation have been shown to be sufficient to predict the development of a range of other medical conditions, such as cardiovascular disease, stroke and diabetes (Pradhan *et al.*, 2001, Raison and Miller, 2011, Ridker, 2007, Ridker *et al.*, 2000).

1.3.2 Medical illness and depression

Depression is two to three times more common in people with a range of chronic medical illnesses characterised by raised inflammatory markers compared to the general population (Haddad, 2009). Depressive symptomatology in physical illness is linked to poorer health outcomes, reduced quality of life (Moussavi *et al.*, 2007), and increased morbidity and mortality (de Groot *et al.*, 2001, Ellis *et al.*, 2010, Katon *et al.*, 2005).

The possible causes of depression in patients with chronic illness are multifaceted. General risk factors associated with depression onset include psychosocial factors, presence of pain and functional disability secondary to illness (Dickens *et al.*, 2002, Dickens *et al.*, 2004, Turvey *et al.*, 2009). However, mounting clinical data indicate the importance of the relationship between inflammation and depression in physically ill patients (Dantzer *et al.*, 2008b). For example, RA is a chronic inflammatory condition that is associated with depressive symptomatology is 13-17% patients (Cavanagh et al., 2010, Dickens et al., 2002), though there are large variations in reported prevalence of depression in RA (Bruce, 2008,

Isik *et al.*, 2007, Matcham *et al*., 2013). In addition, inflammation is recognised as a key contributing factor to coronary heart disease; indeed, depression rates that are three times more common than in the general population may be related to inflammatory mechanisms (Gianaros *et al.*, 2014, Harrison *et al.*, 2013). High rates of neuropsychiatric symptoms are also reported in a range of additional medical diseases, for example multiple sclerosis, Parkinson's disease, human immunodeficiency virus (HIV) and type 2 diabetes - all of which have an inflammatory basis (Feinstein *et al.*, 2014, Lindqvist *et al.*, 2013, Norcini Pala *et al.*, 2016, Swardfager *et al.*, 2016). Taken together, data suggest that innate immune activation during chronic physical illness, in which inflammatory cytokines can be persistently raised, may play a crucial role in the high prevalence of depressive symptomatology in individuals with medical illness.

Encouraging data points to the possibility that anti-inflammatory drugs may be able to reduce depressive symptoms in patients with chronic medical illnesses. For example, a meta-analysis of randomised controlled trials by Abbott *et al.* (2015) reported that TNF-α inhibitor therapy can reduce depression in patients with chronic disease, including RA, psoriasis and ankylosing spondylitis. Antidepressant effects were reported to occur in the absence of changes in clinical disease activity markers, suggesting that inflammatory mechanisms underpin the development and maintenance of depression (Abbott *et al.*, 2015, Arisoy *et al.*, 2013, Ertenli *et al.*, 2012). Peripheral TNF-α antagonism has also been shown to improve depressed mood in patients with psoriasis and Crohn's disease (Persoons *et al.*, 2005, Tyring *et al.*, 2006), and to reduce fatigue in cancer patients (Monk *et al.*, 2006).

1.3.3 Summary

Substantial evidence points to the involvement of pro-inflammatory cytokines in the pathophysiology of depression, both in subtypes of depressed patients who are otherwise

medically healthy and in patients with chronic medical illnesses. Inflammation has also been implicated in well known risk factors of depression, such as childhood trauma and psychological stress, in addition to sleep disturbance that commonly occurs in depression. Moreover, raised inflammatory states are thought to be involved in reduced response to conventional antidepressant treatments. However, the precise mechanisms through which inflammatory cytokines may lead to the development and maintenance of depression are yet to be fully understood, and thus relevant paradigms involving administration of cytokines or cytokine-inducers are required to investigate potential mechanisms involved in inflammation-associated depression.

1.4 Cytokine administration and depression

1.4.1 Interferon-α as a model of cytokine-induced depression

To further explore the relationship between inflammatory cytokines and depression, patients receiving interferon (IFN)-α have been examined as a key model of cytokine-induced depression. IFN-α is an innate immune cytokine that is released early in viral infection since it has antiviral and antiproliferative properties (Capuron and Miller, 2004). For this reason, it is also used as an exogenous cytokine therapy to activate the endogenous pro-inflammatory cytokine network to treat viral infections, such as chronic hepatitis C virus, and certain cancers (Capuron and Miller, 2004). Although this treatment has shown efficacy as an immunotherapy, it is heavily associated with the development of a range of neuropsychiatric symptoms, including depressed mood in 30-50% of patients, anxiety, fatigue, cognitive impairment and sleep disturbance (Hauser *et al.*, 2002, Leutscher *et al.*, 2010, Musselman *et al.*, 2001, Schaefer *et al.*, 2002).

The repeated observation that a pro-inflammatory cytokine treatment can induce symptoms that show marked overlap with symptomatology in idiopathic depression has provided some

of the most compelling evidence supporting the role of inflammation in the development of depressive states (Amodio *et al.*, 2005, Capuron *et al.*, 2009, Capuron *et al.*, 2002a, Fontana *et al.*, 2008, Maddock *et al.*, 2005), although there has been debate over the similarities and differences between IFN-α-induced depression and idiopathic major depression that remains to be fully clarified (discussed in Chapter 7). Furthermore, symptoms of depressed mood in IFN-α have been shown to respond to serotonergic antidepressant treatments (Baraldi *et al.*, 2012, Capuron *et al.*, 2002a, Kraus *et al.*, 2008, Musselman *et al.*, 2001), suggesting that they may be related to cytokine effects on serotonin metabolism (Miller *et al.*, 2013). The ability of peripheral IFN-α administration to activate a central inflammatory response, which has been shown to correlate with changes in neurotransmitter metabolism (particularly serotonin) is discussed later in this chapter.

Thus, IFN-α administration in humans has been suggested to display both face and predictive validity as a model of chronic, low-grade inflammation that may mimic inflammatory mechanisms that occur in subtypes of depression (DellaGioia and Hannestad, 2010). Evidence linking IFN-α to depression pathophysiology is further expanded upon in Chapter 2, where the effects of 6-8 weeks of IFN-α therapy in patients with chronic hepatitis C on emotional processing are investigated.

1.4.2 Experimental models of immune challenge

In addition to data from patients treated with IFN-α, behavioural changes including depressive symptoms have been reported in experimental models of acute innate immune system activation in healthy subjects. There are two main models serving this purpose that involve administration of inflammatory cytokine-inducers; *salmonella typhi* (typhoid) vaccination and endotoxin (lipopolysaccharide). Chapters 3 and 4 have utilised the typhoid vaccination model to investigate the effects of pro-inflammatory cytokines on emotional

processing, cognition and sleep, thus this model is introduced here and expanded upon in later chapters. Endotoxin administration has also contributed to this research field; thus this model is introduced briefly below.

1.4.2.1 *The typhoid vaccination model*

Typhoid vaccination has been shown to stimulate a mild, non-sickness inducing inflammatory response characterised by significantly elevated peripheral IL-6 levels, which has been reported to occur in the absence of flu-like symptoms, or elevations in blood pressure or body temperature (Brydon *et al.*, 2008, Harrison *et al.*, 2009a, Harrison *et al.*, 2009b). Moreover, it has previously been shown to induce reductions in subjective mood ratings and slight increases in fatigue symptoms (Harrison *et al.*, 2009a, Strike *et al.*, 2004, Wright *et al.*, 2005), although Paine *et al.* (2013) did not find evidence of subjective changes, thus further studies are required to explore this. Studies using this model have also provided intriguing evidence that elevated inflammatory cytokines are associated with altered activity in neural circuits that may be relevant to cytokine effects on mood and cognition, which is discussed later and expanded upon in Chapters 3 and 4.

1.4.2.2 *The endotoxin model*

Endotoxin is a component of the outer membrane of gram-negative bacteria that, when administered peripherally, activates an acute systemic inflammatory response (Kullmann *et al.*, 2013). Administration of endotoxin in humans has been shown to induce dose-dependent increases in IL-6 and TNF-α, flu-like symptoms, and concomitant symptoms of depressed mood (Eisenberger *et al.*, 2010b, Krabbe *et al.*, 2005, Kullmann *et al.*, 2013, Reichenberg *et al.*, 2001). Endotoxin has also been shown to disrupt sleep (Mullington *et al.*, 2000), which is discussed in Chapter 4, where the effects of typhoid vaccination on sleep were examined for the first time to our knowledge. This experimental model of acute

immune depression has been reviewed in depth elsewhere, for example by DellaGioia and Hannestad (2010).

1.5 Immune-to-brain communication

Considering the evidence that links increased inflammation and depression, there has been great research interest in elucidating the pathways through which inflammatory signals can be communicated from the periphery to the brain. Cytokines are relatively large molecules (~15-25 kD; Miller *et al.*, 2009), thus earlier work focussed on how they might be able to cross the blood-brain barrier (Miller and Raison, 2015). The three main pathways that have been defined to date include: 1) the 'humoral' route, involving the passage of cytokines through leaky regions in the blood-brain barrier, for instance the circumventricular organs, and active transport via cytokine specific saturable transporter molecules (Dantzer *et al.*, 2000, Miller *et al.*, 2013, Quan and Banks, 2007); 2) the 'neural' pathway, via afferent nerve fibres such as the vagus nerve, which can stimulate ascending catecholaminergic fibres to relay cytokine signals to relevant brain regions or trigger central cytokine signals (Luheshi *et al.*, 2000, Miller *et al.*, 2009, Miller and Raison, 2015, Watkins *et al.*, 1995); and 3) a 'cellular' route, whereby chemokines released by activated microglia (the main immune cell type in the brain) and adhesion molecules expressed within the central nervous system (CNS) can attract activated peripheral immune cells, such as monocytes and T cells, thereby trafficking them to the brain parenchyma and meninges (D'Mello *et al.*, 2009, Miller *et al.*, 2013, Miller and Raison, 2015).

Limitations in detecting subtle inflammatory responses in depressed patients using neuroimaging techniques have made it difficult to investigate immune pathways in humans (Miller *et al.*, 2013); accordingly, the majority of work contributing to immune-to-brain mechanisms outlined above are from animal studies. However, a recent positron-emission

tomography (PET) study using a radiolabelled tracer for translocator protein (TSPO), a protein that shows increased expression when microglia are activated during neuroinflammation (Setiawan *et al.*, 2015), found evidence of microglial activation in patients with major depression, as well as a positive correlation between depression severity and higher TSPO density in the ACC and insula (Setiawan *et al.*, 2015). A previous PET study, in contrast, did not find evidence of neuroinflammation or microglial activation in patients with mild-to-moderate depression (Hannestad *et al.*, 2013), though disparate findings may be due to heterogeneity of the sample (including differences in medication) or lack of depressed patients with elevated inflammation (Hannestad *et al.*, 2013, Setiawan *et al.*, 2015). Torres-Platas *et al.* (2014), however, provide compelling support for low-grade neuroinflammation in major depression, reporting that suicide victims with depression showed evidence of microglial priming in the dorsal ACC and significant upregulation of monocyte chemoattractant protein-1 (MCP-1), a chemokine that is involved in attracting circulating monocytes to the brain via the cellular route mentioned above. Furthermore, a recent study in healthy male subjects following endotoxin administration found evidence of microglial activation throughout the brain, alongside increases in peripheral inflammatory cytokines (Sandiego *et al.*, 2015). Additional evidence from a study in patients with hepatitis C receiving IFN-α therapy supports the ability of peripheral inflammatory signals to induce a central inflammatory response, demonstrated by elevated IL-6 and MCP-1 levels in the CSF (Raison *et al.*, 2009). This study also found that CSF concentrations of IL-6 were negatively correlated with the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), indicating that the CNS inflammatory response can interact with serotonin metabolism - a point that is further discussed in the following section.

1.6 Pathophysiological mechanisms of cytokine effects on the brain

1.6.1 Cytokine effects on neurotransmitter function

Evidence demonstrates that peripheral immune activation can lead to mood and behavioural changes, which is likely to be due to activation of a central inflammatory response via aforementioned pathways. Given the importance of neurotransmission in mood and behaviour, there has been significant research interest into the effects of inflammatory cytokines on monoamines serotonin, norepinephrine and dopamine, and the excitatory amino acid glutamate (Miller and Raison, 2015).

A central hypothesis in depression pathophysiology is the reduction in monoamine availability, particularly serotonin (Sharp and Cowen, 2011). Inflammatory cytokines are thought to be capable of reducing synaptic availability of monoamines via several mechanisms that may be relevant to depression. One frequently discussed mechanism is via activation of the indoleamine 2,3-dioxygenase (IDO) enzyme by inflammatory cytokines. IDO converts the primary amino acid precursor of serotonin, tryptophan, into kynurenine when activated, thereby potentially depleting serotonin availability in the brain (Dantzer *et al.*, 2008b, Felger and Lotrich, 2013, Miller *et al.*, 2013). Once more, animal studies have predominantly provided evidence in support of the activation of IDO in depression-like behaviours (reviewed elsewhere: Haroon *et al.*, 2012, Miller *et al.*, 2009), although human studies in patients receiving IFN-α have also shown significant decreases in tryptophan and increases in kynurenine that correlated with severity of depressive symptoms (Bonaccorso *et al.*, 2002, Capuron *et al.*, 2003). In addition, increases in plasma kynurenine have been shown to be reflected by increased CSF kynurenine levels in IFN-α-treated patients (Raison *et al.*, 2010b).

Cytokines may also affect monoamine metabolism via induction of signalling pathways including p38 mitogen-activated protein kinase (MAPK) that have been found to increase the expression and function of monoamine reuptake transporters. The majority of *in vitro* and *in vivo* studies to date have focused on reduced synaptic availability of serotonin resulting from increased serotonin transporter activity (Haroon *et al.*, 2012, Miller *et al.*, 2013), though cytokine-induced activation of MAPK signalling pathways has also been found to increase activity of the dopamine transporter (Capuron and Miller, 2011). Stimulation of p38 MAPK signalling pathways has been associated with depressive symptoms in patients receiving chronic IFN-α therapy (Felger *et al.*, 2011).

Inflammatory cytokines have been shown to reduce the availability of tetrahydrobiopterin (BH4), an essential enzyme co-factor for tryptophan hydroxylase and tyrosine hydroxylase, the rate limiting enzymes for synthesis of serotonin and dopamine/norepinephrine, respectively (Haroon *et al.*, 2012). Disruption of BH4 can occur as a result of inflammatory cytokines stimulating nitric oxide (NO) synthase, which converts arginine to NO; to do so, it utilises BH4 as an enzyme co-factor, and thus enhanced action of NO synthase reduces BH4 availability. Inflammatory cytokines can also produce reactive oxygen and nitrogen species that can lead to irreversible degradation of BH4, which is highly sensitive to oxidative stress (Miller *et al.*, 2013, Neurauter *et al.*, 2008). This may, therefore, represent a further mechanism through which inflammatory cytokines can reduce monoamine availability in the brain (Haroon *et al.*, 2012). BH4 is necessary for the conversion of phenylalanine (*phe*) to tyrosine (*tyr*) via phenylalanine hydroxylase, thus peripheral blood measures of the *phe*/*tyr* ratio can provide an indirect indicator of BH4 activity (Miller *et al.*, 2013). Indeed, in patients receiving IFN-α therapy, CSF concentrations of BH4 have been shown to negatively correlate with CSF IL-6, and increased plasma *phe*/*tyr* ratio was associated with both reduced dopamine in the CSF and symptoms of fatigue (Felger *et al.*,

2013). Peripheral *phe*, *tyr* and *phe*/*tyr* concentrations were also found to be associated with depressive symptoms including reduced motivation and altered sleep in elderly people with chronic low-grade inflammation (Capuron *et al.*, 2011).

As highlighted above, pro-inflammatory cytokines have been shown to modify the synthesis, release and reuptake of dopamine (Capuron and Miller, 2011). This can occur via degradation of BH4, a key enzyme co-factor for tyrosine hydroxylase (the rate limiting enzyme in dopamine synthesis), as well as via downstream effects of kynurenic acid, which can inhibit the release of glutamate, thereby causing inhibition of dopamine release, whose release is partially regulated by glutamatergic activity (Miller *et al.*, 2009), and via enhanced dopamine transporter activity (Felger and Lotrich, 2013, Moron *et al.*, 2003). Indeed, IFN-α administration in hepatitis C patients has been reported to decrease the release and increase the re-uptake of the radiolabelled dopamine precursor ^{18}F-dopa (Capuron *et al.*, 2012), supporting the notion that alterations in dopamine function may be involved in behavioural changes in inflammation-associated depression. The implications of reduced dopaminergic activity are further discussed with regards to reward circuitry in the next section.

Kynurenine can also be further catabolised into neuroactive metabolites kynurenic acid (in astrocytes) and quinolinic acid (QUIN; largely in activated microglia), which have been suggested to play a key role in behavioural modification, possibly through further effects on other neurotransmitter systems (Maes *et al.*, 2011, Raison *et al.*, 2010b). In particular, quinolinic acid has been found to directly activate glutamatergic *N*-methyl-D-aspartate (NMDA) receptors, stimulate glutamate release and inhibit glutamate re-uptake by astrocytes, leading to elevated synaptic glutamate concentrations (Maes *et al.*, 2011, Miller and Raison, 2015, Tavares *et al.*, 2002). In addition, pro-inflammatory cytokines are capable of reducing expression of astrocytic glutamate re-uptake transporters and stimulating astrocytic glutamate release (Miller *et al.*, 2013, Miller *et al.*, 2009). Together these actions

can contribute to glutamatergic excitotoxicity (Haroon *et al.*, 2012, Muller and Schwarz, 2007). Furthermore, enhanced access of glutamate to extrasynaptic NMDA receptors leads to decreased production of brain-derived neurotrophic factor (BDNF), which is importantly involved in neurogenesis - a key criterion for antidepressant response (Miller and Raison, 2015). Human data supporting these mechanisms include elevated levels of QUIN in microglia within the subgenual ACC of acutely depressed suicidal patients (Steiner *et al.*, 2011). Additionally, Haroon *et al.* (2014) found evidence of significant increases in glutamate in brain regions associated with cytokine-induced behavioural changes, the dorsal ACC and basal ganglia, as measured by magnetic resonance spectroscopy (MRS) in patients receiving IFN-α therapy. Increased glutamine levels have also been observed using MRS in the ACC of IFN-α-treated patients (Taylor *et al.*, 2014). A recent study by Haroon *et al.* (2016) further described increased basal ganglia glutamate levels in depressed patients with elevated inflammation (CRP > 3 mg/L) compared to individuals with low inflammation, which correlated with anhedonia and slower psychomotor speed.

1.6.2 Cytokine effects on neurocircuitry

Considering the effects of pro-inflammatory cytokines on neurotransmitter function, it is perhaps unsurprising that neuroimaging studies have revealed a range of effects on both cortical and subcortical neural activity. Most notably, increased inflammation has been associated with increased activity in the subgenual ACC (sACC), which is strongly implicated in depression, in addition to threat- and anxiety- related neural circuits, such as the dorsal ACC (dACC), insula and amygdala, and the basal ganglia (Capuron and Miller, 2011, Eisenberger and Lieberman, 2004) - regions that are also implicated in emotional processing biases in depression, as discussed previously.

Typhoid vaccination has been shown to increase activity in the sACC during an emotional face processing task, and peripheral IL-6 was associated with reduced connectivity of the sACC to the amygdala, medial prefrontal cortex, nucleus accumbens and superior temporal sulcus (Harrison *et al.*, 2009a). Furthermore, typhoid vaccination increased activity in the anterior insula cortex during performance of a cognitive task (the Stroop task), which was predictive of inflammation-associated fatigue and confusion when compared to placebo (Harrison *et al.*, 2009b). Stress-induced increases in oral soluble TNF-α receptor 2 were also found to correlate with enhanced activation of the dACC and bilateral anterior insula during a social rejection task, and greater activity in the right anterior insula related to increased levels of IL-6 (Slavich *et al.*, 2010). In addition, heightened amygdala activity in response to an acute laboratory-based social stressor was found to be associated with larger stressor-evoked increases in IL-6, along with stronger coupling between the amygdala and dorsomedial prefrontal cortex (DMPFC) (Muscatell *et al.*, 2014) - brain regions thought to be involved in processing social threats, including threatening facial expressions (Eisenberger, 2012, Whalen *et al.*, 2001). Furthermore, IFN-α treatment increased activation of the dACC in hepatitis C patients compared to controls, which correlated with errors in a visuospatial attention task (Capuron *et al.*, 2005). Endotoxin administration has also been shown to increase activity in the right orbitofrontal cortex in response to emotional stimuli (Kullmann *et al.*, 2013) and the amygdala in response to socially threatening images (Inagaki *et al.*, 2012) significantly more than placebo in healthy volunteers.

Consistent with the suggestion that pro-inflammatory cytokines target dopamine neurotransmission, which has a fundamental role in basal ganglia activity, repeated effects of cytokines on activity in the basal ganglia have been reported, particularly reduced activation of the ventral striatum (Capuron *et al.*, 2012, Miller *et al.*, 2013). For instance, increased plasma CRP and inflammatory cytokines in patients with major depression were

associated with reduced connectivity between the ventral striatum and ventromedial prefrontal cortex (vmPFC), which correlated with increased anhedonia (Felger *et al.*, 2015b). Moreover, depressed patients in this study with high inflammation (CRP > 3 mg/L) showed no significant connectivity between the ventral striatum and vmPFC, in contrast to patients with low inflammation who exhibited robust connectivity between these regions (Felger *et al.*, 2015b). IFN-α administration has also been shown to reduce bilateral activity in the ventral striatum during a hedonic reward task, which was correlated with behavioural alterations, such as anhedonia, depression and fatigue (Capuron *et al.*, 2012). Likewise, endotoxin administration has been shown to reduce activity in the ventral striatum in response to monetary reward cues, which was reported to mediate the relationship between inflammatory activity and depressed mood (Eisenberger *et al.*, 2010a). In addition, fMRI studies using typhoid vaccination have demonstrated inflammation-induced blunting of substantia nigra (a dopaminergic structure within the basal ganglia) responses to novelty (Harrison *et al.*, 2015a), and inflammation-induced altered activity in the ventral striatum and anterior insula to reward and punishment, respectively (Harrison *et al.*, 2015c). Taken together, these data provide support for deleterious effects of pro-inflammatory cytokines on basal ganglia nuclei that may be associated with reduced motivation or anhedonia, which is a core symptom of depression (Miller *et al.*, 2013, Miller and Raison, 2015).

1.6.3 Summary

There is substantial evidence indicating that pro-inflammatory cytokines are capable of disrupting neurotransmitter function via diverse mechanisms and pathways, that can, for example, lead to reduced serotonin availability and glutamatergic excitotoxicity. In keeping with such effects on neurotransmission, inflammatory cytokines have been shown to modulate neural activity in brain regions that are implicated in emotional information processing biases in depression, anxiety, motivation and reward. Under conditions of

chronic inflammation, these mechanisms might play important roles in the development of depressive symptomatology.

1.7 Aims of the present thesis

The present chapter has presented robust evidence in support of the involvement of inflammation in depression. Subtypes of depressed patients have been shown to exhibit elevated inflammatory markers, which may be involved in resistance to conventional antidepressant treatments. Furthermore, the prevalence of depression is significantly higher in patients with chronic inflammatory conditions, such as RA, compared to the general population. Administration of the cytokine therapy IFN-α is heavily associated with the onset of depressive symptoms and cognitive dysfunction. In addition, cytokine-inducers, including typhoid vaccination and endotoxin, have demonstrated that activation of the innate immune system can elicit a depression-like syndrome in healthy volunteers, which includes a range of behavioural changes such as cognitive dysfunction, fatigue, anhedonia and sleep disruption. Such observations have led to the hypothesis that pro-inflammatory cytokines are capable of adapting behaviours in ways that may be pertinent to depression pathophysiology.

Negative affective biases in emotional information processing are well-recognised in the aetiology and maintenance of depression, yet cognitive neuropsychological effects of inflammatory cytokines remain under investigated. The current thesis therefore aimed to combine well-validated neuropsychological tasks that have previously been shown to be sensitive to depressive states and a range of pharmacological manipulations (Harmer *et al.*, 2011a, Pringle and Harmer, 2015, Warren *et al.*, 2015), with human models of innate immune system activation to investigate the behavioural effects of inflammatory cytokines in more depth. Overall, we hypothesised that pro-inflammatory cytokines would produce

negative biases in emotional processing that are similar to those observed in depression, which could provide insight into the link between chronic, low-grade inflammation and the development of depressive symptomatology.

Studies were designed to use different approaches to address the overarching research question. Chapter 2 presents the effects of chronic IFN-α administration on emotional processing; findings in this study are compared to those observed following typhoid vaccination in healthy subjects in Chapter 3. Chapter 4 then utilised the typhoid vaccine model once more to investigate the effects of inflammation on sleep, as there is evidence that sleep disruption can modulate mood, emotional processing and inflammatory state. In Chapter 5, the effects of chronic inflammation on emotional processing were investigated in patients with RA, to compare effects in chronic and acute models of inflammation. Chapter 6 investigated an atypical antidepressant drug, tianeptine, which has been proposed to enhance re-uptake of serotonin, thus findings in this study may be of interest when consideration is given to purported inflammatory mechanisms of reduced serotonin function.

Chapter 2

Investigating the Effects of Interferon-α on Emotional Processing in Patients with Hepatitis C

2.1 Introduction

Chronic hepatitis C virus (HCV) is estimated to afflict 170 million people worldwide (Mohd Hanafiah *et al.*, 2013, Schaefer *et al.*, 2012) and represents a major global health concern. Treatment of chronic HCV has, until more recently (see Messina *et al.*, 2015), mainly involved weekly intramuscular pegylated interferon-α (IFN-α) and daily oral polymerase inhibitor ribavirin for up to 48 weeks, largely depending on HCV genotype (Baraldi *et al.*, 2012). IFN-α is itself a pro-inflammatory cytokine, that when given as an exogenous therapy potently activates the endogenous pro-inflammatory cytokine network, especially IL-6, to achieve its antiviral and antiproliferative properties (Capuron and Miller, 2004).

Despite its efficacy as an immunotherapy, IFN-α is also heavily associated with the onset of neuropsychiatric symptoms, including cognitive impairment, anxiety, sleep alterations, fatigue and depressive symptomatology in 30-50% of patients (Hauser *et al.*, 2002, Leutscher *et al.*, 2010, Musselman *et al.*, 2001, Schaefer *et al.*, 2002). The profound clinical observation that IFN-α is capable of inducing symptoms that are strikingly similar to major depression is thought to provide evidence of the potential for endogenous pro-inflammatory cytokines to contribute to the development of depressive states (Raison *et al.*, 2006).

Previous studies on IFN-α-treated patients have revealed two distinctive behavioural syndromes that suggest duration of exposure to treatment impacts on behavioural alterations. Neurovegetative symptoms and somatic symptoms of depression, including fatigue, altered sleep and anorexia, appear to develop early in treatment in a large proportion of patients, in

a seemingly non-specific manner (Capuron *et al.*, 2002a). Neurovegetative symptoms have also been found to be less responsive to antidepressant treatment with paroxetine in cancer patients (Capuron *et al.*, 2002a). In contrast, a mood/cognitive syndrome that is more responsive to antidepressant treatment can appear several days to weeks later in therapy (Baraldi *et al.*, 2012, Capuron and Miller, 2004, Dantzer *et al.*, 2011), however these symptoms appear to develop in a more specific group, particularly patients who develop major depression over the course of IFN-α therapy (Capuron *et al.*, 2002a), and may occur through physiologically distinct mechanisms to neurovegetative symptoms.

Several mechanisms have been proposed for the involvement of inflammation in mood and cognitive symptoms, as introduced in the previous chapter (and reviewed by Felger and Lotrich, 2013). Specific studies in IFN-α-treated patients support an interesting link between depressive symptoms and changes in serotonin metabolism. For example, IFN-α therapy has been shown to significantly reduce serum concentrations of tryptophan, the essential amino acid precursor for serotonin synthesis, which correlated with the development and severity of depressive symptoms, such as loss of concentration, pessimistic thoughts and suicidal ideation in cancer patients (Capuron *et al.*, 2002b). Increases in CSF concentrations of IL-6 have also been shown to be associated with decreases in the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), which were in turn correlated with depressive symptoms after 12 weeks of IFN-α administration for HCV (Raison *et al.*, 2009). A further study by Raison *et al* (2010b) showed that IFN-α increased kynurenine and reduced tryptophan levels in peripheral blood samples of HCV patients, and increased CSF kynurenine (KYN) concentration, which the authors concluded is likely to be secondary to indoleamine-2,3-dioxygenase (IDO) activation together with central cytokine responses. They further hypothesise that IDO and KYN pathways are relevant in promoting behavioural changes following immune activation (Raison *et al.*, 2010b).

IFN-α has also been reported to have varying effects on neurocircuitry, which is perhaps not surprising considering the range of purported effects on neurotransmitter pathways. Of note, increased dorsal anterior cingulate cortex (dACC) activity has been reported in HCV patients receiving IFN-α therapy compared with controls, which was suggested to underlie increased sensitivity to negative events and to contribute to cognitive changes that influence emotion regulation (Capuron *et al.*, 2005). The authors also suggest that cognitive information processing changes may signify a risk factor for the development of behavioural adaptations, including depression. The dACC has been suggested to detect and respond to threatening stimuli in the social domain, in effect acting like a 'neural alarm system' (Eisenberger and Lieberman, 2004), that may confer sensitivity to potentially threatening social conspecifics (Miller *et al.*, 2013). Further suggestions have included the role of the ACC as a psychophysiological bridge between cognitive and biobehavioural systems that may affect behavioural changes (Critchley *et al.*, 2005). Moreover, magnetic resonance spectroscopy (MRS) has been used to show IFN-α-induced elevated glutamate in the dACC (Haroon *et al.*, 2014). In addition, there is evidence that IFN-α can modulate insula and striatal activity (Dowell *et al.*, 2016), which is also believed to play a role in behavioural changes.

As mentioned in the previous chapter, cognitive biases in emotional processing are thought to play a central role in the onset and maintenance of depression. There is also robust evidence demonstrating that monoaminergic modulation can modify emotional processing biases, as measured in psychological tasks, for example reduced serotonin availability following tryptophan depletion (Booij *et al.*, 2005, Harmer *et al.*, 2003b, Hayward *et al.*, 2005, Klaassen *et al.*, 2002) and enhanced serotonin availability following serotonergic antidepressant treatments (Harmer, 2008, Harmer and Cowen, 2013). Importantly, changes in emotional processing have been found prior to changes in subjective mood (Harmer *et al.*, 2009a). Furthermore, the ACC has been suggested to play a key role in the cognitive

model of depression (Disner *et al.*, 2011) and the anterior insula is thought to integrate sensory and cognitive regions involved in emotional processing and interoception (Craig, 2009, Henje Blom *et al.*, 2015).

2.1.1 Aims

Taken together, the above evidence in IFN-α-treated patients provides intriguing rationale for the investigation of the effects of pro-inflammatory cytokines on emotional processing biases. Currently there is a noticeable paucity of literature concerning potential effects of IFN-α on affective biases, and a lack of clarity as to how IFN-α, either directly or indirectly, leads to behavioural changes that can include depression. This study therefore aimed to investigate the effects of pro-inflammatory cytokines induced by IFN-α administration on emotional processing, with a view to improving understanding of its action at a neuropsychological level and to assess whether deterioration in mood could be via effects on emotional processing.

To do so, we tested HCV patients prior to treatment and 6-8 weeks into IFN-α therapy using an established battery of psychological tests that have previously been shown to be sensitive to depressed states and monoaminergic modulation (Warren *et al.*, 2015). We hypothesised, based on previous literature discussed briefly above, that IFN-α could cause negative affective biases in information processing.

2.2 Methods

2.2.1 Participants and study design

We recruited 21 participants, aged between 18 – 65 years, who were scheduled to receive pegylated IFN-α plus ribavirin treatment as part of their routine clinical care for chronic HCV. Four participants did not return for the second study visit, therefore data presented are from 17 participants. Participants were referred from a National Health Service

hepatology clinic at the John Radcliffe Hospital (Oxford, UK). They were screened using the Structured Clinical Interview for DSM-IV (SCID; Spitzer *et al.*, 1995) for history of or current Axis I disorders; past psychiatric history, for example depression, opiate or alcohol dependence, were not exclusion criteria, however participants taking non-stable psychotropic medication were excluded from the study, as were participants with clinical or biochemical evidence of cirrhosis (all participants had a liver biopsy or fibroscan prior to referral). Concomitant medication was maintained at a stable dose throughout the study, therefore results observed in the present study should not be due to changes in other medications. The following supplements or medications were taken by participants: cod liver oil (*n*=1), vitamin C (*n*=1), daily multivitamins (*n*=1), metformin and insulin (*n*=1), ventolin inhaler (*n*=1), lansoprazole (*n*=1), omeprazole (*n*=1), three patients were on stable doses of methadone, and two patients were taking stable doses of the antidepressant mirtazapine. Inclusion criteria also included fluency in English due to the nature of the psychological tasks.

The study received full ethical approval by the Oxfordshire Research Ethics Committee A and was overseen by the Oxford University Clinical Trials and Research Governance team and sponsored by the University of Oxford. All participants gave written informed consent prior to study procedures being performed. Participants were reimbursed for their time and travel expenses.

Participants attended two study visits; one pre-treatment and one after 6-8 weeks of IFN-α treatment. Weeks 6-8 should, according to available literature, begin to capture patients at a stage where mood/cognitive changes may start to differentiate from sickness behaviours in those individuals who might go on to develop major depression (Capuron and Miller, 2004, Capuron *et al.*, 2007, Dowell *et al.*, 2016). During each visit the Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960) was performed, followed by subjective mood

questionnaires, after which participants completed the emotional test battery. In addition, demographic measures were taken at the first visit to characterise the study sample, including age, body mass index (BMI), units of alcohol and cigarettes consumed, if applicable, and verbal IQ was assessed using the National Adult Reading Test (NART) (Nelson, 1982).

2.2.2 HAM-D and subjective state

Participants' mood states were assessed at baseline (pre-treatment) and 6-8 weeks after IFN-α administration using the Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960), Beck Depression Inventory (BDI) (Beck *et al.*, 1961), state component of the State Trait Anxiety Inventory (STAI) (Spielberger *et al.*, 1970), Chalder Fatigue Scale (CFS; Chalder *et al.*, 1993) and Snaith Hamilton Pleasure Scale (SHAPS; Snaith *et al.*, 1995) as a measure of anhedonia. Additionally, participants were asked to complete subjective mood questionnaires (all of the above, except the HAM-D) weekly for 6 weeks after the start of treatment to assess changes over time. Questionnaire data for both study visits are available for all participants, however four participants did not complete the weekly questionnaires in between study visits.

2.2.3 Emotional test battery

2.2.3.1 *Facial expression recognition*

This task featured facial expressions of six basic emotions (happiness, surprise, sadness, fear, anger and disgust) taken from the Pictures of Affect Series (Ekman P, 1976). Faces were morphed between each prototype and neutral to portray varying intensities of each emotion, in 10% steps from 0% (neutral) to 100% (full intensity of emotion). Four examples of each emotion at each intensity were displayed, in addition to ten neutral stimuli, giving a total of 250 stimuli. Facial expressions were presented in random order for 500 ms, followed

by a blank screen. Participants were asked to classify facial expressions as quickly and accurately as possible by pressing one of seven labelled keys on the keyboard. Accuracy (the number of correctly identified faces of a particular emotion divided by the total number of faces containing that emotion), reaction times for correct responses and misclassifications of emotional faces were calculated.

2.2.3.2 *Emotional categorisation and memory*

Emotional Categorisation Task. Sixty personality characteristic words selected to be disagreeable (e.g. domineering, hostile) or agreeable (e.g. optimistic, honest; taken from Anderson, 1968) were presented in a random order on screen for 500 ms. Words were matched in terms of length, ratings of frequency and meaningfulness, and equal numbers of positive and negative words were included. Participants were asked to imagine whether they would be pleased or upset if they were to overhear someone else describing them in this way, so that the judgment was self-referential in nature. Responses were made by pressing a labelled button according to whether they would 'like' or 'dislike' to be described as possessing that characteristic. Subjects were asked to respond as quickly and accurately as possible. Accuracy of categorisation and reaction times for correct identifications were calculated in this task.

Emotional Memory. Fifteen minutes after completion of the emotional categorisation task, during which time participants completed an intervening task (dot probe; see below), subjects were asked to recall as many of the personality trait words as possible. They were given strictly two minutes to do so. This allows the assessment of incidental memory for positive and negative characteristics. The number of positive and negative words recalled were analysed for both accuracy and intrusions (false alarms). Participants were then presented with a mixture of the 60 positive and negative target words that were presented

previously, as well as 60 matched positive and negative distractor words which were not previously presented. Subjects were asked to respond by indicating whether they recognised each word as having been presented previously or not by pressing a key marked 'old' or 'new', as quickly and accurately as possible. Accuracy, reaction times and false alarms were computed.

2.2.3.3 *Dot probe attentional task*

Stimuli were pairs of facial expressions (Matsumoto and Ekman, 1988), each comprising one emotional and one neutral expression of the same individual or two neutral expressions of the same individual (described previously by Murphy *et al.*, 2008). This meant that there were three types of face pairs: neutral-neutral, fearful-neutral and happy-neutral. Fearful and happy faces were presented with equal frequency. A central fixation cross began each trial, followed by pairs of faces, one at the top and one at the bottom of the screen. In the unmasked condition, each face pair was presented for 100 ms, immediately followed by a probe, which appeared in the location of one of the preceding facial expressions. The probe was two dots in a vertical (:) or horizontal (..) orientation. Participants were asked to identify the orientation of the dots by pressing the correspondingly labelled key on a keyboard. The probe remained on the screen until the participant had responded, and participants were asked to respond as quickly and accurately as possible. The masked condition consisted of the same sequence of events, except that the face pair was displayed for 16 ms followed by a mask (scrambled face), which was displayed for 84 ms. Each of the three face pair conditions were presented 32 times in the masked condition and 32 times in the unmasked condition, thus there were 192 trials in total. There were 8 blocks of both the masked and unmasked condition, which were presented in an alternating order. Position of an emotional face, probe position and type were fully counterbalanced. The task design therefore involved congruent trials (where the probe appears in the position of the emotional face) and

incongruent trials (where the probe appears in the position of the neutral face while an emotional face is present).

Attentional vigilance scores were calculated for each participant by subtracting the median reaction time in congruent trials from incongruent trials. Positive values reflect attention towards the emotional face (vigilance), whereas negative values reflect attention away from the emotional face (avoidance).

2.2.4 Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics (version 22). Questionnaire data (HAM-D, BDI, state anxiety, CFQ and SHAPS) were compared before and after IFN-α treatment using paired sample *t*-tests. To assess changes in subjective mood over the 6 week period after the start of treatment, repeated measures ANOVAs were performed with time as a within-subject factor. The facial expression recognition, emotional categorisation, emotional recall, emotional recognition and dot probe tasks were all analysed using repeated measures ANOVA, with time (representing pre-treatment and treatment with IFN-α) and emotion as within-subject factors. In the dot probe task, masking was added as an additional within-subject factor. Post-hoc analyses using paired sample *t*-tests were performed to follow up interactions observed. Where assumptions of equality of variances were broken, Greenhouse-Geisser procedure was used to correct the degrees of freedom. Extreme outlying data (data lying at more than three times the participants' interquartile range above their third or below their first quartile) were removed from all psychological tasks. This resulted in minimal loss of data on any task (< 2%). Data from each task were analysed separately, without correcting for multiple comparisons across tasks.

To assess the relationship between depressed mood and behavioural measures, Pearson's correlation coefficient was computed between depression severity scores (using the BDI for

continuity across studies within this thesis) and neuropsychological task performance. Pearson's correlation coefficients were also examined between pre-treatment and post-treatment HAM-D and subjective mood scores, to assess whether pre-treatment mood was an indicator of depressed mood following IFN-α administration in this cohort.

Since this is the first study, to our knowledge, to assess the effects of IFN-α treatment on a battery of emotional processing tasks, it was intended to be a preliminary exploratory study and power calculations were not performed. Ethics approval was granted for recruitment of 35 patients, however due to slow recruitment of patients this target was not met.

2.3 Results

Data presented herein are from 17 participants (13 males, 4 females; mean age 39.7, SD 10.3, range 22-60 years; mean BMI 26.1 kg/m^2, SD 5.1, range 19.7-36.3). Verbal IQ was also estimated using the NART (mean 111.54, SD 6.3), in addition to alcohol (4 participants consumed alcohol; mean 8±4 units per week) and cigarette (8 participants were smokers; mean 12±5 per day) consumption.

One participant completed the facial expression recognition and dot probe tasks only, thus data for the remaining emotional categorisation and memory tasks are from 16 participants.

2.3.1 HAM-D and subjective state

IFN-α treatment significantly increased HAM-D scores ($t(16)=-4.93$, $p<0.001$). Furthermore, all subjective state measures included in the study were significantly elevated after IFN-α treatment, as shown in Table 1. Mood scores recorded weekly for 6 weeks following the start of treatment increased in a linear manner with time, therefore results presented here are from baseline and 6-8 weeks only.

Table 1. Mood state changes over time. Values are ratings at baseline (pre-treatment) and 6-8 weeks after IFN-α treatment (*n*=17). Means (standard deviations).

	Baseline	**IFN-α**	**Statistical significance**
HAM-D	4.47 (5.75)	13.59 (9.18)	$p < 0.001$
BDI	6.32 (7.79)	14.56 (12.35)	$p = 0.001$
State anxiety	33.35 (8.29)	39.82 (17.27)	$p = 0.045$
CFQ	13.94 (5.54)	21.00 (7.14)	$p < 0.001$
SHAPS	1.06 (2.88)	2.82 (4.29)	$p = 0.031$

HAM-D: Hamilton Depression Rating Scale; BDI: Beck Depression Inventory; CFQ: Chalder Fatigue Questionnaire; SHAPS: Snaith Hamilton Pleasure Scale

According to HAM-D severity (using classifications from Zimmerman *et al.*, 2013), 6/17 participants (35.3%) had a score indicative of mild depression (a score of 8-16), 2/17 participants (11.8%) had moderate depression (a score of 17-23) and 3/17 participants (17.6%) had severe depression (score ≥24) following 6-8 weeks of IFN-α treatment.

Correlations between pre- and post- IFN-α depressed mood scores were found, suggesting that mood state at baseline might be associated with later depressed mood scores following IFN-α administration (HAM-D $r = 0.56$, $p = 0.02$, BDI $r = 0.73$, $p = 0.001$). State anxiety, however, was not significantly correlated with later depressed mood score ($p > 0.1$).

2.3.2 Facial expression recognition

There were significant main effects of emotion ($F(5,70)=14.03$, $p<0.001$) and treatment ($F(1,14)=10.95$, $p=0.005$) on accuracy in this task, as well as a significant interaction between emotion and treatment ($F(3.07, 43.0)=3.41$, $p=0.025$). *Post-hoc* analysis revealed that this was driven by significantly increased accurate recognition of disgust after IFN-α treatment ($t(15)=-4.71$, $p<0.001$; see Figure 1). No significant effects were observed for

other valences (anger p=0.82, fear p=0.25, happy p=0.71, sad p=0.38, surprise p=0.77), hence this appears to be a specific effect on disgust recognition.

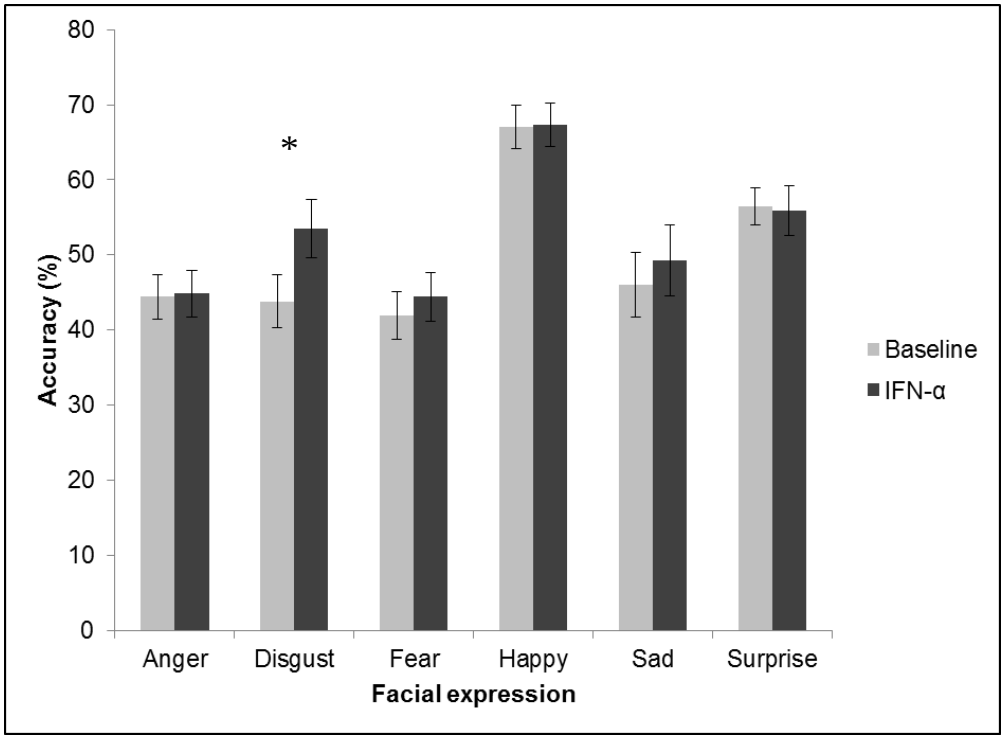


Figure 1. Performance in the facial expression recognition task before (light bars) and following 6-8 weeks IFN-α treatment (dark bars). Values represent the mean percentage correct for each of the six basic emotions summed over the different intensity levels used in this task, with error bars representing standard error of the mean (SEM). * p <0.001.

For emotion discrimination (d'), which controls for differences in response criteria, the results were similar to those seen for accuracy above; there was a significant main effect of emotion (F(5,70)=15.06, p<0.001) and of treatment (F(1,14)=12.16, p=0.004). In addition, there was a significant emotion x treatment interaction (F(3.06,42.81)=3.05, p=0.038). Again, there was a marked increase for disgust sensitivity after IFN-α treatment compared to baseline; *post-hoc* analysis revealed that this difference was highly significant (paired *t*-

test: $t(15)=-5.20$, $p<0.001$). There was a trend for increased sensitivity to fearful faces ($p=0.062$), but no significant effects on other emotions (p values >0.2).

To explore the effect of IFN-α on disgust recognition in more detail, accuracy was analysed over the different morphed intensity levels presented during the task. There was a significant main effect of treatment ($F(1,15)=22.16$, $p<0.001$), with patients after treatment showing significantly elevated recognition of disgust from 30% morphed intensity compared to pre-treatment, as shown in Figure 2. This analysis was extended to other facial expressions over morphed intensity levels, for which there were no significant differences before and after treatment (main effect of treatment: anger $F(1,16)=0.06$, $p=0.82$; fear $F(1,16)=1.42$, $p=0.25$; happy $F(1,16)=0.02$, $p=0.88$; sad $F(1,16)=0.81$, $p=0.38$; surprise $F(1,16)=0.09$, $p=0.77$), confirming a specific effect on disgust recognition.

Moreover, Figure 3 demonstrates a specific effect on the threshold for disgust recognition, whereby IFN-α significantly reduced the threshold of accurate recognition of this emotion (paired t-test: $t(15)=2.35$, $p=0.03$). Once more, this is a highly specific valence effect that was not observed for any other emotion (all p values >0.2). There was no overall emotion x treatment interaction when examining recognition thresholds ($F(5,75)=1.09$, $p=0.38$), though there was a main effect of emotion ($F(5,75)=8.82$, $p<0.001$).

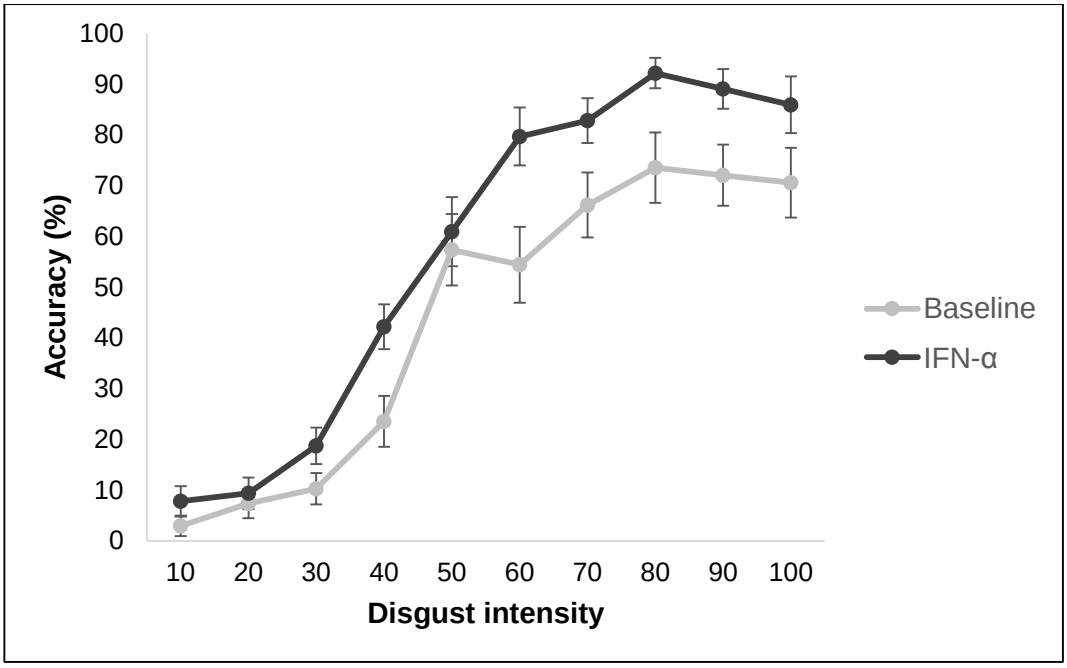


Figure 2. Recognition accuracy over the different intensity levels for disgust before (light grey) and after IFN-α treatment (dark grey). Values represent mean percent accuracy, with error bars representing SEM.

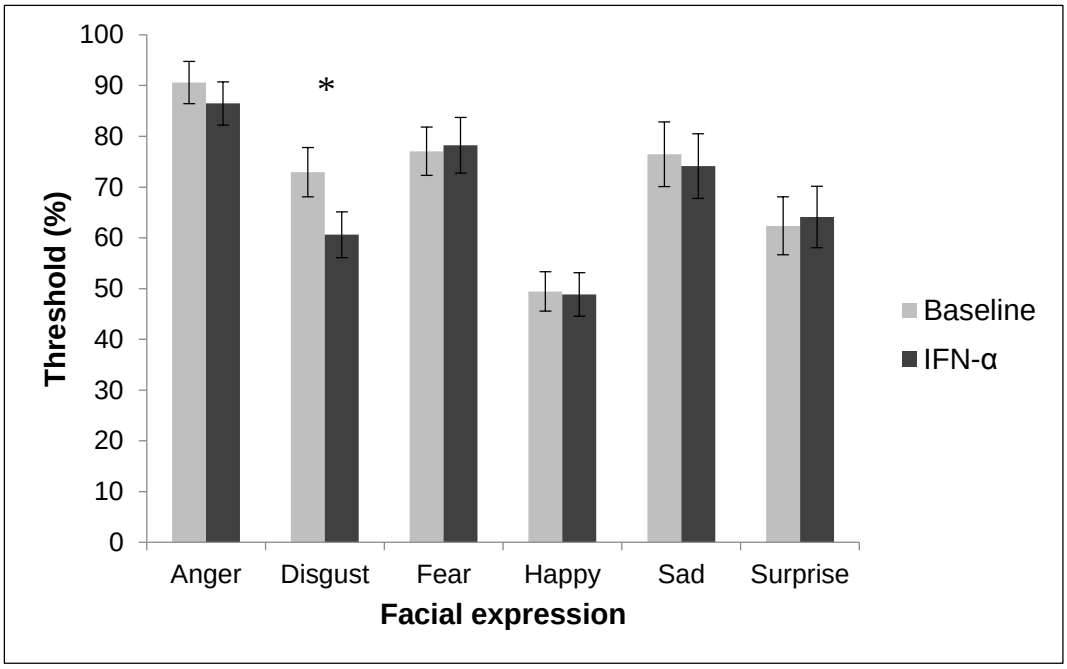


Figure 3. Threshold for accurate detection of facial expressions before (light bars) and after IFN-α treatment (dark bars). Values represent mean percentage of thresholds, at which emotional expressions were accurately identified. Error bars show SEM. * p=0.03

There were main effects of emotion ($F(5,75)=7.60$, $p<0.001$) and treatment ($F(1,15)=22.53$, $p<0.001$) on reaction times in the facial expression recognition task, however there was no interaction between emotion and treatment ($F(5,75)=0.91$, $p=0.48$). *Post-hoc* analysis using paired *t*-tests showed significantly reduced reaction times for both fearful ($t(16)=2.39$, $p=0.029$) and sad faces ($t(16)=2.43$, $p=0.027$) after treatment, and a trend towards significantly faster responses to disgusted faces ($t(15)=1.98$, $p=0.067$), but not angry, happy or surprised faces (p values >0.1). This observation suggests that there could be an effect of IFN-α reducing reaction times for negative valences more than positive valences; thus reaction times were pooled in this way (Figure 4) to reveal a significant emotion x treatment interaction ($F(1,16)=8.52$, $p=0.01$), in addition to significant main effects of treatment ($F(1,16)=22.81$, $p<0.001$) and emotion ($F(1,16)=365.12$, $p<0.001$). It is possible that reaction times may have been reduced due to a practice effect of the task being repeated at the second visit, although it is interesting that there was not a global reduction in reaction times for all emotions, rather reaction times were only significantly reduced for negative emotional stimuli.

There were main effects of emotion ($F(2.86,40.04)=5.10$, $p=0.005$) and treatment ($F(1,14)=5.50$, $p=0.034$) on misclassifications in this task, however there was no emotion x treatment interaction ($F(5,70)=1.73$, $p=0.14$). *Post-hoc* analysis revealed a trend towards reduced misclassification of fearful faces ($t(16)=2.11$, $p=0.051$), but no further valence specific effects of treatment (all p >0.1).

There were no significant correlations between depression rating (using the BDI) and accuracy or reaction times in the facial expression recognition task either pre-treatment or following IFN-α administration (all p values >0.1). A positive correlation between

depression score and anger misclassifications (r = 0.664, p = 0.007) was found after IFN-α (for all other valences p values >0.1).

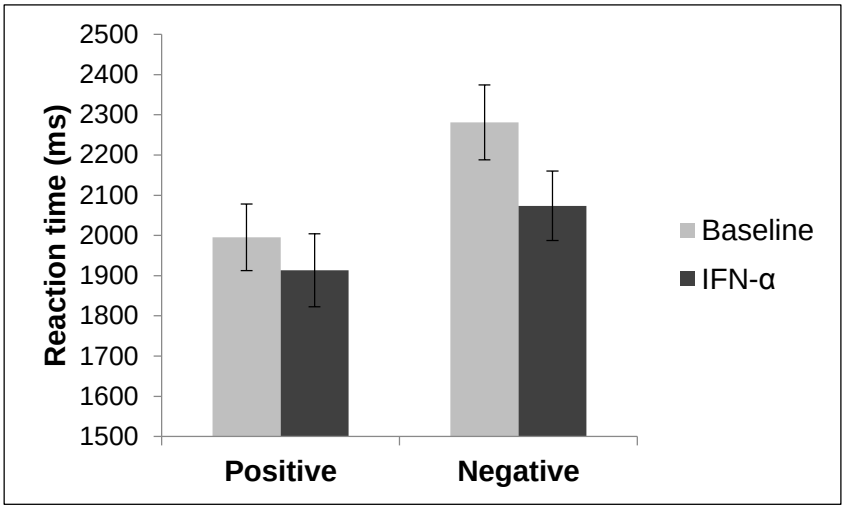


Figure 4. Reaction times for accurate detection of positive and negative emotional facial expressions before (light bars) and after IFN-α treatment (dark bars). Values represent mean reaction times, with error bars representing SEM. Emotion x treatment interaction: p=0.01.

2.3.3 Emotional categorisation task

There was a significant main effect of emotion (F(1,14)=8.74, p=0.01) and of treatment (F(1,14)=9.6, p=0.02) on accuracy in categorising self-referent personality characteristics, but no interaction between emotion and treatment (F(1,14)=4.27, p=0.24). Figure 5 shows that IFN-α treatment appears to increase accuracy in categorising negative characteristics, which is a significant difference to baseline (t(14)=-2.70, p=0.017), whereas there was no significant difference in positive categorisation between the two visits (t(14)=-0.48, p=0.64).

There were no treatment effects on reaction times to categorise self-referent personality characteristics in terms of a main effect of treatment (F(1,14)=0.84, p=0.38) or emotion x treatment interaction (F(1,14)=2.04, p=0.18), though there was a main effect of emotion (F(1,14)=10.17, p=0.007).

There were no correlations between depression score and accuracy or reaction times in the emotional categorisation task (all *p* values >0.1).

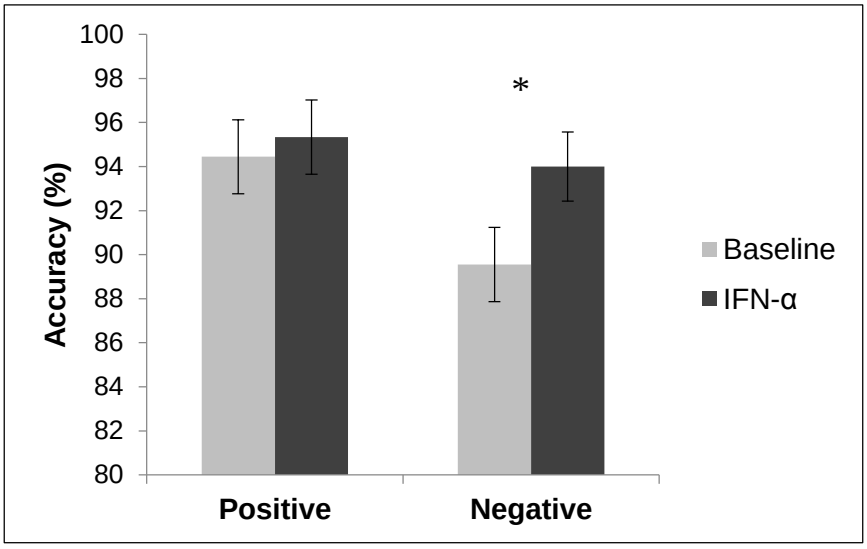


Figure 5. Accuracy in the emotional categorisation task before (light bars) and after IFN-α administration (dark bars). Values represent the mean percentage correct categorisation of positive and negative personality characteristic words used in this task, with error bars representing SEM. * p=0.017.

2.3.4 Emotional memory

The total number of correct words recalled differed significantly between the two visits before and after IFN-α treatment (F(1,15)=5.29, p=0.04), which is likely to be a practice effect since this task assessed incidental emotional memory at the first visit, whereas by the second visit the surprise element of this task was removed. It is still interesting to note, however, that negative emotional recall nearly reached significance (t(15)=-2.08, p=0.056; see Figure 6), in contrast to positive emotional recall (t(15)=-1.46, p=0.17). No significant main effect of emotion (F(1,15)=0.36, p=0.56) or emotion x treatment interaction (F(1,15)=0.36, p=0.56) was observed.

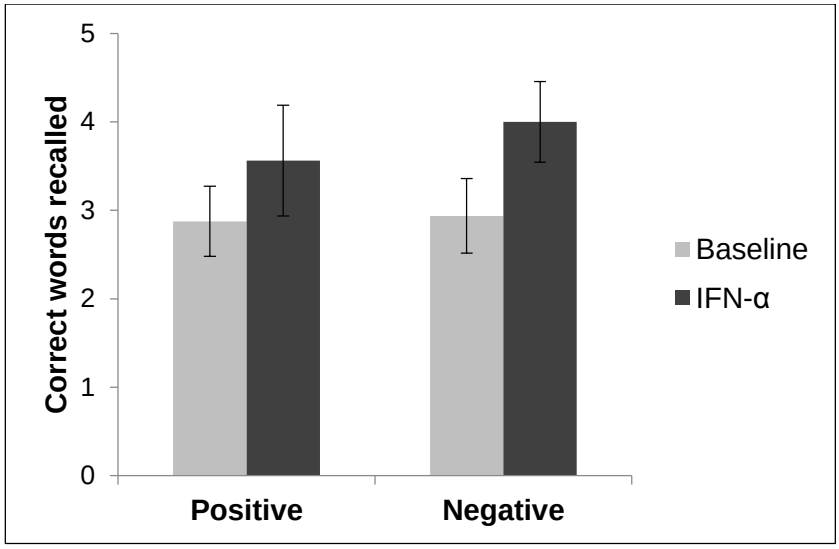


Figure 6. Recall of positive and negative personality characteristics before (lights bars) and after 6-8 weeks IFN-α treatment (dark bars). Values represent the mean number of correct trait words recalled, with error bars representing SEM.

There was no main effect of treatment ($F(1,15)=0.34$, $p=0.57$) or emotion x treatment interaction ($F(1,15)=1.06$, $p=0.32$) for false alarms in this task, though there was a significant main effect of emotion ($F(1,15)=28.88$, $p<0.001$).

There was no main effect of IFN-α treatment on accuracy in the emotional recognition memory task ($F(1,14)=2.31$, $p=0.15$), and no emotion x treatment interaction ($F(1,14)=0.047$, $p=0.83$). There was, however, a main effect of emotion ($F(1,14)=16.92$, $p=0.001$). Similarly, there was a main effect of emotion on reaction times ($F(1,14)=6.71$, $p=0.021$), but no main effect of IFN-α on reaction time performance ($F(1,14)=0.27$, $p=0.61$) or emotion x treatment interaction ($F(1,14)=0.59$, $p=0.45$). There were no effects of IFN-α on false alarms or correct rejections (p values >0.4) in this task.

No correlations were found between depressed mood score and emotional recall or recognition (all p values >0.1).

2.3.5 Dot probe attentional vigilance

There was a significant interaction between emotion, mask and treatment ($F(1,16)=8.32$, $p=0.01$), where mask was included as an additional within-subject factor (see Figure 7). When masked and unmasked trials were considered separately, there was a significant emotion x treatment interaction in the unmasked ($F(1,16)=5.46$, $p=0.03$), but not masked ($F(1,16)=1.08$, $p=0.31$), condition. *Post-hoc* analysis revealed that this was driven by a significant reduction in vigilance scores for happy faces in the unmasked condition following IFN-α treatment ($t(16)=2.10$, $p=0.05$). Figure 7 also shows that attentional vigilance towards fearful faces appears to be enhanced in the unmasked condition, however this was not statistically significant ($t(16)=-0.87$, $p=0.4$).

There were no general effects of IFN-α on reaction times in this task (all p values >0.1).

There were no correlations between depression scores and vigilance to happy or fearful faces in the dot probe task (all p values >0.1).

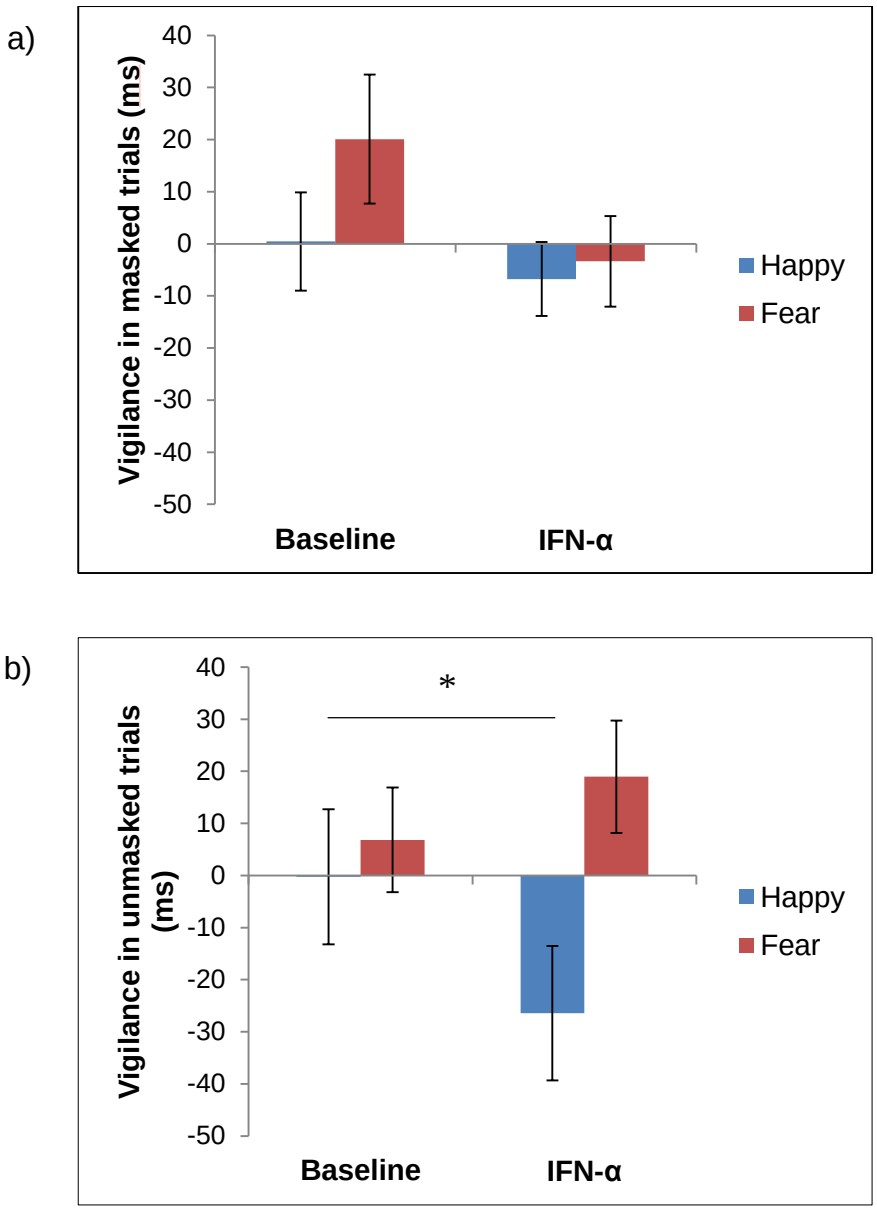


Figure 7. Effects of IFN-α on attentional vigilance for happy and fearful facial expressions in the a) masked condition and b) unmasked condition of the attentional dot probe task. Values are attentional scores before (baseline) and after IFN-α treatment. Attentional vigilance scores were calculated by subtracting the median reaction time from congruent trials (when the probe appeared in the same position as the emotional face) from incongruent trials (when the probe appeared in the opposite position to the emotional face, i.e. in the position of the neutral face). A positive score indicates vigilance towards the emotional face, whereas a negative score reflects avoidance of the emotional face. Error bars show the SEM. *p=0.05.

2.4 Discussion

IFN-α significantly increased depression severity ratings, as well as subjective ratings of state anxiety, fatigue and anhedonia by 6-8 weeks, which is in keeping with numerous previous reports of induction of depressive symptoms by this treatment (Amodio *et al.*, 2005, Capuron *et al.*, 2009, Capuron *et al.*, 2002a, Capuron and Miller, 2004, Fontana *et al.*, 2008, Maddock *et al.*, 2005). Pre-treatment depressed mood scores were positively correlated with depressed mood following IFN-α, which also supports previous reports suggesting prediction of severity of depressive symptoms during IFN-α treatment may be possible based on baseline depression scores (Capuron and Ravaud, 1999, Dieperink *et al.*, 2003, Musselman *et al.*, 2001). Indeed, it is thought to be the most reliable predictor of depressive symptoms and the development of major depression with IFN-α therapy (Raison *et al.*, 2005). Depressed mood scores measured by the BDI were not, however, significantly correlated with emotional processing task performance in the current study, for example depressed mood was not associated with disgust recognition. This suggests that behavioural results presented here are not simply determined by changes in depressed mood; further work discussed in the limitations section below could help to investigate this.

In the facial expression recognition task, IFN-α had a highly specific effect of enhancing accurate recognition of disgusted faces. Indeed, when disgust recognition was analysed over varying morphed intensities of faces presented, there was a significant elevation of recognition compared to pre-treatment, which was also reflected in significant lowering of the threshold at which disgusted faces were accurately detected following IFN-α administration. Reaction times in this task were faster for recognition of negative faces more generally, compared to positive faces. Furthermore, IFN-α increased accuracy for negative, but not positive, self-referent categorisation of emotional trait words. There was also a trend towards more accurate negative emotional memory as assessed by the emotional recall task,

however no effects were observed in the emotional recognition task. IFN-α significantly affected attentional vigilance in the unmasked condition of the dot probe task by increasing avoidance of positive stimuli and enhancing vigilance towards negative stimuli, though this latter effect was not statistically significant. Taken together, neuropsychological tasks employed within this study suggest that IFN-α induces negative biases in emotional processing.

2.4.1 Facial expression recognition

The present study showed highly specific effects of IFN-α treatment on sensitivity to disgusted faces, including enhanced ability to discriminate disgusted faces, paired with reduced threshold of disgust detection. There were no effects of treatment on processing of other key emotions, and elevated disgust accuracy was not accompanied by enhancement of mislabelling other facial expressions as disgust. Several previous studies have reported increased disgust recognition that might indicate a negative perceptual bias that is relevant to mood disorders, for example elevated discrimination of disgusted facial expressions has been observed in euthymic medicated patients with bipolar disorder (Harmer *et al.*, 2002b). Hayward *et al.* (2005) also observed enhanced recognition of disgusted faces in a recovered depressed sample following tryptophan depletion. Similarly, tryptophan depletion in remitted depressed individuals speeded the recognition of disgust faces (Merens *et al.*, 2008). In contrast, tryptophan supplementation, as opposed to depletion, reduced recognition of disgusted faces in healthy female volunteers (Murphy *et al.*, 2006).

Previous studies have investigated neural responses underlying perception of disgusted facial expressions and shown frequent activation of the insula cortex (Anderson *et al.*, 2003, Anderson *et al.*, 2007, Elliott *et al.*, 2011, Phillips *et al.*, 2004, Phillips *et al.*, 1998, Posamentier and Abdi, 2003), which is distinct to activation of the amygdala to fearful faces

(Anderson *et al.*, 2003, Phillips *et al.*, 2004, Sprengelmeyer *et al.*, 1998). The anterior insula does, however, have close connections to the amygdala, and also forms a network along with the ventromedial prefrontal cortex, hypothalamus and periaqueductal grey that is thought to be involved in identification of and response to potentially threatening or aversive stimuli (Anderson *et al.*, 2007). Surguladze *et al.* (2010) demonstrated that patients with depression showed greater activation in the left insula, left orbito-frontal gyrus, and left and right middle/inferior temporal gyrus to expressions of disgust. Furthermore, Anderson *et al.* (2007) found that disgusted faces resulted in the most widespread activation of three negative emotions (disgust, anger, fear); in particular, increased activity in the posterior insula, amygdala and putamen were found in response to disgusted faces. Interestingly, serotonergic modulation of facial emotion processing using citalopram activated the left posterior insula and dampened left amygdala activity (Anderson *et al.*, 2007). Additionally, lesion to the insula and putamen has been documented to reduce ability to recognise social signals of disgust (including Ekman faces) (Calder *et al.*, 2000), and stimulation of the left insula has been shown to interfere with disgust processing (Papagno *et al.*, 2016). Disgusted facial expressions have also been associated with activation of the dorsolateral prefrontal cortex, posterior cingulate cortex, thalamus, inferior frontal cortex and temporal cortex (Anderson *et al.*, 2007, Phillips *et al.*, 2004, Posamentier and Abdi, 2003).

Despite considerable evidence of neural substrates involved in disgust processing and growing appreciation of disgust as an important feature in depression (Surguladze *et al.*, 2010), interpretation of the role of disgust processing in depressive disorders lacks clarity. Emotional processing biases in disgust processing have been suggested to serve an evolutionary purpose of behavioural modification to avoid threats of infectious disease (Curtis *et al.*, 2004), which could be related to evidence that suggests environmental and social disgusted stimuli are attended to and appraised for more time than fearful faces (Zhang

et al., 2014). Enhanced disgust recognition may also be associated with visceral symptoms such as nausea (Anderson *et al.,* 2007). For example, increased recognition of disgusted faces was seen following duloxetine treatment, which might have been due to poor tolerability of a high dose, including nausea (Harmer *et al.*, 2008). Insula activity is purported to be involved in the emotional response to interoceptive sensory stimuli, body sensations or potentially distressing stimuli (Husted *et al.*, 2006, Papagno *et al.*, 2016), which could tie together the hypotheses stated above. There are suggestions, however, that disgust processing can extend into the social domain, and that heightened disgust sensitivity might reflect an emotional processing bias that constitutes social and self-related disgust, feelings of social rejection (Rozin *et al.*, 1994), as well as feelings of shame and guilt (Giner-Sorolla and Espinosa, 2011, Surguladze *et al.*, 2010), all of which are highly relevant to depression.

It should be noted that although discussion here has focussed on enhanced disgust sensitivity, some studies report no difference in disgust processing in depression (e.g. Bediou *et al.*, 2005), while others have reported impaired disgust recognition (e.g. Douglas and Porter, 2010). When consideration is given to the heterogeneity of symptomatology in depression, in addition to differing compensatory adaptations (as described by Mayberg, 2009), perhaps this variability in disgust processing is not surprising.

Such divergent evidence makes it difficult to reliably conclude the meaning of enhanced disgust recognition following immune system activation in the current study. It seems conceivable, however, that effects on disgust recognition observed could represent a complex interplay between inflammatory pathways and neurocircuits that result in modified information processing of aversive disgusted facial emotions, which may in part be modulated by serotonin function (Anderson *et al.*, 2007). Furthermore, it seems plausible that hypotheses regarding evolutionary purposes of disgust recognition related to the insula

cortex (Curtis *et al.*, 2004) and evolutionary theories of behavioural modification by inflammatory mechanisms are related, yet in modern times such interactions may play a role in the development of depression (Miller and Raison, 2015) or at least some core symptoms of depression (Sprengelmeyer *et al.*, 2011).

On another note, reaction times to respond to negative faces (particularly fearful and sad faces) were faster than to positive faces following IFN-α. It appeared at the second study visit that reaction times overall were reduced, which might have been due to a practice effect. Reaction times have generally been thought to increase with IFN-α treatment due to psychomotor slowing (Capuron *et al.*, 2005, Fontana *et al.*, 2008, Majer *et al.*, 2008); in this light a reduction in reaction times for negative valences is curious and may support a more general negative bias in emotional facial expression processing. No further alterations in valence specific reaction times were observed in the present study, which could be in keeping with observations by Capuron *et al.* (2002a), who found that psychomotor slowing did not occur until week 12 (and not earlier at week 8).

2.4.2 Emotional categorisation

Increased accurate categorisation of negative self-referent personality words observed following IFN-α therapy is suggestive of a negative bias in emotional processing. Negative biases in emotional categorisation have previously been reported in depressed patients, reversal of which have been associated with later antidepressant response. For example, slower reaction times to positive trait words were found in depressed patients compared to healthy controls, however after a single dose of reboxetine reaction times to positive adjectives were faster, and the magnitude of this effect corresponded to antidepressant response six weeks later (Harmer *et al.*, 2009b). Further studies have reported similar antidepressant effects on emotional categorisation with reboxetine (Harmer *et al.*, 2003a,

Harmer *et al.*, 2004), citalopram (Harmer *et al.*, 2004), and mirtazapine (Arnone *et al.*, 2009).

Interestingly, the insula has also previously been reported to be involved in processing of self-description words (Kircher *et al.*, 2000), and studies have shown the importance of the ACC, prefrontal cortex, precuneus and inferior parietal cortex in performance in both this task (Fossati *et al.*, 2003, Miskowiak *et al.*, 2007, Norbury *et al.*, 2008), and more widely in the pathogenesis of depression (Komulainen *et al.*, 2016). Regardless of the precise neural substrates, this finding with IFN-α is in line with negative biases in emotional categorisation that are believed to occur in depression.

2.4.3 Emotional memory

IFN-α administration produced a trend towards increased recall of negative self-referential characteristics, whereas positive recall remained unaffected, and there were no effects on emotional recognition. Once more, the left insula has been implicated in emotional memory in depressed patients (Ai *et al.*, 2015) and negative biases have been found in depression by several studies, whereby a higher proportion of negative words or a lower proportion of positive words are recalled (Gilboa-Schechtman *et al.*, 2002, Leppanen, 2006). Such studies are in line with hypotheses of the key involvement of negative emotional memory biases in maintaining depression (Bower, 1981, Disner *et al.*, 2011). Emotional memory appears to be sensitive to monoaminergic modulation; antidepressant treatments have been shown to remediate negative memory bias in depressed patients (Harmer *et al.*, 2009b) and improve positive affective recall in healthy volunteer studies, including with citalopram (Browning *et al.*, 2011, Harmer *et al.*, 2004), agomelatine (Harmer *et al.*, 2011b), reboxetine (Harmer *et al.*, 2003a), duloxetine (Harmer *et al.*, 2008) and mirtazapine (Arnone *et al.*, 2009). In contrast, tryptophan depletion has been found to decrease memory for positive words in

participants displaying a depressive response (Booij *et al.*, 2005) and in healthy volunteers tryptophan depletion reduced recall of positive and neutral words (Klaassen *et al.*, 2002). The atypical antidepressant tianeptine, which is purported to have serotonergic re-uptake enhancing properties, was also shown to reduce positive recall (this study is detailed in Chapter 6) (Cooper *et al.*, 2015). Rimonabant, a cannabinoid CB1 receptor antagonist, similarly reduced positive recall in healthy volunteers, which was thought to be indicative of depressogenic effects of this drug (Horder *et al.*, 2009).

In summary, negative biases in emotional memory are thought to be central to depression (Leppanen, 2006), however no previous studies, to our knowledge, have investigated effects of IFN-α or inflammation on emotional memory. Results observed in this study perhaps indicate a negative bias in emotional recall, that could play a role in the depressive phenotype induced by IFN-α, however this effect was not strong, it did not emerge as an emotion x treatment interaction, and no effects were observed in the emotional recognition task.

2.4.4 Attentional dot probe

The current findings in the attentional dot probe task suggest that administration of IFN-α produces negative biases in attentional vigilance, particularly by increasing attentional avoidance of happy facial expressions in the unmasked condition. IFN-α also appeared to enhance attention towards negative, fearful faces, although this was not statistically significant. Negative biases in emotional processing include both vigilance towards negative stimuli and away from positive stimuli (Disner *et al.*, 2011). Depressed patients have previously been shown to display an attentional bias away from positive (happy) faces and towards negative (sad) faces (Fritzsche *et al.*, 2010, Gotlib *et al.*, 2004) and negative words (Bradley *et al.*, 1997).

It has been previously proposed that in the neutral-happy face pair presented in this task, the neutral face signifies the more threatening facial stimuli when compared to the happy face. Therefore, avoidance of the happy face in the current study indicates attention towards the neutral face, which may be relevant to threat-related processing biases (Cooper and Langton, 2006). This would also be in keeping with the (non-significant) observation of enhanced vigilance to threatening fearful faces with IFN-α treatment.

Tianeptine, an antidepressant drug with suggested properties of reducing serotonin function early in treatment (discussed further in Chapter 6) was also shown to significantly enhance avoidance to happy faces (Cooper *et al.*, 2015) in a very similar manner to effects seen here with IFN-α. Tryptophan supplementation, in contrast, has been shown to reduce vigilance to negative words in healthy female volunteers (Murphy *et al.*, 2006). It is too soon to conclude the mechanism through which IFN-α may be exerting this effect, though it is intriguing to note that once again supporting literature suggests that this might be associated with reduced serotonin availability.

2.4.5 Limitations and further work

There are some important limitations in the present study that are worth noting. Firstly, a sample size of 17 participants is relatively small, particularly given known variances in mood and behavioural response to IFN-α treatment as not all patients become depressed. The sample size is also limiting since it does not allow reliable stratification of those patients who did exhibit depressive symptoms, compared to those who did not. This would be helpful to investigate whether baseline neuropsychological task performance is able to predict mood response to treatment, and thereby identify those at elevated risk of developing IFN-α-associated depression. Further limitations of this study include the lack of control subjects with HCV receiving placebo and not IFN-α treatment, which would have helped to

examine cause and effect in relation to depression symptoms, as well as learning effects on task performance, although the test-retest reliability of the emotional test battery has been investigated in healthy subjects and shown no significant effects on specific emotional stimuli (Adams *et al.*, 2015). Accurate accounts of somatic symptoms on testing days could have also been useful with regards to accounting for visceral symptoms such as nausea (Anderson *et al.*, 2007), which can unfortunately be a side effect of IFN-α treatment and, as discussed above, may be involved in altered disgust recognition.

The present study does not have any biological markers, including inflammatory cytokines or tryptophan and its metabolites, which could have provided insight into the biological mechanisms associated with behavioural effects observed. Furthermore, mediation analysis factoring in inflammatory cytokine measures could have facilitated assessment of the mediator of psychological effects observed and potentially informed the lack of correlation between mood scores and behavioural findings. It would have additionally been interesting to test baseline inflammatory markers to assess correlation with later mood symptoms (Loftis *et al.*, 2013, Wichers *et al.*, 2006) and to be able to discuss the potential roles of specific cytokines. For instance, elevated IL-6 has been strongly associated with IFN-α and might play a more important role in IFN-induced depression (Hoyo-Becerra *et al.*, 2014). Although a limitation in the present study, the following chapter attempts to investigate the effects of elevated IL-6 on emotional processing in healthy volunteers. The current study also did not include a reward task, which would have been interesting given the literature surrounding inflammation/IFN-α and reward circuitry (Capuron *et al.*, 2012, Felger *et al.*, 2015b), and again this is addressed in the next chapter.

2.5 Conclusions

The present study provides intriguing evidence that IFN-α, either directly or perhaps indirectly via pro-inflammatory cytokine pathways, is capable of producing negative emotional processing biases that are widely thought to be important in the onset and maintenance of depression. As such, these results highlight that exposure to inflammation by immune system activation may alter affective information processing, and that this could play a role in the development of depression over time in subgroups of patients.

Whilst there are many similarities between the present findings and the depression literature, there are some interesting differences; particularly the highly specific enhancement of disgust recognition. Although this has previously been seen in mood disorders, it could represent an effect that is more specific to inflammatory pathways. Further investigation of the effects of pro-inflammatory cytokines is undoubtedly required to clarify the current hypotheses. The following chapter will utilise an inflammatory model in healthy volunteers to examine whether effects reported here can be replicated.

Chapter 3

Investigating the Effects of Typhoid Vaccine on Emotional Processing and Cognition

3.1 Introduction

The previous chapter presented intriguing evidence that activation of the innate immune system with IFN-α treatment induced negative affective biases in emotional processing, which may represent a mechanism through which inflammation could contribute to the pathogenesis of depression. In order to examine the relationship between inflammation and depression in more detail, and without potential confounds of physical illness symptoms, experimental models of inflammation have been developed that involve administration of cytokine-inducers, such as *salmonella typhi* (typhoid) vaccination and endotoxin (lipopolysaccharide) to healthy subjects. Such models are thought to elicit a depression-like syndrome in healthy participants, including a range of behavioural changes such as negative mood, cognitive dysfunction, fatigue, anhedonia and sleep disruption (Brydon *et al.*, 2009, Eisenberger *et al.*, 2010a, Harrison *et al.*, 2009a, Mullington *et al.*, 2000, Strike *et al.*, 2004, Wright *et al.*, 2005).

Previous studies investigating the neuroanatomical basis of the inflammatory hypothesis of depression have utilised these models of experimental immune challenge and demonstrated that pro-inflammatory cytokines can modulate neural circuits involved in emotional processing. For example, typhoid vaccination has been shown to increase activity in the ACC (Harrison *et al.*, 2009a) and insula (Harrison *et al.*, 2009b), the latter of which is discussed in the previous chapter for its potential role in enhanced disgust recognition observed following IFN-α, in response to emotional cues. Furthermore, evidence suggests endotoxin administration enhances activity in the orbitofrontal cortex (Kullmann *et al.*,

2013) and amygdala (Inagaki *et al.*, 2012) during visual presentation of emotional stimuli. Taken together, such evidence suggests that acute inflammatory stimuli are capable of modulating neural circuitry implicated in emotional processing, however behavioural effects of inflammation on affective information processing remain largely unknown.

There is also evidence to suggest that inflammation may have deleterious effects on non-emotional cognition, such as memory function, which has been shown to be impaired following endotoxin administration in healthy participants (Reichenberg *et al.*, 2001). In addition, inflammation has been implicated in reward learning, since endotoxin (Eisenberger *et al.*, 2010a) and IFN-α treatment (Capuron *et al.*, 2012) have been shown to reduce activity in the ventral striatum in response to reward outcomes, and a recent study found that typhoid vaccination enhanced sensitivity to punishments compared to rewards in healthy subjects (Harrison *et al.*, 2015c). Despite this intriguing evidence, data are still limited and the behavioural effects of inflammation on cognitive function remain under investigated.

The present study utilised typhoid vaccination as a model of acute inflammatory challenge, since it has previously been shown to stimulate a mild, non-sickness inducing inflammatory response that significantly increases levels of the pro-inflammatory cytokine, interleukin (IL)-6, in a safe manner without increasing symptoms of illness, body temperature and blood pressure (Brydon *et al.*, 2008, Harrison *et al.*, 2009b). Peripheral IL-6 is purported to increase two- or three-fold, peaking between 2-3 hours post-vaccine (Brydon *et al.*, 2008, Harrison *et al.*, 2015b). This should allow investigation of acute behavioural effects without confounds of sickness symptoms or increased body temperature that have been observed following endotoxin administration (Eisenberger *et al.*, 2010b, Reichenberg *et al.*, 2001). Typhoid vaccine has also previously been shown to affect subjective ratings of mood and fatigue (Harrison *et al.*, 2009a, Strike *et al.*, 2004, Wright *et al.*, 2005).

3.1.1 Aims

Considering the established relevance of perturbed emotional processing in depression, together with the growing appreciation that pro-inflammatory cytokines can modulate motivational and emotional behaviour, we investigated neuropsychological function in the typhoid vaccine experimental inflammatory model. The present exploratory study therefore aimed to examine the effects of typhoid vaccination on emotional processing and cognitive function using a well-validated battery of psychological tasks, as well as detailed mood assessment, inflammatory marker analysis and salivary cortisol measurements. We hypothesised that typhoid vaccination would produce similar negative biases in emotional processing to those observed following IFN-α therapy (Chapter 2), including enhanced recognition of disgusted emotional faces and negative biases in emotional categorisation and attentional vigilance. Given the literature on inflammation and cognition (discussed in more depth in Chapter 5), we hypothesised that typhoid vaccination would reduce cognitive function.

3.2 Methods

3.2.1 Participants and study design

This was a double-blind, placebo-controlled parallel group study with 1:1 randomisation. 42 healthy volunteers (21 male, 21 female; mean age 21.2, SD 2.2, range 18-27 years; mean BMI 21.9 kg/m^2, SD 2.3, range 19-28) were recruited through local and online advertising. 16/21 participants randomized to the vaccine group had never received the typhoid vaccine; hence this should represent a primary immune response. Participants were screened using the Structured Clinical Interview for DSM IV (Spitzer *et al.*, 1995) to ensure no current or past history of any Axis I psychiatric disorder. Participants were willing and able to give informed consent, were physically in good health, as assessed by a medical doctor, and not

currently taking any medications (except the contraceptive pill). Inclusion criteria also included fluency in English (due to the nature of the tasks), age between 18-55 and body mass index (BMI) within 19-30 kg/m^2. Exclusion criteria included: typhoid vaccination within the last three years, any vaccination within the last six months, current or recent physical illness or infection within two weeks of the research visit, steroidal or non-steroidal anti-inflammatory medication within two weeks of the research visit, any previous history of allergies to drugs or vaccines, or to any component of the typhoid vaccine specifically, in addition to a congenital or acquired immune deficiency, bleeding disorder, smoking, current substance misuse or psychotropic medication, and pregnancy or breastfeeding. Participants were also unable to take part if they had been involved in a psychological or medical study where they had been given a drug within the last three months, or if they had ever taken part in a study that involved the same battery of emotional processing tasks used in the present study.

The study was approved by the University of Oxford Medical Sciences Inter Divisional Research Ethics Committee, sponsored by the University of Oxford and overseen by Oxford University Clinical Trials and Research Governance. All participants signed and dated the Informed Consent Form prior to any study procedures.

Participants undertook the National Adult Reading Test (NART) (Nelson, 1982), a measure of verbal IQ, and Eysenck Personality Questionnaire (EPQ) (Eysenck and Eysenck, 1975) at screening to characterise the sample.

3.2.2 Experimental model of inflammation

Participants attended the research visit at approximately 12 – 12.30 pm, to control for diurnal variance. Participants were asked not to consume any alcohol, caffeinated drinks, or high-fat content meals and avoid intensive exercise for 12 hours prior to this visit. Upon arrival

for the research visit, a brief interview ensured that there had been no changes in participants' health or medication since the screening visit.

The randomization schedule was drawn up by a member of the group not otherwise involved in the study to maintain allocation concealment, and this randomisation code was not broken until study analyses were complete. According to the randomization schedule, participants received either a single 0.5mL injection of typhoid polysaccharide vaccine (25 micrograms Typhim Vi®, Sanofi Pasteur MSD Ltd) or placebo (0.9% sodium chloride saline solution) intramuscularly into the non-dominant deltoid muscle in the arm. This was administered by a medical doctor or nurse. To maintain participant blinding, labels were placed over the syringes just prior to administration, and participants were asked to look away while the injection was given.

Typhoid vaccine is usually recommended for travel to parts of the world where typhoid fever is present. Each 0.5mL dose of typhoid vaccine contains 25 micrograms of the active substance, purified Vi capsular polysaccharide of Salmonella typhi (Ty2 strain). The other ingredients are phenol, sodium chloride, disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate and water for injection. It is indicated for active immunisation against typhoid fever caused by *Salmonella enterica serovar typhi*. The vaccine is generally well-tolerated, but some individuals can experience temporary soreness, swelling, redness or hardness at the site of the injection. Less common side effects can include high temperature, headache, nausea, diarrhoea, generally feeling unwell, abdominal pain and asthma. Severe reactions are rare, but necessary precautions were taken in the present study to reduce the risk of severe allergic reaction as much as possible. All participants were closely monitored throughout the study.

The placebo injection consisted of 0.5mL of sterile sodium chloride 0.9% w/v, which is suitable for injection; this was drawn up immediately prior to administration.

3.2.3 Interleukin-6 (IL-6) immunoassay

The pro-inflammatory cytokine IL-6 was analysed since it is one of the most commonly investigated immune markers in major depression (Mossner *et al.*, 2007) and has repeatedly been shown to be elevated in depressed patients (Dowlati *et al.*, 2010, Howren *et al.*, 2009, Liu *et al.*, 2012a, Valkanova *et al.*, 2013). IL-6 has also previously been shown to be elevated three hours after typhoid vaccine (Brydon *et al.*, 2008, Harrison *et al.*, 2009a).

5mL blood samples were collected into ethylenediaminetetraacetic acid anticoagulant (EDTA) tubes via separate venepunctures at baseline, prior to the injection, and 4 hours post-dose. Samples were centrifuged at 1250 x g for 10 minutes immediately after collection. Plasma was then extracted, aliquoted and stored in an anonymised form at -30^{o}C until analysis. On completion of the study, all samples were analysed using Human IL-6 Quantikine® HS (high sensitivity) ELISA kits (R&D Systems, Abingdon, UK) on the same day. The intra-assay and inter-assay coefficients of variation are 7.4% and 7.8%, respectively, and the limit of detection of the assay is 0.039 pg/mL.

3.2.4 Physiological measurements and side effects

Blood pressure (BP), heart rate (HR) and body temperature were measured at baseline, then at 1, 2 and 4 hours post-dose.

Participants completed a side effects questionnaire that assessed the presence of: soreness around the injection site, muscle pain, tiredness, fever, headache, dizziness, nausea, loss of alertness and feeling unwell. In addition, participants were asked to report any symptoms experienced that were not captured in the above and finally to guess whether they had

received the typhoid vaccine or placebo injection at the end of the research visit, including an indication of how certain they felt using a visual analogue scale.

3.2.5 Salivary cortisol

Participants collected saliva samples upon waking on two separate days; once at baseline on the morning of the research visit and once the morning after testing. This was included to assess salivary cortisol awakening response (Pruessner *et al.*, 1997) as an indication of hypothalamic-pituitary-adrenal (HPA) axis activity, since HPA abnormalities are widely observed in depressed patients (Mossner *et al.*, 2007, Pariante, 2003, Zunszain *et al.*, 2011). Samples were collected into pre-labelled salivette tubes (Sarstedt, Leicester, United Kingdom), following standardised instructions given to each participant; three samples were taken immediately upon waking, 15 minutes after waking and 30 minutes after waking in their own home. Samples were stored at 4^{o}C until analysis within 7 days of sample collection. Cortisol was analysed using a Salimetrics Salivary Cortisol ELISA kit (Salimetrics Europe Ltd., Newmarket, UK). The limit of detection of this assay was 0.19nmol/L, the overall inter-assay coefficient of variation was 7.8%, and overall intra-assay coefficient of variation was 6.3%.

One participant did not provide baseline cortisol samples and, hence, was not included in baseline or change from baseline analyses.

3.2.6 Subjective mood questionnaires

Participants completed the following questionnaires at baseline, prior to receiving the typhoid vaccine or placebo injection: Beck Depression Inventory (BDI) (Beck *et al.*, 1961); Positive and Negative Affective Schedules (PANAS) (Watson *et al.*, 1988); State Trait Anxiety Inventory (STAI) (Spielberger *et al.*, 1970); Befindlichkeits Scale (BFS) (Von Zerssen *et al.*, 1974) and visual analogue scales (VAS) (Bond and Lader, 1974), which

consisted of sixteen 100 mm scales anchored by antonyms, for example Alert-Drowsy, Lethargic-Energetic. VAS scales were combined to form three mood factors, as advised by the authors: alertness, contentedness and calmness, with higher scores indicating higher subjective feelings of stress (Bond and Lader, 1974). PANAS and VAS scales were additionally completed each hour up to 4 hours post-dose, when participants repeated all mood questionnaires once more.

3.2.7 Emotional test battery

The tasks constituting the emotional test battery (facial expression recognition, emotional categorisation, emotional memory and dot probe) are described in full in the previous chapter. The same versions of these tasks were used in this study to allow direct comparison of effects.

3.2.8 Cognitive tasks

3.2.8.1 *N-back task*

Working memory was studied using a latter variant of the *n*-back task (Harvey *et al.*, 2005) that used three levels of complexity: 1-, 2- and 3- back, and a control task (0-back). Subjects were asked to respond to a series of stimuli (letters), indicating whether they were the same as or different to a cue by pressing a corresponding button. Stimuli consisted of the following phonologically closed characters: b, B, d, D, g, G, p, P, t, T, v and V. Participants were advised to ignore the case of the letter. In the 0-back, subjects were asked to look out for an additional target letter 'X', pressing 'same' when this stimulus appeared and 'different' for all other stimuli. In the remaining blocks there was no specific target letter; they were asked to press 'same' when the presented stimulus was the same letter as that presented 1, 2 or 3 letters previously for the 1-, 2-, and 3- back blocks, respectively. Ten stimuli were presented in a block for 500 ms each with a fixed interstimulus interval of 1500

ms. An instruction screen (0-, 1-, 2-, 3- back) was presented for 2000 ms prior to each task, followed by a 4000 ms blank screen before the onset of the first letter. Task blocks were separated by 1000 ms of fixation cross. Four blocks of each condition matched for number of target stimuli and upper/lower case letters were presented in a fixed pseudorandom order (0-, 1-, 2-, 3-, 1-, 3-, 2-, 0-, 2-, 1-, 0-, 3-, 1-, 0-, 3- and 2-back). Accuracy and latency of response were recorded.

3.2.8.2 *Rapid visual information processing task*

The Rapid Visual Information Processing (RVIP) is a serial discrimination task in which performance is thought to assess sustained attention (Neale *et al.*, 2015), whilst also providing an indication of psychomotor speed. Working memory is also required for successful execution of the task (Camfield *et al.*, 2013, Coull *et al.*, 1996). This task was programmed using ePrime (version 2.0, Psychological Software Tools, Inc.), based on the RVIP task that is included in the Cambridge Neuropsychological Test Automated Battery (CANTAB; Sahakian and Owen, 1992). Single digits ranging from 2 – 9 appear consecutively in the centre of the screen for 600 ms, without an inter-stimulus interval. Participants are asked to respond by pressing the space bar as soon as they see one of three target sequences of numbers (2-4-6, 4-6-8 or 3-5-7). Target sequences are displayed in the corner of the screen throughout the task. Only responses within 1800 ms after the onset of the last digit in the target sequence are counted as correct hits. Digits are presented at a rate of 100 digits per minute, and there are eight target sequences to detect per minute. The task lasts 12 minutes in total. Accuracy (target detection hits), response latency for correct hits and false alarms were computed in this task.

3.2.8.3 *Reward task*

This task was based upon the instrumental learning task devised by Pessiglione *et al.* (2006). Pairs of abstract symbols were presented side by side on screen. Each pair was associated with either potential monetary gain or loss. Subjects were instructed to try to maximise their financial gains, and for each trial choose the symbol (by pressing a key indicating left or right) that they believed was most likely to win money or least likely to lose money. Within the gain pairs, the probabilities of winning £0.50/nil were 0.7/0.3 for one stimulus and 0.3/0.7 for the other. Similarly, within the loss pairs the probabilities of losing £0.50/nil were 0.7/0.3 for one stimulus and 0.3/0.7 for the other. After each trial the outcome was presented on screen. Subjects therefore learnt by trial and error the associated outcomes for the stimuli, modifying their behaviour accordingly. The gain pairs modelled subjects' ability to learn from rewarding choices, allowing comparison with their learning behaviour from aversive stimuli (loss pairs). Subjects first completed an example run of 10 trials, followed by two runs of 60 trials with two different sets of abstract stimuli. Participants were able to keep the total of their gains from the two tasks, starting with £5. Data were collected on the outcome of the choice and outcome of each trial allowing analysis of both gain and loss conditions.

3.2.8.4 *Auditory verbal learning task*

The Rey Auditory Verbal Learning Test (AVLT) (Rey, 1964) was used to assess declarative verbal memory. Participants were read a list of 15 words at an even pace of 1 second per word (List A) before being asked to recall as many words as possible in any order (immediate free recall). This was repeated on four further consecutive occasions with the same list of words giving a total of five presentations. A second list of words (List B) was then read and tested on just one occasion. Participants were then asked to recall words from

List A again (short delay free recall). Intervening tasks (the reward task and *n*-back task) were then undertaken, before participants were asked to recall words from List A (long delay free recall). Correct responses, intrusions and repetitions were also recorded. Finally, a recognition task was undertaken with a list of words read out containing all 15 items from List A, as well as 30 filler words consisting of words from List B and other distractor words. Participants were asked to indicate whether each word was in List A or not. Correctly recognised words and false alarms were recorded. Signal detection theory was also applied to recognition test data to provide a measure of accuracy corrected for subjects' response tendency using: A'=1–1/4(fr/cr+(1–cr)/(1–fr)), where the proportion of correctly recognised words (cr) and falsely recognised words (fr) were factored in to this non-parametric sensitivity measure (as in Harmer *et al.*, 2002a).

3.2.9 Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics (version 22). Extreme outlying data (data lying at more than three times the participants' interquartile range above their third or below their first quartile) were removed from all psychological tasks. This resulted in minimal data (<2%) being lost as outliers on any task. Demographic and baseline characteristics of the two groups, in addition to IL-6, the emotional memory task and RVIP task, were analysed using one-way ANOVAs. Mood and energy scales completed before and after vaccine administration were compared between the two groups using repeated measures ANOVA, with treatment group (typhoid vaccine or placebo injection) as the between-subject factor and time as the within-subject factor. Salivary cortisol was measured using repeated measures ANOVA with time point of sampling (0, 15 and 30 min after waking) and day of sampling (baseline vs morning after treatment) as within-subject factors and treatment group (placebo, vaccine) as a between-subjects variable. Data from the facial expression recognition task, emotional categorisation task, emotional recognition task, dot

probe task and reward task were also analysed using repeated measures ANOVA, with treatment group as the between-subject factor and valence as the within-subject factor. In the dot probe task, masking was added as an additional within-subject factor. The *n*-back task was also analysed using repeated measures ANOVA, with treatment as the between-subject factor and complexity as the within-subject factor. For the AVLT task, repeated measures ANOVA was used to assess learning over trials, with trial as a within-subject factor and group as the between-subjects factor. Statistically significant interactions were followed up using simple main effects analyses. Where assumptions of equality of variances were broken, Greenhouse-Geisser procedure was used to correct the degrees of freedom. Data from each task were analysed separately, without correcting for multiple comparisons across tasks.

Correlational analysis was performed to assess the relationship between IL-6 response and behavioural measures in the vaccine treatment group. To do so, Pearson's correlation coefficient was computed between IL-6 raw values (in pg/mL) and neuropsychological task performance.

Power calculations were performed prior to ethics submission based on previous behavioural studies conducted within the lab using the same validated battery of tasks. This indicated that the number of participants recruited into this study had a statistical power of 0.9 to detect a 20% difference in emotional processing. As a specific example, in the facial expression recognition task, one of the main outcome variables would be accuracy in recognizing fearful facial expressions; a total sample size of 40 would give power of 0.95 to detect changes of the magnitude of those we have seen in a previous study (drug mean 17.29 (SD 6.68) vs placebo mean 23.29 (SD 4.25), from Harmer *et al.*, 2004). A second outcome variable of importance on this task would be the misclassification of expressions as happy; here a total sample size of 40 would give power of 0.86 to detect changes of the magnitude

of those we have seen in a previous study (drug mean 10.64 (SD 9.77) vs placebo mean 3.36 (SD 5.96), from Harmer *et al.*, 2004).

3.3 Results

3.3.1 Group matching

The vaccine and placebo groups were well matched in terms of demographics, including verbal IQ as measured by the NART and personality traits measured by the EPQ (see Table 1).

Table 1. Demographic, mood and personality measures at baseline. Means (standard deviations).

	Placebo (*n*=21)	**Vaccine (*n*=21)**	**Statistical significance**
Age	20.7 (2.2)	21.7 (2.2)	$p = 0.15$
Gender	11F:10M	11F:10M	N/A
BMI	21.7 (2.0)	22.2 (2.5)	$p = 0.42$
Verbal IQ	119.9 (4.1)	120.2 (3.3)	$p = 0.76$
Trait anxiety	32.1 (7.9)	31.0 (6.6)	$p = 0.63$
BDI	2.4 (2.8)	1.8 (2.2)	$p = 0.42$
EPQ: E	14.8 (4.8)	15.8 (3.7)	$p = 0.48$
EPQ: N	5.8 (4.5)	6.7 (4.3)	$p = 0.49$
EPQ: P	2.4 (2.3)	2.7 (2.1)	$p = 0.73$
EPQ: L	9.0 (4.1)	8.2 (3.5)	$p = 0.47$

BMI: body mass index; BDI: Beck Depression Inventory; EPQ: Eysenck Personality Questionnaire, where E: extroversion, N: neuroticism, P: psychoticism, L: lie

3.3.2 Interleukin-6 (IL-6)

There were no baseline differences in IL-6 levels between groups ($F(1,38)=0.04$, $p=0.84$), however at four hours after administration of the vaccination or placebo injection there was a significant increase in IL-6 in the typhoid vaccine group compared to the placebo group ($F(1,35)=24.27$, $p<0.001$; see Figure 1).

Samples were not obtained at baseline for two participants (one in each group), and for five participants (one in the vaccine group, four in the placebo group) at 4 hours post-dose due to unsuccessful venepuncture.

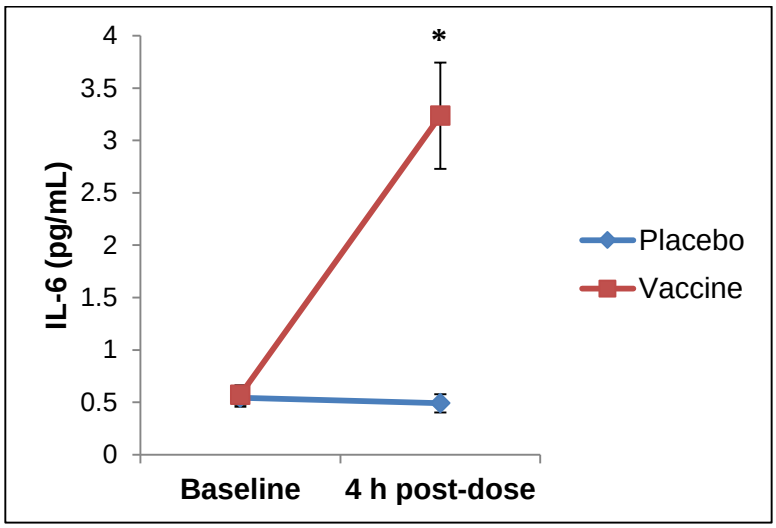


Figure 1. Interleukin-6 (IL-6) measured at baseline and 4 hours after placebo injection (blue line) and typhoid vaccine (red line). Raw values are presented, with error bars representing standard error of the mean (SEM). * p <0.001.

3.3.3 Physiological measurements and side effects

Typhoid vaccine had no effect on systolic or diastolic blood pressure, heart rate or body temperature (all p values >0.5). Soreness around the injection site ($F(1,40)=92.4$, $p<0.001$) and muscle pain ($F(1,40)=9.52$, $p=0.004$) were reported significantly more in the vaccine

group than the placebo group. There were no effects of typhoid vaccine on any other side effect (tiredness p=0.69, headache p=0.57, dizziness p=0.56, nausea p=0.32, alertness p=0.61, feeling unwell p=0.32; no symptoms of fever were reported). 14/21 (67%) participants in the vaccine group correctly guessed that they received the typhoid vaccination, with an average certainty of 28±5%.

3.3.4 Salivary cortisol

There was no interaction effect between day of sampling (pre- vs post-treatment), treatment group (placebo vs vaccine) and sampling time point (0, 15 and 30 mins) on salivary cortisol levels (F(1.39, 54.21)=1.66, p=0.21). Main effects analyses confirmed that cortisol levels were significantly increased post-waking in both groups (main effect of time: F(1.67, 65.07)=56.32, p<0.001), however there were no main effects of treatment group (F(1,39)=0.08, p=0.78) or day of sampling (F(1,39)=0.83, p=0.37).

There were no correlations between IL-6 response and cortisol awakening response in the typhoid vaccine group (p values >0.6).

3.3.5 Subjective state

The following subjective state measures were unaffected by typhoid vaccine administration: BDI ratings, state anxiety, BFS scores, PANAS and VAS of alertness, contentedness and calmness (p values >0.1, see Table 2). PANAS and VAS scores were measured at additional time points (baseline, 1, 2 and 4 hours post-dose); no effects of treatment group were observed over this time course (all p values >0.1).

There were no correlations found between IL-6 and subjective mood scores at 4 hours post-dose (all p values >0.1).

Table 2. Subjective state changes over time. Values are ratings at 4 hours after treatment minus baseline ratings. Means (standard deviations).

	Placebo (*n*=21)	**Vaccine (*n*=21)**	**Statistical significance**
BDI	-0.05 (0.30)	-0.67 (0.33)	$p = 0.17$
State anxiety	-0.48 (1.72)	-0.24 (1.49)	$p = 0.90$
BFS: Mood	-1.71 (1.00)	0.10 (1.96)	$p = 0.41$
BFS: Energy	0.90 (0.36)	2.65 (1.33)	$p = 0.21$
BFS: Total	-0.81 (1.18)	2.75 (2.92)	$p = 0.26$
PANAS: Positive	-3.71 (1.04)	-2.48 (1.14)	$p = 0.43$
PANAS: Negative	-1.33 (0.41)	-1.48 (0.64)	$p = 0.85$
VAS: Alertness	0.86 (1.00)	0.28 (1.04)	$p = 0.69$
VAS: Contentedness	1.86 (1.18)	0.65 (1.30)	$p = 0.50$
VAS: Calmness	-1.17 (2.76)	0.65 (2.59)	$p = 0.64$

BDI: Beck Depression Inventory; BFS: Befindlichkeits Scale; PANAS: Positive and Negative Affective Schedules; VAS: Visual Analogue Scales.

3.3.6 Emotional test battery

3.3.6.1 *Facial expression recognition*

There was no main effect of treatment group on accuracy in this task (see Figure 2: $F(1,39)=0.06$, $p=0.81$), and there was no interaction between intervention group and emotion ($F(5,195)=0.82$, $p=0.53$). Discriminability index d', which controls for differences in response criteria, failed to show any significant differences between the typhoid vaccine or placebo groups (main effect of group $F(1,40)=<0.001$, $p=1.0$, emotion x group $F(4.1,164.4)=0.7$, $p=0.6$). Similarly, there was no main effect of treatment ($F(1,38)=1.86$, $p=0.18$) or an emotion x treatment interaction ($F(3.96,150.31)=0.95$, $p=0.44$) using conservative beta analysis. However, post-hoc analysis using one-way ANOVA revealed a

significant reduction in disgust for the vaccine group compared to the placebo group ($F(1,39)=6.85$, $p=0.013$), which was not found with other emotions (p values >0.3). This indicates a less conservative response style specifically for disgusted faces in the vaccine group compared to the placebo group, which is characterised by more false alarms for disgusted faces.

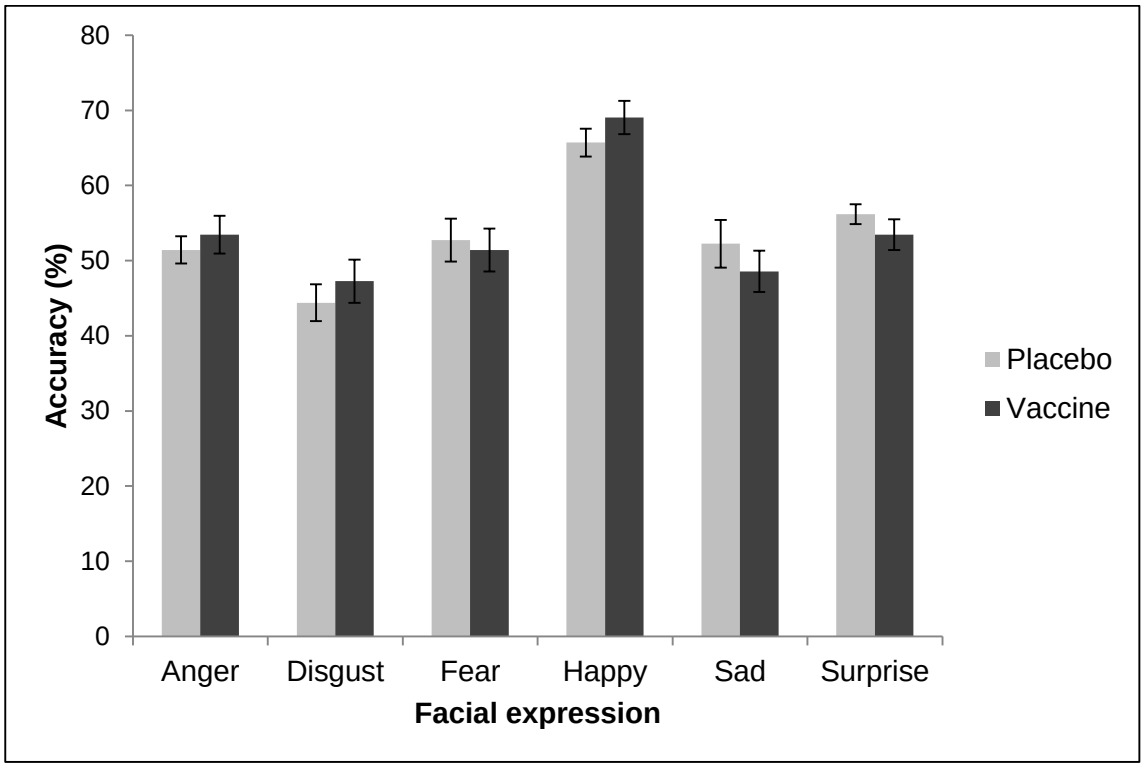


Figure 2. Performance in the facial expression recognition task following placebo (light bars) and typhoid vaccine (dark bars). Values represent the mean percentage correct for each of the six basic emotions summed over the different intensity levels used in this task, with error bars representing SEM. p >0.05.

Another way to investigate facial expression recognition accuracy is to look at correct recognition of individual emotions displayed at varying morphed intensities, and the threshold at which each emotion is accurately detected by treatment groups. There were no effects of vaccine on anger (main effect of group $F(1,40)=0.43$, $p=0.52$, emotion x group

$F(7,266)=0.9$, $p=0.5$), disgust (main effect of group $F(1,40)=0.58$, $p=0.45$, emotion x group $F(6,235)=1.07$, $p=0.38$), fear (main effect of group $F(1,40)=0.11$, $p=0.75$, emotion x group $F(9, 351)=1.63$, $p=0.11$), happy (main effect of group $F(1,40)=1.32$, $p=0.26$, emotion x group $F(6,244)=0.46$, $p=0.84$), sad (main effect of group $F(1,40)=0.78$, $p=0.38$, emotion x group $F(6,245)=0.48$, $p=0.83$) or surprise (main effect of group $F(1,40)=1.27$, $p=0.27$, emotion x group $F(6,241)=0.58$, $p=0.75$). Similarly, there was no main effect of treatment on the threshold at which the groups were able to accurately detect emotion ($F(1,39)=1.59$, $p=0.21$), or an emotion x group interaction ($F(4,154)=1.26$, $p=0.29$). There was a main effect of emotion ($F(4,154)=17.35$, $p<0.001$), as would be expected.

There were no effects of typhoid vaccine on this task in terms of misclassifications (main effect of group $F(1,40)=0.25$, $p=0.62$; emotion x group $F(3,129)=0.68$, $p=0.58$, main effect of emotion $F(3,129)=21.26$, $p=<0.001$). Though post-hoc analysis revealed that the typhoid vaccine group misclassified more disgusted faces than the placebo group ($F(1,39)=5.91$, $p=0.02$), as shown in Figure 3.

There was no main effect of group ($F(1,39)=0.59$, $p=0.45$), and no emotion x group interaction ($F(5,195)=0.04$, $p=0.99$) for reaction times in this task. There was, as expected, a main effect of emotion ($F(5,195)=11.09$, $p<0.001$).

Additionally, analyses were conducted with the typhoid vaccine group to examine whether there were any correlations between IL-6 and all FERT measures, however no significant correlations were found (p values >0.1). Thus, there did not appear to be a relationship between the magnitude of IL-6 response and behavioural responses following typhoid vaccination in this task.

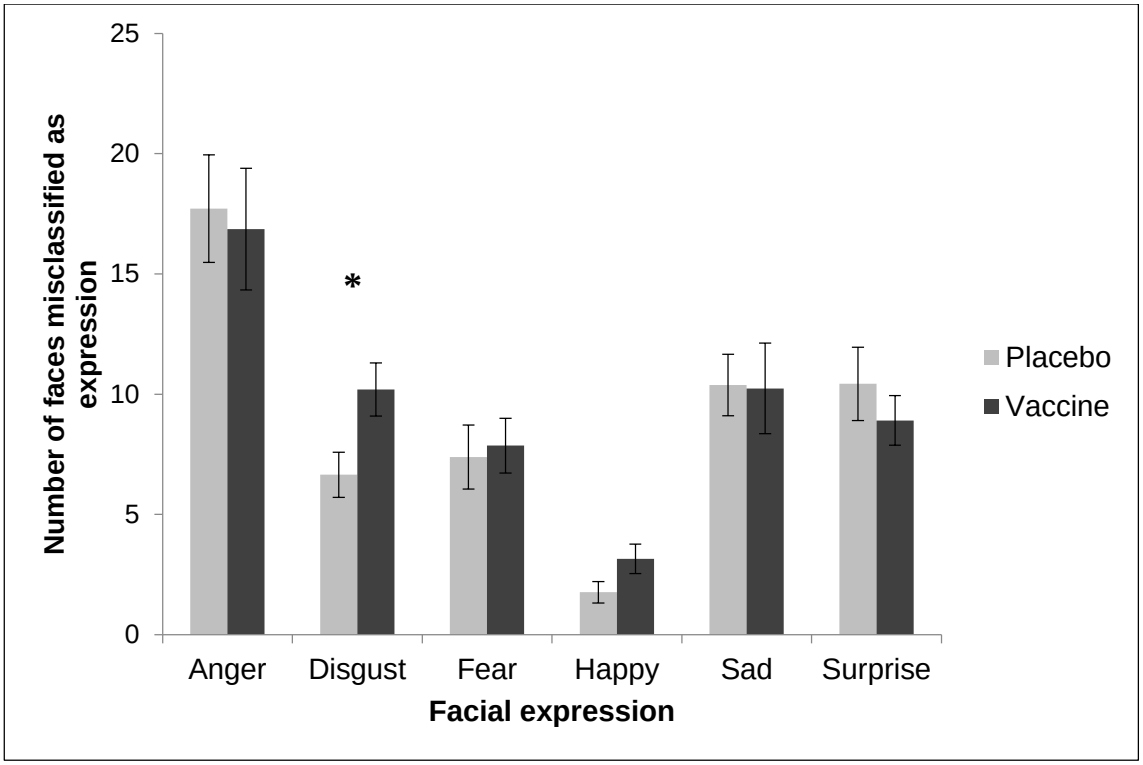


Figure 3. Misclassifications in the facial expression recognition task following placebo (light bars) and typhoid vaccine (dark bars). Values represent the mean percentage correct for each of the six basic emotions summed over the different intensity levels used in this task, with error bars representing SEM. * $p = 0.02$.

3.3.6.2 *Emotional categorisation task*

There were no between-group differences in accuracy to categorise self-referent personality characteristics in terms of an effect of group ($F(1,39)=0.65$, $p=0.42$) or emotion x group interaction ($F(1,39)=0.03$, $p=0.88$), though there was a main effect of emotion ($F(1,39)=11.13$, $p=0.002$; see Figure 4).

Similarly, there were no between-group differences in reaction times to categorise self-referent personality characteristics (main effect of group $F(1,40)=0.39$, $p=0.54$, emotion x group $F(1,40)=0.05$, $p=0.83$, main effect of emotion $F(1,40)=41.23$, $p<0.001$).

No significant correlations were found between IL-6 and accuracy or reaction times in this task within the typhoid vaccine group (p >0.1).

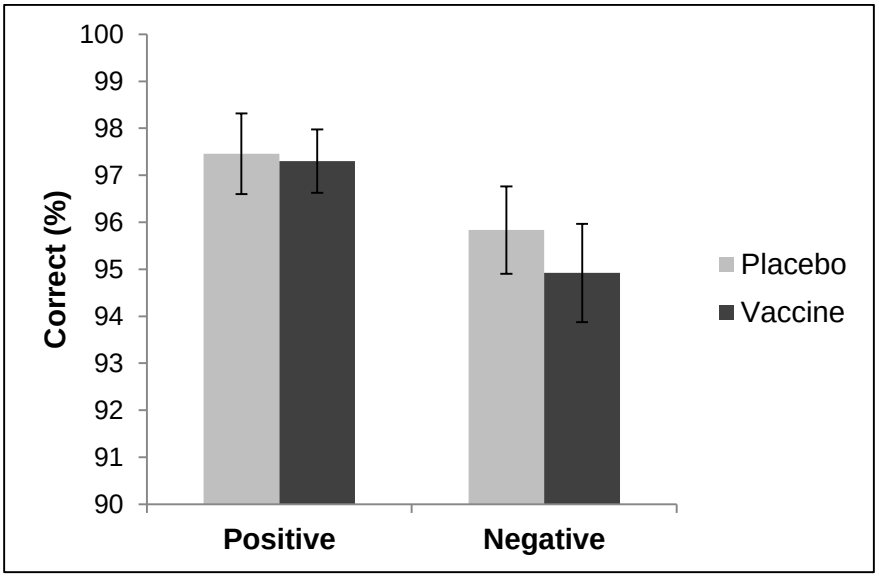


Figure 4. Accuracy in the emotional categorisation task following placebo (light bars) and typhoid vaccine (dark bars). Values represent the mean percentage correct categorisation of positive and negative personality characteristic words used in this task, with error bars representing SEM.

3.3.6.3 *Emotional memory*

The total number of correct words recalled did not differ significantly between the groups (F(1,40)=<0.001, p=1.0), nor were there any differences in accurate recall of positive or negative valence words (main effect of group F(1,39)=<0.001, p=0.99; see Figure 5) or false intrusions (main effect of group F(1,40)=0.09, p=0.77).

Interestingly, IL-6 in the vaccine group was positively correlated with accurate total recall (r = 0.54, p = 0.014), with trends towards improved recall for both positive (r = 0.44, p = 0.058) and negative (r = 0.43, p = 0.058) valence words in this group. There were no correlations between IL-6 and false intrusions (p values >0.3).

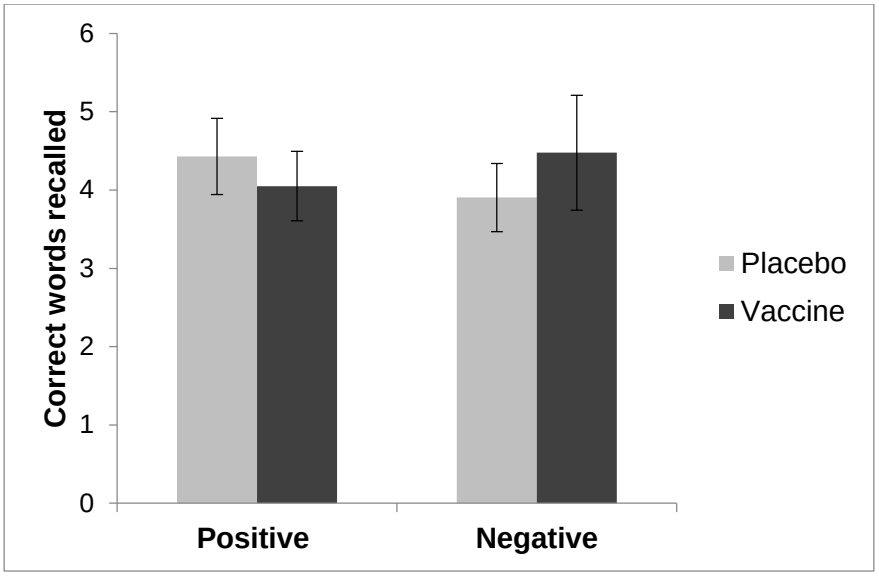


Figure 5. Recall of positive and negative personality characteristics following placebo (light bars) and typhoid vaccine (dark bars). Values represent the mean number of correct trait words recalled, with error bars representing SEM.

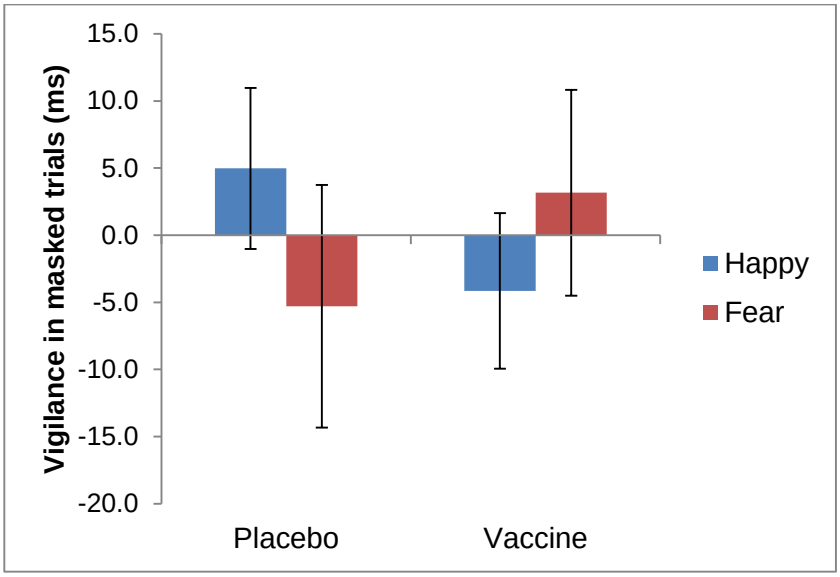
There was no effect of treatment group on accuracy in the emotional recognition memory task (main effect of group $F(1,40)=0.16$, $p=0.69$, emotion x group $F(1,40)=0.13$, $p=0.72$). No difference was found between groups when positive words (one-way ANOVA $F(1,40)=0.02$, $p=0.89$) and negative words (one-way ANOVA $F(1,40)=0.24$, $p=0.63$) were considered separately. In terms of false alarms in this task, there were no between-group differences (main effect of group $F(1,38)=0.04$, $p=0.84$, emotion x group $F(1,38)=0.49$, $p=0.49$). Nor were there any effects of typhoid vaccine on reaction times (main effect of group $F(1,40)=0.28$, $p=0.60$, emotion x group $F(1,40)=0.02$, $p=0.89$). Furthermore, typhoid vaccine did not affect participants' ability to deduce correct rejections or reaction times on this task (p values >0.5).

No significant correlations between IL-6 and any emotional recognition parameters were found (p values >0.1).

3.3.6.4 *Dot probe attentional vigilance*

There was a mask x emotion x treatment group interaction ($F(1,39)=6.59$, $p=0.014$), including mask as a within-subject factor. Although when masked ($F(1,39)=1.41$, $p=0.24$) and unmasked ($F(1,40)=1.01$, $p=0.32$) trials were considered separately, no significant emotion x group interaction was found in either condition. Moreover, post-hoc analyses revealed no between-group differences in vigilance to happy or fearful faces. The interaction effect when masking was included as a within-subject factor is, in fact, a result of changes in vigilance to happy faces in the placebo group between the masked and unmasked condition, as can been seen in Figure 6. No further differences were found (all p values >0.1). This suggests typhoid vaccination has not modulated attentional vigilance compared to placebo injection. Furthermore, there were no correlations between IL-6 and attentional vigilance scores (p values >0.1) in the vaccine group.

a)



b)

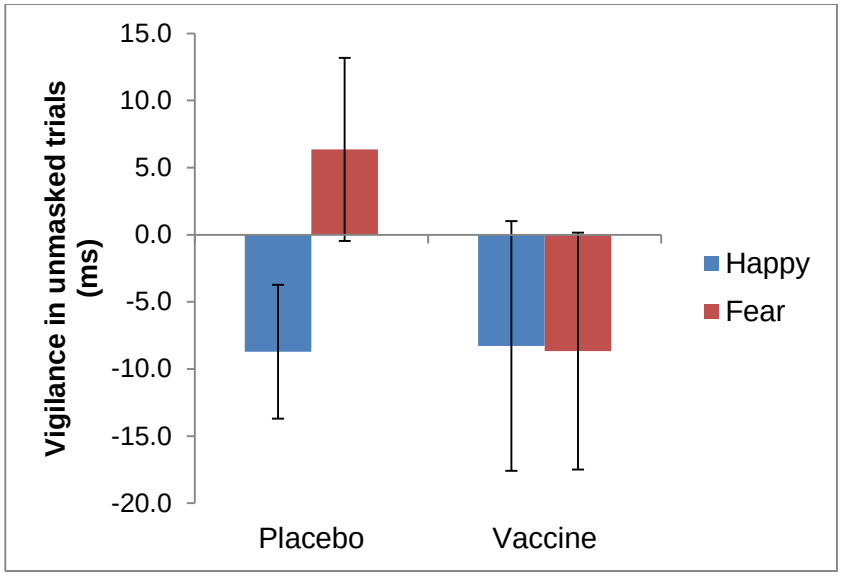


Figure 6. Effects of placebo and typhoid vaccine on attentional vigilance for happy and fearful facial expressions in the a) masked condition and b) unmasked condition of the dot probe task. Attentional vigilance scores were calculated by subtracting the median reaction time from congruent trials (when the probe appeared in the same position as the emotional face) from incongruent trials (when the probe appeared in the opposite position to the emotional face, i.e. in the position of the neutral face). A positive score indicates vigilance towards the emotional face, whereas a negative score reflects avoidance of the emotional face. Error bars show SEM.

3.3.7 Cognitive tasks

3.3.7.1 *N-back*

There were no between-group differences in accuracy ($F(1,37)=0.15$, $p=0.70$) on the control task, nor was there a main effect of treatment group ($F(1,37)=0.16$, $p=0.69$) or a group x complexity interaction ($F(2.5,94.2)=1.69$, $p=0.18$). There was, as expected, a main effect of complexity ($F(2.5,94.2)=89.2$, $p<0.001$) on accuracy in this task.

There was a trend for an effect of vaccine on latency in the control task (zero-back) ($F(1,40)=3.99$, $p=0.053$), whereby the vaccine group were slower than the placebo group.

No overall main effect of group was found, however (F(1,40)=1.34, p=0.25), nor complexity x group interaction (F(1.73,69.0)=0.23, p=0.76), though once more there was a main effect of complexity (F(1.73,69.0)=65.19, p<0.001). Post-hoc analysis of reaction times for the one-, two- and three- back levels found no differences between the two groups (all p values >0.2). Factoring in control task reaction times had no effect of the lack of interaction or main effects in this task (p >0.2).

There were no correlations between IL-6 and *n*-back performance (all p values >0.1) in the typhoid vaccine group.

3.3.7.2 *Rapid visual information processing task*

There were no effects of typhoid vaccine compared to placebo on accuracy (F(1,40)=0.007, p=0.93), false alarms (F(1,40)=0.67, p=0.42) or reaction times for correct responses (F(1,40)=2.33, p=0.14) in this task.

There were no correlations between IL-6 and performance in the RVIP task (all p values >0.1) in the typhoid vaccine group.

3.3.7.3 *Reward task*

There was no inflammation (placebo, vaccine) by valence (reward, punishment) interaction (F(1,29)=0.13, p=0.72) across trials; Figure 7a appears to show that the vaccine group selected more high probability reward stimuli and avoided more high probability punishment stimuli compared to the placebo group. Furthermore, no inflammation by valence interaction (F(1,15)=1.19, p=0.29) was found when the last 50% of choices were analysed as in Harrison *et al.* (2015c). There was, however, a main effect of inflammation (F(1,29)=45.22, p<0.001). Post-hoc t tests revealed significant differences between vaccine and placebo groups for reward (t(15)=3.77, p=0.002) and punishment (t(15)=3.45, p=0.004)

conditions in the last 50% of trials. Figure 7b shows that the vaccine group responded correctly to more high probability reward and punishment stimuli compared to the placebo group.

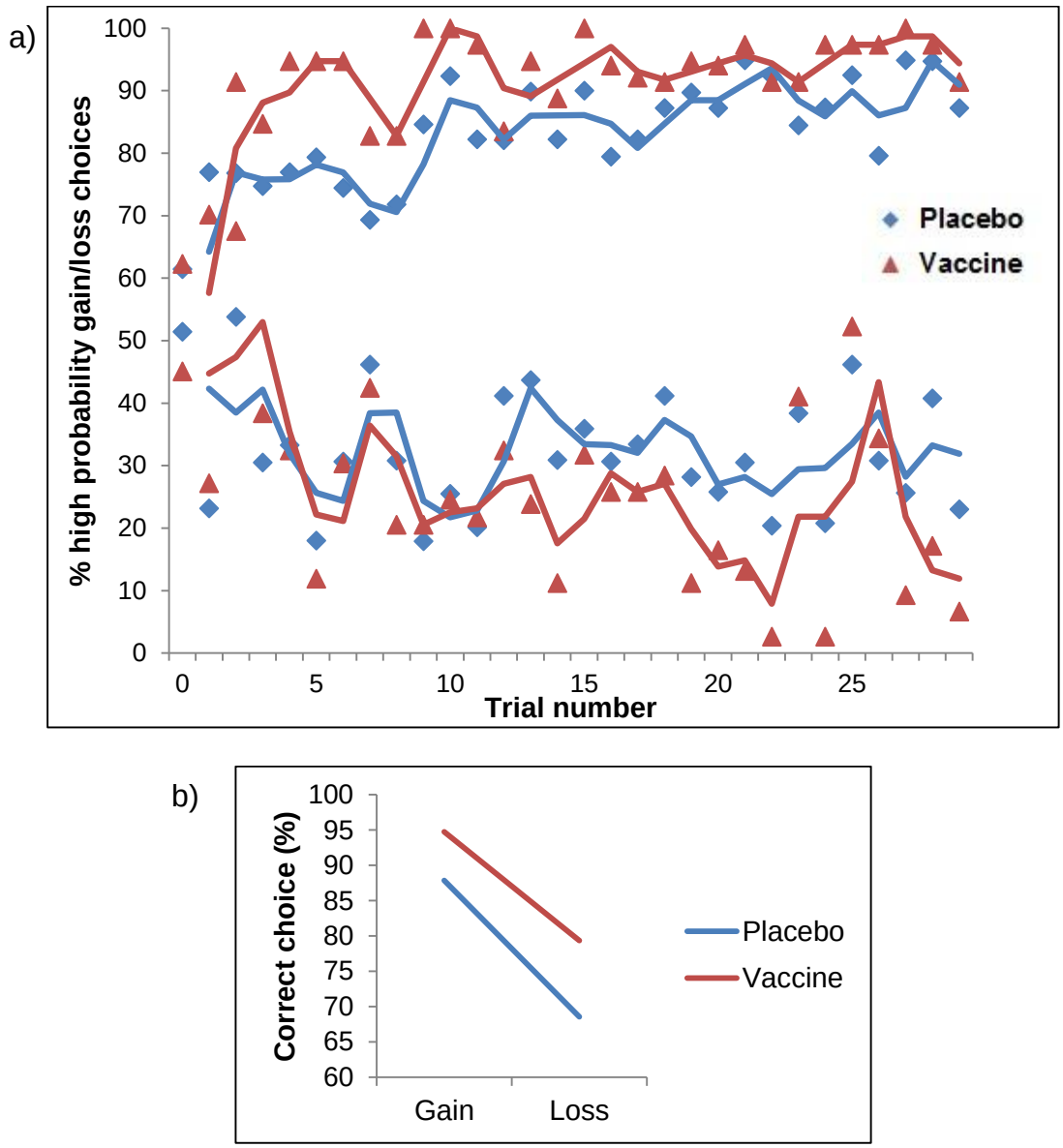


Figure 7. a) Responses on the reward task following placebo (blue) and typhoid vaccine (red). Points depict the percentage of times participants chose the correct stimulus in the gain condition (upper lines on graph) and the incorrect stimulus in the loss condition (lower lines on graph). Average values for the two runs in each trial are shown with learning curves (moving average). b) Correct choices in gain and loss conditions in the last 50% of trials after placebo (blue line) and typhoid vaccine (red line). Values are percentage means across two task runs completed.

3.3.7.4 *AVLT*

There was no significant difference between treatment groups for initial recall of either List A ($F(1,40)=2.27$, $p=0.14$) or List B ($F(1,40)=0.14$, $p=0.72$). Learning over trials (LOT), which takes into account learning over trials 2 – 5 for the recall of the 15-item List A, improved over the trials for both treatment groups (main effect of trial: $F(2.01, 80.54)=33.72$, $p<0.001$). There was no main effect of group ($F(1,40)=2.89$, $p=0.097$), however there was a significant treatment group by trial interaction ($F(2.01, 80.54)=4.17$, $p=0.019$). Post-hoc analysis revealed that recall in the second and third trials was greater in the vaccine group (R2: $F(1,40)=5.99$, $p=0.019$; R3: $F(1,40)=4.04$, $p=0.051$) compared to the placebo group, as shown in Figure 8. This suggests that the vaccine group learned at a slightly faster rate than the placebo group, however the vaccine group also had a significantly higher total number of repetitions ($F(1,40)=5.40$, $p=0.025$). There were no between-group differences in total number of intrusions ($F(1,40)=0.01$, $p=0.92$).

Delayed recall following the distracter List B ($F(1,40)=1.52$, $p=0.22$) and the longer 40-minute delay ($F(1,40)=0.26$, $p=0.61$) showed no differences between the two groups. Signal detection analysis (A') also revealed no between-group differences in recognition score ($F(1,40)=2.04$, $p=0.16$).

There were no correlations between IL-6 and performance in the AVLT task (all p values >0.1) in the typhoid vaccine group.

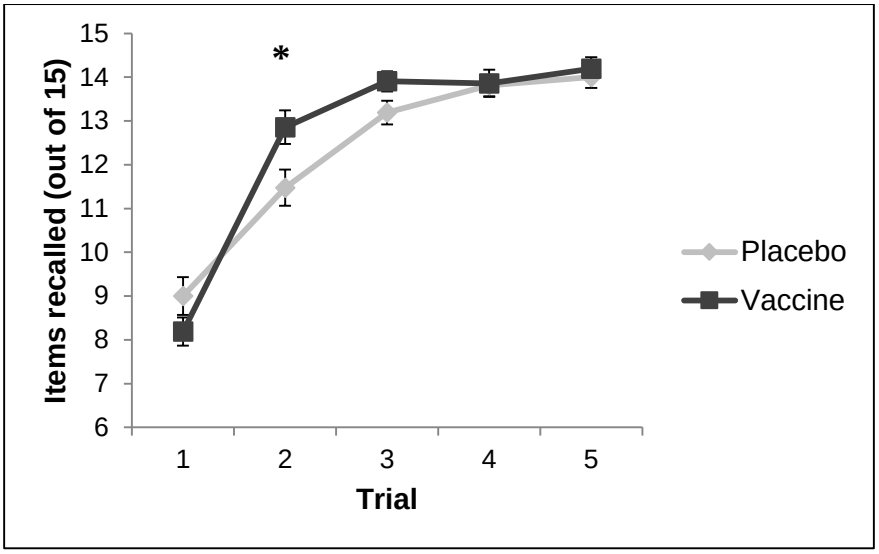


Figure 8. Recall over the five learning trials following placebo (light grey) and typhoid vaccine (dark grey). Values represent mean number correct, with error bars representing SEM. * $p = 0.02$.

3.4 Discussion

Typhoid vaccination significantly elevated levels of IL-6 compared to placebo injection, thereby replicating previous findings (Harrison *et al.*, 2009a, Harrison *et al.*, 2014, Harrison *et al.*, 2015c) and providing the intended model of mild systemic inflammation for the current investigation. Typhoid vaccine did not significantly affect mood compared to placebo; these findings are contrary to previous reports in this same model (Harrison *et al.*, 2009a, Wright *et al.*, 2005), though Paine *et al.* (2013) also did not observe mood changes following typhoid vaccine, and Strike *et al.* (2004) found that mood improved with time in the placebo group, but not the vaccine group, thus more negative mood was reported relative to the placebo condition. Correlations between subjective mood state and IL-6 response were not found, which is in line with some studies (e.g. Harrison *et al.*, 2009a), and contrary to others (e.g. Wright *et al.*, 2005). This may be owing to different sample sizes or

questionnaires used to assess mood, although the array of mood measures employed in the current study would be expected to detect mood alterations.

The lack of effects on illness symptoms and physiological measures (blood pressure, heart rate, body temperature) are in line with previous studies (Brydon *et al.*, 2008, Harrison *et al.*, 2009a, Strike *et al.*, 2004). Harrison *et al.* (2009a) also found no effects on salivary cortisol three hours following typhoid vaccine; we extended our investigation of cortisol to measure morning awakening response, however this also remained unaltered. Further work could investigate the diurnal cortisol slope as evidence suggests that this is altered in depression (Jarcho *et al.*, 2013, Lamers *et al.*, 2013) and it has been shown to be flattened by IFN-α therapy in hepatitis C patients (Felger *et al.*, 2015a, Raison *et al.*, 2010a).

3.4.1 Emotional processing

3.4.1.1 *Facial expression recognition*

There were no differences in accuracy or reaction times in the facial expression recognition task. However, the typhoid vaccine group misclassified more faces as disgusted and displayed a significantly less conservative response style for disgusted faces compared to the placebo group. Effects were not observed with other emotions, hence this represents a specific effect of false discrimination of disgust. These observations were not correlated with IL-6 increases in the vaccine group.

The present effect on misclassification of disgust is interesting in the context of interferon-α (IFN-α) results shown in the previous chapter. Since the potential meaning of increased sensitivity to disgust is explored in the preceding chapter, it will not be discussed in depth again here; however, two further points are worth noting in light of the typhoid vaccination model results. Firstly, the previous chapter raised the possibility that disgust recognition could be affected by physical sickness symptoms such as nausea; disgust findings in the

present study (although slightly different to those observed with IFN-α) occurred in the absence of subjective feelings of nausea, so this could help to inform this argument. Secondly, a study by Harrison *et al.* (2009b) that utilised typhoid vaccination, found that activity in interoceptive brain regions such as the dorsal-mid and anterior insula predicted inflammation-associated (measured by IL-6) fatigue within three hours post-vaccination. As discussed in the previous chapter, the insula is implicated in emotional processing of disgust; hence it is plausible that the effects seen in this study with disgusted faces may provide further support for the ability of inflammatory cytokines to rapidly modulate neural activity in this region, potentially leading to changes in behaviour.

Harrison *et al.* (2009a) previously reported neural activity in response to emotional face processing following typhoid vaccination. They found increased subgenual ACC (sACC) activity when viewing emotional facial expressions, which correlated with inflammation-associated mood deterioration three hours following typhoid vaccine. They suggest that this demonstrates the ability of inflammation to recruit neural circuitry that is implicated in the aetiology of depression. They also found that inflammation-associated mood change reduced functional connectivity of the sACC to the amygdala, medical prefrontal cortex, nucleus accumbens and superior temporal sulcus, and that this was moderated by IL-6 (Harrison *et al.*, 2009a). Furthermore, endotoxin (an alternative inflammatory model) has been shown to activate the right orbitofrontal cortex in response to emotional stimuli (Kullmann *et al.*, 2013) and the amygdala in response to socially threatening images (Inagaki *et al.*, 2012) significantly more than placebo in healthy volunteers. The sACC, orbitofrontal regions and amygdala are all thought to be involved in emotional information processing (Disner *et al.*, 2011, Elliott *et al.*, 2011, Kullmann *et al.*, 2013). For this reason, behavioural effects in both the facial expression recognition and dot probe tasks may have been expected in the present study. It is possible that although acute inflammatory stimuli are able to

modulate neural circuitry implicated in emotional processing, behavioural tasks may be less sensitive to this modulation due to recruitment of additional neural resources to maintain equal task performance (reported for cognitive performance in Harrison *et al.*, 2009b).

3.4.1.2 *Emotional categorisation*

The current study found no effects on emotional categorisation in terms of accuracy or reaction times, and there were no correlations between these measures and IL-6. This suggests that this mild inflammatory stimulus is not acutely able to modulate self-referent categorisation of personality characteristics. These results are in contrast to the negative bias we observed in the previous chapter following IFN-α therapy, and they are not in line with negative biases previously shown in depressed patients (Harmer *et al.*, 2009b).

3.4.1.3 *Emotional memory*

In the emotional recall task, typhoid vaccine had no effects on accurate recall of positive or negative self-referent personality words or false intrusions, however a larger IL-6 response was associated with enhanced accurate total (positive and negative) recall. This effect was not expected, since it does not correspond with negative emotional memory biases observed in depression (Gilboa-Schechtman *et al.*, 2002, Leppanen, 2006), nor does it mirror the trend towards a negative memory bias presented in Chapter 2. There are no published data on effects of acute inflammation on emotional memory, however non-emotional memory function has been shown to be impaired following endotoxin administration in healthy volunteers (Reichenberg *et al.*, 2001). Conversely, memory performance has also been shown to improve with acutely higher IL-6 levels; this is discussed within later discourse on cognitive function.

There were also no effects on emotional recognition following typhoid vaccination. The current results therefore suggest that this mild inflammatory model does not modulate

emotional memory. As discussed in the previous chapter, emotional memory changes are thought to be sensitive to acute alterations in monoaminergic availability, so it is possible that the lack of behavioural modification in this task following typhoid vaccination reflects limited effects on monoaminergic function.

3.4.1.4 *Attentional vigilance*

Typhoid vaccine had no effect on attentional vigilance to happy or fearful faces compared to the placebo group. Vigilance scores also did not correlate with IL-6 levels in the experimental inflammatory model. Once more, this finding is not in line with observations of negative biases in vigilance following IFN-α presented within this thesis, or with attentional biases reported in depressed patients (Bradley *et al.*, 1997, Fritzsche *et al.*, 2010, Gotlib *et al.*, 2004). It is interesting to note here that experimental inflammatory challenge with endotoxin selectively enhanced amygdala activity to fearful faces to a larger extent than any other socially non-threatening (happy faces) or non-social non-threatening/threatening images (e.g. household objects or guns) compared to placebo (Inagaki *et al.*, 2012). The absence of effects in the current study are perhaps surprising in this light, though data are insufficient to conclude whether or not inflammatory cytokines are capable of adapting attentional behaviours. Potential reasons for null findings and suggestions of further work are discussed below.

3.4.2 Cognitive function

Inflammation is also thought to be able to modulate cognitive function in ways that might be pertinent to inflammation-associated depression (Dantzer *et al.*, 2008b, Felger and Lotrich, 2013, Yirmiya and Goshen, 2011). However the precise mechanisms are not clearly delineated and may benefit from further investigation in healthy volunteer studies, without

interferences from chronic illness or physical sickness symptoms that result from large doses of inflammatory stimuli.

3.4.2.1 *Working memory, declarative memory and sustained attention*

Working memory deficits have been observed in clinical depression (Doumas *et al.*, 2012) and as residual symptoms in remitted patients (Hoorelbeke *et al.*, 2016). Effects of inflammation on working memory, however, are ill-defined. Typhoid vaccination did not significantly alter performance in the *n*-back task in terms of accuracy. Similarly, a previous study utilising endotoxin in healthy volunteers showed no effect of inflammatory stimulus on accuracy in this task (Grigoleit *et al.*, 2011).

The AVLT task, which assesses different components of declarative memory, including initial and delayed recall, learning and recognition (Rey, 1964), showed some curious effects; neither initial nor delayed recall were altered by typhoid vaccination, yet the vaccine group showed a slightly faster rate of learning over trials compared to the placebo group, as well as a higher number of repetitions over all trials. In the recognition component of the task, sensitivity analysis found no significant differences between groups. In general, therefore, typhoid vaccine did not impair declarative memory, if anything it slightly improved memory performance compared to placebo. This is in contrast to endotoxin-induced impairments in verbal memory (Reichenberg *et al.*, 2001), as well as studies in depressed patients that point to impairments in initial recall (Query and Megran, 1984, Sweeney *et al.*, 1989) and impairments in all components of this task (Austin *et al.*, 1992, Brown *et al.*, 1994).

Typhoid vaccination also showed no effects on sustained attention, as measured by the RVIP task. Previous literature supports impaired sustained attention in remitted depressed patients (Hasselbalch *et al.*, 2011, Weiland-Fiedler *et al.*, 2004), and sustained attention deficits have

been shown to be positively correlated with clinical symptoms in depressed patients (Yang *et al.*, 2015). The lack of findings in the current healthy volunteer model therefore suggest that acute immune system activation does not alter sustained attention. Interestingly however, curcumin, which is thought to have anti-inflammatory properties, has been shown to improve sustained attention just one hour after administration (Cox *et al.*, 2015). Further studies are needed to explore these potential effects in more detail.

3.4.2.2 *Psychomotor speed*

Psychomotor speed has been reported to be reduced in current and remitted depressed patients (Hasselbalch *et al.*, 2011, Lee *et al.*, 2012, Weiland-Fiedler *et al.*, 2004). Furthermore, inflammation is thought to reduce psychomotor performance, for example in patients receiving IFN-α treatment (Capuron and Miller, 2004, Majer *et al.*, 2008). The current study found a trend towards an effect of slower reaction time performance on the control task of the *n*-back, which is in contrast to faster reaction times observed in this task following high-dose endotoxin (which was suggested to indicate an adaptive state of alertness; Grigoleit *et al.*, 2011). This difference could be related to stronger inflammatory responses evoked by endotoxin, however that would not be in line with reports of associations between slower reaction times and increased IL-6 responses three hours after typhoid vaccination (recorded in the stroop task; Brydon *et al.*, 2008). Of course task differences may account for such discrepancies, though it should be noted that IL-6 responses reported by Brydon *et al.* (2008) are similar to those reported here, and taken in a similar time frame.

3.4.2.3 *Reward learning*

The typhoid vaccine group were significantly more accurate in choosing high probability reward and avoiding high probability punishment; therefore this group demonstrated

generally better task performance in both gain and loss conditions compared to the placebo group. This task was analysed in a similar manner to Harrison *et al.* (2015c) in order to compare results between comparable probabilistic learning tasks tested in the same experimental inflammatory model, both carried out at roughly the same time point (three hours after typhoid vaccination). Harrison *et al.* (2015c) showed that inflammation enhanced sensitivity to punishments (loss condition) compared with rewards (gain condition); the authors suggest this indicates acutely biased behaviours that lead to potential rewards being less attractive and punishments more aversive. They posit that this could provide a mechanism underpinning motivational changes associated between chronic inflammation and depression. However, the results in the present study are not aligned with Harrison *et al.* (2015c), as no significant interaction between inflammation and valence was observed, hence the finding that typhoid vaccine enhanced sensitivity to punishment versus reward was not replicated. There are some methodological differences that might account for this dissimilarity; for example, Harrison *et al.* (2015c) tested 24 healthy participants in a cross-over design – it is possible that the present study was insufficiently powered since 42 participants were tested in a parallel design that is more sensitive to inter-subject variability. Small additional differences between the studies, such as reciprocal probabilities (0.8/0.2 and 0.2/0.8), three trial runs and £1 gain/loss in the Harrison study, compared to 0.7/0.3 and 0.3/0.7 reciprocal probabilities, two trial runs and £0.50 gain/loss in the current study, may also contribute to inconsistencies. Though it is noteworthy that probabilities used in the current reward task make the task more difficult, which is intriguing considering the vaccine group performed better by choosing the correct stimulus in the gain condition (probability = 0.7 of winning £0.50) more and the incorrect stimulus in the loss condition (probability = 0.7 of losing £0.50) less than the placebo group.

Inflammation has previously been shown to reduce activity in the ventral striatum in response to reward outcomes following 4-6 weeks of IFN-α treatment for hepatitis C, where ventral striatum activity correlated with anhedonia, depression and fatigue (Capuron *et al.*, 2012). Furthermore, ventral striatal reactivity to reward cues was reduced following endotoxin administration, though interestingly this study did not observe between-group differences (endotoxin vs placebo) in behavioural responses in a monetary reward task (Eisenberger *et al.*, 2010a). In addition, typhoid vaccine has been shown to reduce encoding of reward prediction errors within the ventral striatum, and enhance encoding of punishment prediction errors in the anterior insula (Harrison *et al.*, 2015c). Decreased connectivity between the ventral striatum and ventromedial prefrontal cortex has also been observed in depressed patients, and was associated with increased c-reactive protein (CRP), IL-6, IL-1β and IL-1 receptor antagonist, as well as increased anhedonia and psychomotor slowing (Felger *et al.*, 2015b).

Given growing evidence that points to the modulatory capability of inflammation on reward-related neural circuitry and the potential importance of these mechanisms in anhedonia, fatigue and depressive symptomatology, it seems that there is good rationale for conducting further work in healthy volunteer models of inflammation. However, for reliable insight it will be important to improve understanding of variances within the same model presented above and to validate the model by replicating findings between different research groups.

3.4.2.4 *The varying effect of IL-6 on cognition*

Immune modulation of cognitive function is complex, appearing to have beneficial effects under normal conditions such as promoting learning and memory; yet when conditions such as infections, injury or chronic stress disrupt this well co-ordinated system, impaired learning, memory and neuroplasticity can arise (reviewed by Yirmiya and Goshen, 2011).

This means that the role of IL-6 in cognition is far more complex than simply elevated levels resulting in poorer performance, which some evidence from healthy volunteers models of inflammation may suggest. Indeed, there are (albeit limited) data that suggest IL-6 may have protective effects on cognition under some circumstances. For example, higher levels of IL-6 in patients with lupus erythematosus were associated with greater learning scores (Kozora *et al.*, 2001) and higher IL-6 levels in patients one day after surgery were associated with smaller declines in declarative memory (Shapira-Lichter *et al.*, 2008). Furthermore, exogenous IL-6 administered to patients with chronic fatigue syndrome and healthy controls promoted improved performance in a memory test; this could have been due to a practice effect but it is also possible that IL-6 itself caused the improvement (Arnold *et al.*, 2002, Yirmiya and Goshen, 2011). Intriguingly, low-dose endotoxin has been suggested to improve declarative memory, without affecting working memory, whereas higher doses of endotoxin have been shown to impair declarative memory and conversely improve working memory (Cohen *et al.*, 2003, Krabbe *et al.*, 2005, Reichenberg *et al.*, 2001). It seems plausible therefore that the modulatory role of IL-6 on cognitive function is not constant, and may have opposing effects depending on cytokine levels in the brain, in addition to length of exposure. This might have important implications on the way in which acute, healthy volunteer models are used to investigate aberrant mechanisms in relation to chronic conditions.

3.4.3 Limitations and further work

Results in the present study do not provide strong evidence of the ability of typhoid vaccination as an acute experimental inflammatory model to modulate emotional processing or cognitive function. It is therefore important to consider limiting factors that may contribute to current findings that are not in keeping with previous published studies, or the depression literature in general. Firstly, consideration should be given to the timeline of

investigation in the present study; tasks were performed between two and four hours after the typhoid vaccine or placebo, at time points that have previously shown significant changes in neural activity that are relevant to emotional processing and cognition. However, it is possible that changes at a behavioural level may have a longer time course. Indeed, it may be that tasks were performed too early, and that behavioural adaptations may occur 6-8 hours after inflammatory challenge, when increases in central cytokine levels may be greater (Konsman *et al.*, 1999, Paine *et al.*, 2013). Peripheral measures of IL-6 do not adequately inform the central inflammatory processes that promote behavioural modifications; therefore further work should investigate acute effects slightly later after peripheral inflammatory challenge to examine whether longer exposure produces differing effects on emotional processing. It is also possible that downstream effects of immune system activation, such as sleep disruption (Cho *et al.*, 2016), could lead to indirect or augmenting effects on emotional processing that may be worthy of investigation.

Although IL-6 serves as a useful marker of inflammation given its importance in inflammatory responses (Schaper and Rose-John, 2015), coupled with evidence that supports a key role of IL-6 in depression (Dowlati *et al.*, 2010), simply measuring one pro-inflammatory cytokine (in the periphery) and looking for associations with behaviour seems crude. IL-6 acts as part of a complex network of both pro- and anti- inflammatory cytokines that respond to pathogens, therefore future studies in this field as a whole should examine a range of inflammatory markers.

It is also important to remember that under normal circumstances immune system activity is cleverly orchestrated and tightly controlled, therefore it is not surprising that inflammatory cytokines are thought to act as neuromodulators to induce protective and energy conserving sickness behaviours (Dantzer *et al.*, 2008b). This could also mean that variances in cognitive performance following experimental induction of inflammation could reflect complex and

sometimes protective cytokine signalling mechanisms. When this system becomes chronically maladaptive, deleterious effects may ensue. Thus, while the typhoid vaccine model is thought to provide insightful investigation of behavioural changes that may, under conditions of chronic inflammation, be involved in the development of depression, further studies are undoubtedly required to replicate and extend current literature. Caution should also be taken when extrapolating meaning from data collected in young, healthy participants and applying to patients with chronic inflammation.

As mentioned in the introduction, there are two main experimental models of immune system activation that have been used in healthy participants; typhoid vaccination and endotoxin. Endotoxin appears to induce a comparatively stronger inflammatory response, that can include increases in other pro-inflammatory cytokines, TNF-α and IL-1 (Eisenberger *et al.*, 2010b, Krabbe *et al.*, 2005, Kullmann *et al.*, 2013, Reichenberg *et al.*, 2001), in addition to cortisol at higher doses (Kullmann *et al.*, 2013, Reichenberg *et al.*, 2001) - all of which have not been shown to be elevated after typhoid vaccination, yet they have been shown to be elevated in depression (Cowen, 2002, Howren *et al.*, 2009, Liu *et al.*, 2012a). It would be interesting to investigate whether a stronger inflammatory stimulus like endotoxin might produce differing effects on emotional processing.

There is also intriguing evidence that stress, combined with typhoid vaccine, can upregulate cytokine responses and induce negative mood (Brydon *et al.*, 2009, Reichenberg *et al.*, 2001, Strike *et al.*, 2004) in a manner that is arguably more reflective of the combination of immune and psychological stressors that might culminate in vulnerability to depression (Slavich and Irwin, 2014). Further studies could develop this model.

3.5 Conclusions

This study supports previous data exemplifying the increase in the pro-inflammatory cytokine IL-6 following typhoid vaccination in healthy volunteers. Typhoid vaccination had limited effects on emotional processing, except an intriguing increase in false discrimination of disgusted faces. Negative biases in emotional processing overall, however, were not found. In non-emotional cognition tasks, typhoid vaccine had no effects on sustained attention, no marked effects on working memory, mild effects on improving learning in an auditory verbal learning task, as well as improved task performance in a probabilistic learning task. These results are at variance with some reported associations between inflammation and general impairments in cognitive function, though they are consistent with other reports. Further work is required to provide more robust empirical data to inform whether this experimental inflammatory model is capable of modifying emotional information processing that is strongly implicated in the onset and maintenance of depression. Future investigations should also assess effects on emotional and cognitive function with longer exposure to raised inflammatory profiles (this is discussed further in Chapter 5), and consideration should be given to possible downstream effects of cytokine activation, such as sleep disruption, that could enhance effects on emotional processing. This latter topic is explored in the subsequent chapter.

Chapter 4

Investigating the Effects of Typhoid Vaccine on Sleep in Healthy Participants

4.1 Introduction

Sleep alterations are commonly experienced in major depression (Nutt *et al.*, 2008) and are frequently reported as a key symptom of sickness behaviour (introduced in Chapter 1), occurring during infections (Imeri and Opp, 2009), and as a neurovegetative symptom following IFN-α administration (Capuron and Miller, 2011). Although pro-inflammatory cytokines are thought to have a role in homeostatic regulation of sleep (Imeri and Opp, 2009, Irwin and Cole, 2011, Krueger *et al.*, 2001, Opp, 2005), chronic inflammatory mechanisms may play a pathophysiologic role in sleep changes that are relevant to depression (Dantzer *et al.*, 2008b, Motivala *et al.*, 2005). Sleep disturbance has been associated with increased risk of depression (Cho *et al.*, 2016, Felger and Lotrich, 2013, Irwin, 2015) and suicide (Wojnar *et al.*, 2009), as well as compromised response to conventional antidepressant treatments (Weinberger *et al.*, 2015), all of which have also been associated with inflammation (Kiecolt-Glaser *et al.*, 2015, Miller *et al.*, 2009, Miller and Raison, 2015, Uher *et al.*, 2014).

Polysomnography permits examination of sleep continuity, sleep architecture and rapid eye movement (REM) sleep. Sleep continuity parameters assess the duration of sleep, sleep onset latency and sleep efficiency, denoting the degree of continuity of sleep throughout the night (Irwin, 2015). Sleep architecture is divided into REM sleep and non-REM (NREM) sleep, which is further separated into stages 1 – 3 (stages N1 – N3) based upon electroencephalogram characteristics (Benca and Peterson, 2008, Bryant *et al.*, 2004, DellaGioia and Hannestad, 2010, Irwin, 2015). More recent research has combined stages

3 and 4 due to difficulties in delineating one from the other and together they are classified as slow wave sleep (SWS; stage N3, characterised by slow delta waves) (Benca and Peterson, 2008, Irwin, 2015). Sleep disturbances that are characteristic of depression include diminished sleep continuity, such as reduced sleep efficiency and increased wake after sleep onset (WASO), decreased SWS and REM latency, in addition to increased REM density (Benca and Peterson, 2008, Riemann, 2007, Thase, 2006, Tsuno *et al.*, 2005).

Previous animal and human studies have demonstrated that inflammatory cytokines, including IL-6, TNF-α and IL-1β, can produce changes in sleep (Imeri and Opp, 2009, Krueger, 2008, Opp, 2005, Raison *et al.*, 2010c). For example, high doses of endotoxin have been shown to disrupt NREM sleep and to reduce SWS (Mullington *et al.*, 2000). In addition, IFN-α administration can reduce sleep efficiency and SWS (Raison *et al.*, 2010c), and poor sleep quality prior to IFN-α treatment has been demonstrated to be predictive of depression during therapy (Franzen *et al.*, 2010, Prather *et al.*, 2009). Moreover, similar disruptions observed following IFN-α treatment have also been seen in patients with rheumatoid arthritis (Abad *et al.*, 2008, Ranjbaran *et al.*, 2007), a chronic inflammatory condition that is discussed in the next chapter. A recent study in patients with treatment resistant depression and high inflammation (> 5 mg/L C-reactive protein; CRP) found that blockade of the pro-inflammatory cytokine TNF-α using infliximab increased sleep efficiency, suggesting that peripheral inhibition of inflammation may improve sleep disruption in patients with depression (Weinberger *et al.*, 2015). Evidence therefore supports the ability of innate immune activity to mediate sleep alterations both in chronic illnesses and in depression (Raison *et al.*, 2010c), which may be ameliorated by anti-inflammatory treatment.

In addition to immune activity altering sleep, evidence from sleep deprivation studies demonstrates the ability of sleep disruption to increase inflammatory markers, such as IL-6

(Irwin, 2015, Irwin *et al.*, 2006, Irwin *et al.*, 2008). Thus, neuroimmune interactions appear to have a bidirectional relationship whereby sleep loss can modify immune function and immune challenges (for example with endotoxin; Mullington *et al.*, 2000, Pollmacher *et al.*, 2000) can alter sleep (Imeri and Opp, 2009). This could have important ramifications in patients with chronic inflammation, including depressed and medically ill patients (Dantzer *et al.*, 2008b). However, there are still limited data on the effects of pro-inflammatory cytokines on sleep investigated using objective measures such as polysomnography and, to our knowledge, no previous study has investigated effects of typhoid vaccination on sleep parameters. Therefore, the current study used typhoid vaccination as a model of acute inflammatory challenge to examine effects of inflammation on sleep polysomnography. As outlined in the previous chapter, typhoid vaccination induces a mild innate inflammatory response, characterised by significantly raised IL-6, in the absence of sickness symptoms that have been reported following the comparatively stronger inflammatory stimulus, endotoxin (Eisenberger *et al.*, 2010b, Reichenberg *et al.*, 2001).

4.1.1 Aims

The aim of the present study was to examine the effects of typhoid vaccination on sleep polysomnography in healthy subjects, to aid the understanding of innate immune system modification of sleep architecture, which may be relevant to sleep changes associated with chronic inflammation in depression and medical conditions such as rheumatoid arthritis. Based on previous literature, we hypothesised that this acute inflammatory stimulus could disrupt sleep parameters in a similar way to aberrations frequently documented in depression.

4.2 Methods

4.2.1 Participants and study design

This was a double-blind, placebo-controlled, cross-over design study, in which 16 healthy participants were recruited (9 female, 7 male; mean age 26.6, range 20-38 years; mean BMI 23 kg/m^2; range 18.8-26.4) through local and online advertising. 11/16 participants had never received the typhoid vaccine; hence this should represent a primary immune response in these subjects. Inclusion criteria were the same as in the preceding chapter, with the addition of self-report as a good sleeper. A sleep disorders screening interview was also conducted by a sleep specialist within the lab (Dr Ann Sharpley) and current sleep disorders were an exclusion criteria.

The study was approved by the University of Oxford Medical Sciences Inter Divisional Research Ethics Committee, sponsored by the University of Oxford and overseen by Oxford University Clinical Trials and Research Governance. All participants signed and dated the Informed Consent Form prior to any study procedures.

Participants completed the Eysenck Personality Questionnaire (EPQ) (Eysenck and Eysenck, 1975), State Trait Anxiety Inventory (STAI) (Spielberger *et al.*, 1970) and Beck Depression Inventory (BDI-II) (Beck *et al.*, 1996) at screening to characterise the sample, as in the previous chapter.

4.2.2 Experimental model of inflammation

Participants attended two study visits, between 2.45 – 5.30 pm on both occasions. Participants were asked not to consume any alcohol or high-fat content meals and to avoid intensive exercise for 24 hours prior to each study visit. Upon arrival for the research visit, a brief interview ensured that there had been no changes in participants' health or medication since the screening visit.

According to the randomisation schedule (drawn up by a member of the group not otherwise involved in the study), participants were given either a single 0.5mL injection of typhoid polysaccharide vaccine (25 micrograms Typhim Vi®, Sanofi Pasteur MSD Ltd) or placebo (0.9% sodium chloride saline solution) intramuscularly into the non-dominant deltoid muscle in the arm. This was administered by a medical doctor or nurse. To maintain participant blinding, labels were placed over the syringes just prior to administration, and participants were asked to look away while the injection was given. The randomisation code was not broken until study analyses were complete. As this was a double-blind, balanced order, cross-over design study, participants received both the placebo and vaccine, with a 7-14 day washout period between each study visit.

The vaccine constituents are detailed in Chapter 3.

4.2.3 Interleukin-6 (IL-6) immunoassay

Interleukin-6 (IL-6) was analysed using the same methodology as in the previous chapter. 5mL blood samples were collected into ethylenediaminetetraacetic acid anticoagulant (EDTA) tubes two hours post-injection. Samples were centrifuged at 1250 x g for 10 minutes within 30 minutes after collection. Plasma was then extracted, aliquoted and stored in an anonymised form at -30^{o}C until analysis. On completion of the study, all samples were analysed using Human IL-6 Quantikine® HS (high sensitivity) ELISA kits (R&D Systems, Abingdon, UK) on the same day. The intra-assay and inter-assay coefficients of variation are 7.4% and 7.8%, respectively, and the limit of detection of the assay is 0.039 pg/mL.

4.2.4 Subjective mood questionnaires and side effects

Participants completed the Positive and Negative Affective Schedules (PANAS) (Watson *et al.*, 1988) and visual analogue scales (VAS) (Bond and Lader, 1974) hourly for 4 hours post-dose. VAS scales were combined to form three mood factors, as advised by the authors and

in keeping with the previous chapter: alertness, contentedness and calmness (Bond and Lader, 1974).

Participants completed an adverse effects questionnaire that assessed the presence of: soreness around the injection site, headache, dizziness, nausea, loss of alertness, drowsiness and appetite changes hourly for four hours post-dose, then again the following morning for each treatment condition, when they were also asked to guess which treatment (placebo or typhoid vaccine) they had received, indicating their certainty using a visual analogue scale. In addition, participants were asked to report any symptoms experienced that were not captured in the above.

4.2.5 Polysomnograms

Polysomnograms (PSGs) were conducted by an experienced sleep physiologist (Dr Ann Sharpley) within the research group. Electrodes were fitted during each study visit, then participants returned home to sleep as usual. On each study night, PSGs were recorded using the Embla titanium recording system (Natus Neurology Incorporated, Middleton, WI, USA). Participants were asked to go to bed and awaken at their usual time for both study nights and the preceding nights prior to study visits. The industry standard montage, American Academy of Sleep Medicine (AASM), was used which comprises six electroencephalogram (EEG) channels (C3-M2, C4-M1, O1-M2, O2-M1, F3-M2, F4-M1), two electrooculogram (EOG) channels (E1-M2 and E2-M2), and submentalis electromyogram (EMG) channels using three electrodes (Iber *et al.*, 2007). PSGs were staged in 30-second epochs using the Embla RemLogic sleep diagnostic software (Natus Neurology Incorporated, Middleton, WI, USA). RemLogic adheres to the AASM scoring criteria (Iber *et al.*, 2007). Additionally, the PSGs were edited by Dr Ann Sharpley, who was blind to treatment status.

The following morning, participants removed the electrodes themselves and, within 20 minutes of waking, participants completed the 10-item Leeds Sleep Evaluation Questionnaire (LSEQ) (Parrott and Hindmarch, 1980). This is a standardised VAS looking at four aspects of sleep: getting to sleep (GTS), quality of sleep (QOS), awake following sleep (AFS) and behaviour following awakening (BFW) following a treatment compared to the participants' usual sleep.

4.2.6 Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics (version 22). Mood state and side effects questionnaire data were analysed using repeated measures ANOVA, factoring in treatment (placebo or typhoid vaccine). Where assumptions of equality of variances were broken, Greenhouse-Geisser procedure was used to correct the degrees of freedom. Statistically significant interactions were followed up using post-hoc *t* tests. Differences between pairs of sleep nights and IL-6 measurements were assessed using two-tailed paired samples *t* tests. Correlational analysis was conducted between significant sleep parameter measures and IL-6 raw values (in pg/mL) using Pearson's correlation coefficient.

Power calculations were performed prior to study commencement. Sixteen participants were recruited in the present study; based on previous PSG studies, this study had a statistical power of 0.8 to detect a 5% change in sleep efficiency.

4.3 Results

4.3.1 Interleukin-6 (IL-6)

Typhoid vaccination significantly elevated IL-6 levels (placebo: 0.53 ± 0.15 pg/mL; vaccine 0.90 ± 0.61 pg/mL; $t(14)=-2.49$, $p=0.026$. Values are mean ± standard deviation). This increase was lower than the IL-6 response in the previous chapter, which was to be expected since it was measured at two hours post-dose (compared to four hours post-dose in the

previous study). Samples were not obtained from one participant due to unsuccessful venepuncture.

4.3.2 Subjective state and side effects

Baseline mood and personality questionnaire data are shown in Table 1 and are in the normal range expected in healthy subjects.

Table 1. Mood and personality measures at baseline. Means (standard deviations). *n*=16

	Mean score (SD)	**Questionnaire range**
BDI	1.2 (2.2)	0-63
State anxiety	26.5 (5.1)	20-80
EPQ: E	13.2 (5.1)	0-23
EPQ: N	6.9 (3.0)	0-24
EPQ: P	2.2 (2.4)	0-32
EPQ: L	7.1 (2.7)	0-21

BDI: Beck Depression Inventory; EPQ: Eysenck Personality Questionnaire, where E: extroversion, N: neuroticism, P: psychoticism, L: lie

Typhoid vaccination had no effects on mood state measures (PANAS and VAS) or participants' subjective evaluation of sleep quality, measured by the LSEQ (all *p* values >0.4), shown in Table 2 and Table 3, respectively.

Side effects reported were not significantly different in vaccine and placebo groups, except for soreness around the injection site (main effect of treatment: $F(1,15)=6.45$, $p=0.023$), which is consistent with the preceding vaccine study. Post-hoc analysis also revealed this symptom was reported significantly more the following morning in the vaccine group ($t(15)=2.24$, $p=0.041$; all other *p* values >0.1). To expand on this side effect in more detail,

no participants reported soreness following the placebo injection, whereas at 4 hours post-dose three participants reported soreness following the typhoid vaccine, rising to five participants by the following morning. 13/16 (81%) participants correctly guessed which treatment they received following both nights, though they were more certain on average following placebo (62.6%) compared to typhoid vaccine (55.2%).

Table 2. Subjective state changes over time using the PANAS and VAS. Means (standard deviations) one, two, three and four hours post-dose. *n*=16

	Placebo				**Vaccine**				
	1h	**2h**	**3h**	**4h**	**1h**	**2h**	**3h**	**4h**	***P* value**
PANAS: Positive	30.0 (6.2)	28.4 (6.2)	27.9 (6.1)	27.9 (5.6)	30.2 (6.3)	29.1 (6.5)	25.9 (6.9)	26.5 (8.2)	$p = 0.8$
PANAS: Negative	12.0 (3.2)	11.9 (2.6)	10.9 (1.7)	10.9 (1.8)	11.2 (1.9)	10.6 (1.1)	10.9 (1.6)	10.9 (1.8)	$p = 0.4$
VAS: Alertness	47.9 (4.4)	47.7 (3.0)	47.0 (4.4)	48.0 (4.5)	46.5 (3.6)	46.9 (4.6)	48.5 (3.1)	50.3 (3.0)	$p = 0.7$
VAS: Contentedness	52.0 (7.0)	49.8 (8.1)	49.5 (11.7)	54.8 (7.6)	52.7 (8.2)	50.6 (8.5)	52.2 (9.2)	53.9 (8.5)	$p = 0.7$
VAS: Calmness	50.5 (5.6)	50.8 (4.3)	52.5 (4.5)	52.6 (4.6)	50.1 (5.5)	51.9 (4.1)	53.3 (4.9)	51.2 (5.3)	$p = 1.0$

PANAS: Positive and Negative Affective Schedules; VAS: Visual Analogue Scales

Table 3. Subjective sleep measured by the Leeds Sleep Evaluation Questionnaire (LSEQ). Means (standard deviations). *n*=16

	Placebo	**Vaccine**	**Difference of means (SD)**	**Statistical significance**
GTS	47.7 (7.0)	49.0 (11.4)	1.3 (12.6)	$t(15)=0.42$, $p=0.68$
QOS	43.3 (11.1)	45.3 (9.2)	2.0 (9.4)	$t(15)=0.85$, $p=0.41$
AFS	47.5 (9.9)	51.0 (11.4)	3.4 (15.5)	$t(15)=0.87$, $p=0.39$
BFW	53.4 (14.6)	53.4 (12.2)	-0.02(10.6)	$t(15)=-0.008$, $p=1.0$

GTS: Getting to Sleep; QOS: Quality of Sleep; AFS: Awake following Sleep; BFW: Behaviour Following Wakening

4.3.3 Sleep polysomnography

One participant's data was removed from this analysis due to external noise that prevented sleep on one night.

Typhoid vaccine produced several changes in sleep continuity parameters compared to placebo (see Table 4). Total sleep time (TST, in minutes; $t(14)=3.35$, $p=0.005$) and sleep efficiency ($t(14)=3.17$, $p=0.007$; Figure 1) were significantly decreased; consistent with this, there were significant increases in wake after sleep onset (WASO, $t(14)=-3.18$, $p=0.007$; see Figure 2), as well as total wake ($t(14)=-3.37$, $p=0.005$), sleep stage transitions ($t(14)=-2.49$, $p=0.026$), number of awakenings ($t(14)=-3.15$, $p=0.007$) and awakening index ($t(14)=-3.34$, $p=0.005$) following typhoid vaccine compared to placebo. No significant correlations were found between changes in IL-6 levels two hours following vaccine administration and any of the significant sleep continuity measures (all p values >0.1). Furthermore, no other sleep parameters were significantly altered by typhoid vaccination, including NREM SWS (Figure 3) and REM sleep (all p values >0.1; shown in Table 4).

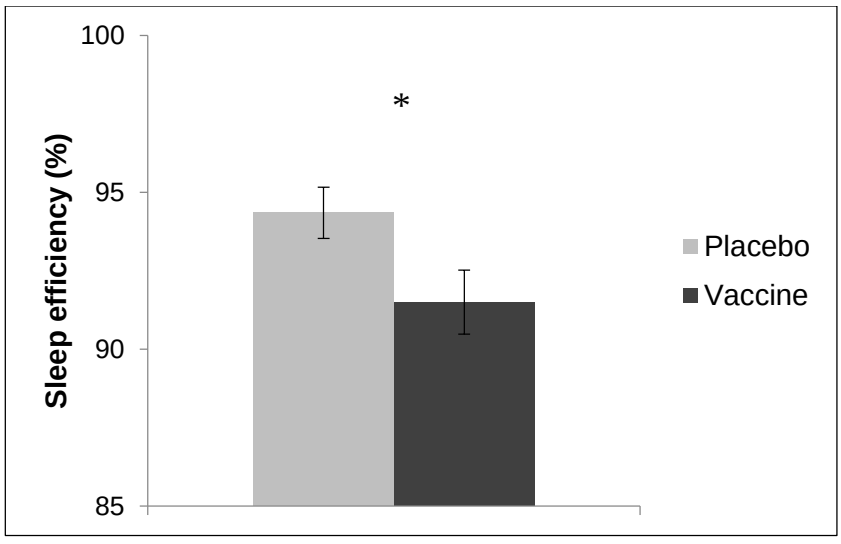


Figure 1. Sleep efficiency following placebo (light bar) and typhoid vaccine (dark bar). Values are mean percentages (actual sleep time/time in bed x 100), with error bars representing SEM. *p = 0.007.

Table 4. Effects of typhoid vaccine and placebo on selected sleep parameters. *n*=15

Selected Sleep Parameters [a]	Placebo	Vaccine	Difference of means (SD)	Statistical significance
Sleep continuity measures				
Total sleep time	426.1 (50.2)	410.7 (47.4)	-15.4 (17.8)	*p* = 0.005
Sleep efficiency (%) [b]	94.3 (3.2)	91.5 (4.0)	-2.8 (3.5)	*p* = 0.007
Wake after sleep onset	25.5 (13.5)	38.8 (18.8)	13.2 (16.1)	*p* = 0.007
Total wake	34.9 (13.4)	50.3 (19.5)	15.4 (17.8)	*p* = 0.005
Sleep stage transitions	155.9 (31.1)	173.1 (35.5)	17.3 (26.9)	*p* = 0.026
No. awakenings	27.2 (7.6)	36.1 (13.7)	8.9 (11.0)	*p* = 0.007
Awakening index	3.8 (1.2)	5.3 (1.9)	1.4 (1.6)	*p* = 0.005
Sleep onset latency	9.4 (4.8)	11.6 (10.2)	2.2 (10.0)	*p* = 0.4
NREM sleep measures				
N1	37.8 (10.7)	45.0 (20.2)	7.2 (19.8)	*p* = 0.2
N2	140.7 **(**33.9)	133.0 **(**31.6)	-7.7 (25.6)	*p* = 0.3
N3 (SWS)	140.8 **(**26.2)	128.7 **(**26.7)	-12.1 (29.6)	*p* = 0.1
REM sleep measures				
REM latency	65.6 **(**19.0)	69.5 **(**18.3)	3.9 (22.3)	*p* = 0.5
REM sleep	106.8 **(**26.4)	103.6 **(**20.9)	-3.2 (16.9)	*p* = 0.4

[a] Sleep parameters expressed in minutes unless stated otherwise; [b] Sleep efficiency % = actual sleep time/time in bed x 100; NREM: non-rapid eye movement; SWS: slow wave sleep; REM: rapid eye movement

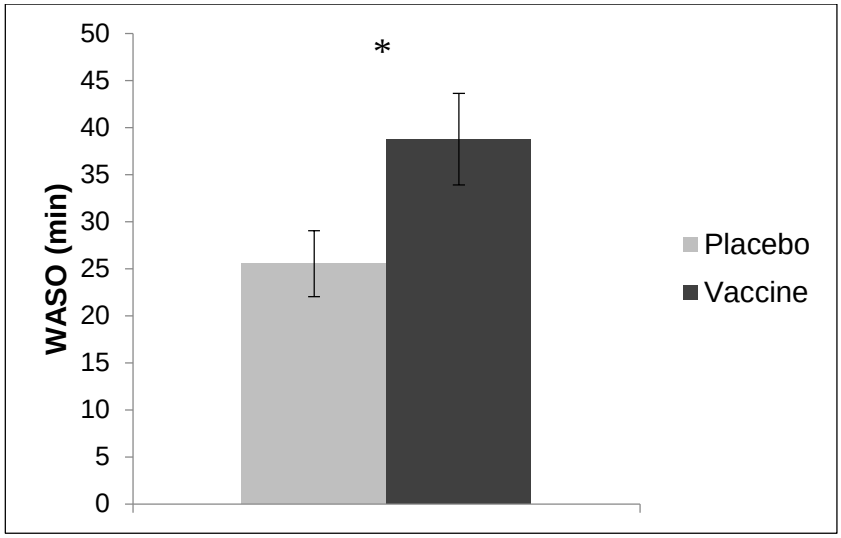


Figure 2. Wake after sleep onset (WASO) in minutes following placebo (light bar) and typhoid vaccine (dark bar). Values are mean values, with error bars representing SEM. *p = 0.007.

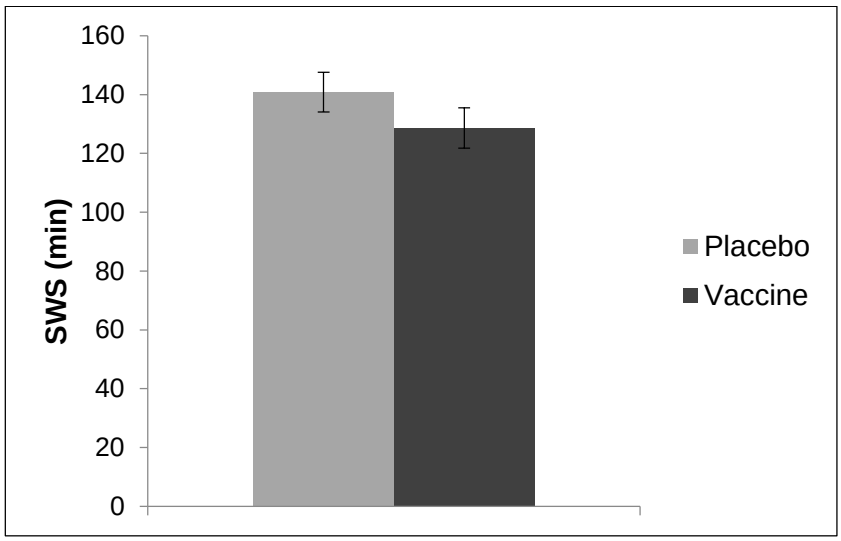


Figure 3. Slow wave sleep (SWS) in minutes following placebo (light bar) and typhoid vaccine (dark bar). Values are mean values, with error bars representing SEM. p >0.05.

4.4 Discussion

Typhoid vaccination significantly elevated IL-6 levels compared to placebo injection, thereby replicating the previous vaccine study presented within this thesis and previous reports in the literature (Harrison *et al.*, 2009a, Harrison *et al.*, 2014, Harrison *et al.*, 2015c). The increase was smaller than that seen in the previous study, as it was taken at an earlier time point, yet it is in line with previous measures at this time (Brydon *et al.*, 2008). This demonstrates that the typhoid vaccine model produced the intended mild systemic inflammatory response.

Typhoid vaccine did not affect mood compared to placebo; this replicated the findings from the previous chapter, and once more the breadth of visual analogue scales in particular would be expected to detect changes in subjective mood state if present. Similarly to the previous study, there were few adverse effects following the typhoid vaccine, hence results discussed here should not be due to physical sickness symptoms, such as nausea. Participants reported soreness around the injection site more following the vaccine compared to the placebo - though only a minority of participants experienced this soreness, therefore it is unlikely that significant sleep continuity effects observed are due to this symptom alone. It is also noteworthy that there were no effects of the vaccine on subjective reports of sleep (measured by the LSEQ). Although moderately good correlations have been reported in the literature between subjective sleep estimates and polysomnography (Ferrie *et al.*, 2013), subjective reporting of sleep efficiency and latency has been shown to be poorly correlated with polysomnography (Signal *et al.*, 2005). The finding here of objective sleep continuity changes in the absence of subjective changes suggests that at least when investigating acute inflammatory effects on sleep it is worth including objective measures.

4.4.1 Sleep Continuity and NREM Sleep

The current findings indicate that typhoid vaccine disrupted several sleep continuity parameters, by significantly reducing sleep efficiency and increasing WASO, as well as increasing total wake, sleep stage transitions, number of awakenings and awakening index compared to placebo. These findings may be relevant to depression, particularly since sleep continuity has been shown to be a state dependent measure in depression that can improve with antidepressant treatment (Kupfer and Ehlers, 1989, Weinberger *et al.*, 2015).

This is the first study to our knowledge to examine the effects of typhoid vaccination on sleep polysomnography, although effects of high doses of endotoxin have shown analogous disruption of sleep continuity in healthy subjects (Mullington *et al.*, 2000). Similar sleep continuity disruptions have also been shown following chronic administration of IFN-α. Indeed, IFN-α treatment significantly increased WASO, which was associated with higher evening plasma cortisol levels, and significantly decreased sleep efficiency compared to no treatment in patients with hepatitis C (Raison *et al.*, 2010c). This evidence is intriguing, since polysomnography revealed such effects after ~12 weeks of IFN-α treatment, whereas the sleep continuity impairments in the current study were produced following a single inflammatory stimulus. This suggests that the experimental inflammatory model in the present study is capable of mimicking deleterious effects of chronic cytokine treatment on sleep continuity, which may be linked to fatigue and psychomotor slowing in depression (Raison *et al.*, 2010c).

Further evidence has shown that improvements in sleep continuity measures can occur with the anti-inflammatory drug, infliximab, a TNF-α antagonist. Weinberger *et al.* (2015) measured sleep parameters using polysomnography in patients with treatment resistant depression before and after infliximab treatment or placebo. They found that infliximab

significantly improved sleep continuity measures, including decreased WASO and increased sleep efficiency, as well as decreased sleep period time in patients with high (CRP >5 mg/L, $n = 9$) compared to low (CRP ≤5 mg/L, $n = 10$) inflammation. Moreover, WASO reduction and sleep efficiency increases in patients with high inflammation correlated with soluble tumour necrosis factor receptor 1 (sTNFR1) (Weinberger *et al.*, 2015). Infliximab is a highly specific TNF antagonist, therefore the authors suggest that these data provide evidence that blockade of inflammation may improve sleep continuity in depressed patients, particularly patients with treatment resistance and high inflammation. Moreover, it should be noted that infliximab is not readily able to cross the blood-brain barrier owing to its large size (Raison *et al.*, 2013, Weinberger *et al.*, 2015); these data consequently suggest that infliximab may be able to modulate sleep parameters through anti-inflammatory effects in the periphery. In addition, sleep efficiency has been shown to improve with infliximab treatment in patients with rheumatoid arthritis (discussed in the next chapter), which was independent of improvements in joint symptoms (Zamarron *et al.*, 2004). Typhoid vaccination in the present study also activated peripheral inflammatory signals which appear to have modulated central mechanisms involved in sleep continuity. Together, such evidence supports the propensity for immune-brain communication (covered in Chapter 1) to influence central mechanisms involved in sleep continuity, and advocates further study into anti-inflammatory treatments for sleep impairments in depression and other chronic inflammatory illnesses.

No correlations between IL-6 response and sleep continuity measures were found in the present study, similar to the lack of association between IL-6 and behavioural measures reported in the previous chapter. The potential reason for this is discussed in the limitations section below, though sleep effects are likely to be due to inflammatory activity since there are comparative sleep data for the same participants following placebo injection. Significant

associations between inflammatory markers and sleep changes have been found elsewhere. For example, a longitudinal study in the London-based Whitehall II study found that reduced sleep duration is associated with higher levels of IL-6 and C-reactive protein (CRP) over a five year period (Ferrie *et al.*, 2013), and experimental sleep deprivation studies have determined that sleep disturbance is associated with increases in markers of systemic inflammation, including CRP and IL-6 (Haack *et al.*, 2007, Irwin, 2015, Irwin *et al.*, 2006, Meier-Ewert *et al.*, 2004). Further, in depressed patients sleep disturbance has been associated with increased IL-6 (Motivala *et al.*, 2005). Thus, although the current study was unable to report any correlations between IL-6 and sleep modification, results strongly suggest that effects observed were due to innate immune system activation and associations previously examined in the literature support the relationship between immune markers and sleep disruption.

In contrast to clear effects of typhoid vaccine on sleep continuity, the current study did not find any effects of mild inflammation on sleep architecture, including NREM SWS. SWS has been shown to be decreased in depression (Benca *et al.*, 1992, Riemann *et al.*, 2001), however reported effects of inflammatory modulation of SWS are variable. Exogenous IL-6 has been found to decrease SWS in the first part of the night and to enhance it in the second half of the night (Benedict *et al.*, 2009, Spath-Schwalbe *et al.*, 1998), and administration of IFN-α has been reported to suppress SWS (Raison *et al.*, 2010c, Spath-Schwalbe *et al.*, 2000); these observations support the ability of innate immune activity to cause NREM sleep disturbances in SWS that may be relevant to depression. Acute low doses of the pro-inflammatory cytokine IL-2, conversely, had no effect on sleep architecture in healthy males when given exogenously (Lange *et al.*, 2002), and no effects on SWS were observed following anti-inflammatory treatment with infliximab in depressed patients (Weinberger *et al.*, 2015). Varied effects of endotoxin administration have been reported; some studies have

not demonstrated modulation of SWS by endotoxin (Trachsel *et al.*, 1994), whereas Mullington *et al.* (2000) investigated dose-dependent effects of endotoxin on sleep and found that high doses of endotoxin increased wakefulness and reduced NREM sleep during the first half of the night (that was then enhanced in the latter part of the night). In contrast, a lower dose of endotoxin had no such effects on wakefulness or SWS, meanwhile the lowest dose enhanced sleep (Mullington *et al.*, 2000). These differential dose-dependent effects are interesting and mirror the discussion in the previous chapter regarding opposing effects of IL-6 concentrations on cognition. It seems plausible that typhoid vaccination did not promote a sufficiently substantial immune response required to modify SWS, though it would be interesting to examine how even low levels of inflammation may have deleterious effects over time.

In summary, NREM effects on sleep continuity measures appear to be well aligned in terms of reported effects of inflammation and impairments observed in depression, however effects on SWS remain comparatively ambiguous and require further delineation. Nonetheless, the current study adds to intriguing evidence that inflammatory mechanisms may be involved in sleep continuity disruption in some depressed patients.

4.4.2 REM Sleep

The current study did not find any effects of typhoid vaccination on REM sleep. Reduced REM latency is thought to be a biological marker of depression, although studies have suggested that disruptions in NREM sleep discussed above, particularly sleep continuity as well as reductions in SWS, may be more reliably altered in depression (Benca and Peterson, 2008, Raison *et al.*, 2010c, Thase, 2006). Mullington *et al.* (2000) found that endotoxin suppressed REM sleep and increased REM latency - an effect that was most prominent following the high dose of endotoxin, and one which is in keeping with earlier findings

(Bauer *et al.*, 1995, Korth *et al.*, 1996). The authors hypothesise the reduction in REM sleep may have been related to increased IL-6, as this has previously been shown to reduce REM sleep (Spath-Schwalbe *et al.*, 1998), although this mirrors effects previously observed with antidepressant treatments, not REM sleep alterations reported in depression (Tsuno *et al.*, 2005). IFN-α administration has also been found to increase REM latency, which was associated with enhanced fatigue (Raison *et al.*, 2010c), however this is also in opposition to decreased REM latency in major depression (Benca *et al.*, 1992, Riemann, 2007). Infliximab was not shown to change REM latency (Weinberger *et al.*, 2015), whereas etanercept, an alternative TNF antagonist, has been shown to induce short-term normalisation (i.e. reduction) of REM sleep in alcohol-dependent patients with high levels of REM sleep (Irwin *et al.*, 2009).

The absence of typhoid vaccine effects on REM sleep implies that mild activation of the innate immune system is insufficient to modify this sleep parameter, although the literature regarding inflammatory modulation of REM sleep in general lacks clarity, particularly when effects of inflammation are opposite to the nature of REM sleep aberrations in depressed patients, such as with IFN-α therapy. It is possible that differences in this sleep parameter may exist depending on depressive phenotypes; depression is a heterogeneous condition in which not all patients have elevated inflammatory profiles, so REM sleep in depressed patients without inflammation may differ to inflammatory-associated depression, including medically ill patients with depression (Raison *et al.*, 2010c). Currently, REM sleep characteristics in depressed patients with elevated inflammatory markers are under investigated. Further investigation of the differences between REM sleep associated with inflammation and depression therefore seems reasonable, particularly if it transpired to be possible to define clinical subgroups based upon REM phenotype (Raison *et al.*, 2010c).

4.4.3 Limitations and further work

The present study utilised an objective measure of sleep architecture, representing a significant strength of the study design over subjective measures used in many studies. A further strength is that this was a balanced order cross-over design study, and previous work has shown that sleep patterns are stable over time in healthy subjects using this methodology (Sharpley *et al.*, 1990). Home recordings also offer the advantage of enabling subjects to continue their normal evening routine, making them more likely to have a typical night's sleep (Sharpley *et al.*, 1990). However, there are some weaknesses of the study that warrant consideration. Firstly, blood sampling for cytokine analysis was taken two hours post-vaccination, which is several hours before participants went to sleep. As a result, the inflammatory status of participants during the night is unknown. This limitation likely accounts for the absence of correlations between IL-6 and sleep parameters in this study. As discussed in the previous chapter, increases in central cytokine levels may be greater 6-8 hours after inflammatory challenge (Konsman *et al.*, 1999, Paine *et al.*, 2013), which should represent an advantage of the current study design, however later blood samples would have been advantageous to support this suggestion. Furthermore, examination of a range of inflammatory markers could have been more informative, particularly TNF-α and IL-1β (Imeri and Opp, 2009, Krueger, 2008), and future studies should consider hypothalamic-pituitary-adrenal (HPA) axis activity effects on sleep (Imeri and Opp, 2009, Steiger, 2002). There is also evidence of gender differences in inflammation after sleep disturbance (Irwin *et al.*, 2010); thus it is possible that gender differences in sleep alterations following innate immune activation may exist, however this study was underpowered to distinguish effects in males and females reliably. Once more, as in the previous chapter, caution should also be taken when extrapolating findings in healthy subjects into patients with chronic illness.

Further work could aim to better define the bidirectional relationship between inflammation and sleep, including a feedforward cascade evidenced by the ability of chronic inflammation to impair sleep, which in turn can further increase inflammatory markers including IL-6 and CRP (Raison *et al.*, 2010c), as shown by sleep deprivation studies (Haack *et al.*, 2007, Meier-Ewert *et al.*, 2004, Vgontzas *et al.*, 2004). This represents a possible avenue for further investigation, perhaps following IFN-α therapy, that unfortunately was not assessed in the study presented in Chapter 2. In light of the current findings, it would also be interesting to examine whether sleep disruption following typhoid vaccine could lead to further heightened inflammation.

The present thesis provides intriguing evidence that pro-inflammatory cytokines are capable of modulating emotional processing, and the current study supports previous evidence that innate immune cytokines can alter sleep. Intriguingly, Cho *et al.* (2016) found that sleep disturbance appeared to increase affective sensitivity to pro-inflammatory cytokines induced by endotoxin in healthy females. Sleep deprivation studies have also shown that one night of sleep loss can impair processing of emotional facial expressions (Cote *et al.*, 2014, van der Helm *et al.*, 2010), enhance neural reactivity to threat-related negative emotions (Cote *et al.*, 2014) and reduce facial reactivity to emotional stimuli (Schwarz *et al.*, 2013). Furthermore, REM sleep deprivation has been found to enhance reactivity to threatening emotional stimuli (Rosales-Lagarde *et al.*, 2012). The findings presented in Chapter 2 following IFN-α administration were not in keeping with emotional processing impairments, instead the presence of negative biases in emotional processing were demonstrated, though it would have been intriguing to examine effects of IFN-α administration on sleep in that study. In future, therefore, it could be interesting to combine methodology employed within this thesis to investigate effects of sleep on emotional processing in the context of inflammation. The following chapter examined emotional processing in patients with RA,

a chronic inflammatory condition that is also commonly associated with sleep disturbance (Abad *et al.*, 2008).

Evidence in this chapter supports suggestions that sleep disturbance combined with an inflammatory stimulus might provide an interesting model that could be relevant to vulnerability factors in depression, referred to as a 'two-hit' model of depression by Cho *et al.* (2016). This is similar to the idea expressed in Chapter 3 that combining typhoid vaccination and psychological stressors might serve as a model of depression with potentially improved construct validity. Perhaps further work could investigate neuropsychological effects of interactions between vulnerability factors, such as sleep, inflammation and stress, since it appears that there is significant interplay and reciprocity between these pathways.

4.5 Conclusions

In conclusion, our findings indicate that an acute, mild inflammatory stimulus produces impairments in sleep continuity, including decreased sleep efficiency and increased wake after sleep onset. Decreased sleep continuity and efficiency are hallmarks of depression, therefore the present findings, combined with evidence from previous studies involving endotoxin and IFN-α administration, suggest that innate immune cytokine activation can disrupt sleep continuity in ways that may be pertinent to depression. It is plausible that deleterious effects of pro-inflammatory mechanisms on sleep may, over time, contribute to the onset and maintenance of depression, or at least that inflammatory mechanisms may play a role in sleep disturbance experienced by subgroups of depressed patients. Furthermore, previous studies provide promising data that anti-inflammatory treatment may improve sleep continuity, thereby suggesting that this could be a viable treatment target to improve sleep impairments that are thought to contribute substantially to depressive

symptomatology. Such mechanisms may also be relevant to a range of chronic medical illnesses, including patients with RA; the next chapter presents emotional processing data from patients with RA, a chronic inflammatory condition that is also commonly associated with impaired sleep.

Chapter 5

Investigating Emotional Processing in a Model of Chronic Inflammation in Patients with Rheumatoid Arthritis

5.1 Introduction

The previous chapters in this thesis investigated the effects of pro-inflammatory cytokines on emotional processing, as low-grade chronic inflammation is hypothesised to play a role in the pathogenesis of depression (Raison *et al.*, 2006). Administration of typhoid vaccination to provoke an acute immune response in healthy subjects had limited effects on emotional processing (Chapter 3), whereas chronic administration of IFN-α in patients with hepatitis C appeared to induce marked negative biases in emotional processing (Chapter 2). From a mechanistic perspective such studies are of great interest, however it is difficult to extrapolate these findings into patients with chronically elevated inflammatory states, thus deleterious effects resulting from chronic exposure to inflammation remain largely unclear.

Consistent with hypotheses regarding the link between inflammation and depression, depression is two to three times more common in chronic physical illnesses than in the general population (Abbott *et al.*, 2015), which has significant negative ramifications on quality of life, as well as increased mortality and morbidity (Ellis *et al.*, 2010, Katon *et al.*, 2005, Meijer *et al.*, 2011, Moussavi *et al.*, 2007). An example of a chronic medical illness that is frequently associated with depression is rheumatoid arthritis (RA), an autoimmune disease characterised by chronic inflammation of multiple joints and high levels of circulating pro-inflammatory markers (Cavanagh *et al.*, 2010, Iaquinta and McCrone, 2015, Ozkan *et al.*, 2012).

RA afflicts 0.3-1.0% of the global population (Sheehy *et al.*, 2006, Taylor *et al.*, 2016), the average age of diagnosis is 66.8 years and females are afflicted 2-3 times more commonly

than males (CDC, 2012, Iaquinta and McCrone, 2015). Conservative estimates suggest that depression occurs in approximately 13-17% patients with RA (Cavanagh *et al.*, 2010, Dickens *et al.*, 2002), though some studies have suggested the prevalence of depressive symptomatology can exceed 40% (Bruce, 2008, Isik *et al.*, 2007, Matcham *et al.*, 2013).

Depression in RA contributes substantially to the burden of illness, it can result in poorer health outcomes, interfere with treatment adherence, enhance pain perception and negatively impact patients' perception of and ability to cope with their illness (CDC, 2012, Dickens and Creed, 2001, Iaquinta and McCrone, 2015, Rathbun *et al.*, 2013, Sheehy *et al.*, 2006). Both the physical and psychological impacts of RA are well-documented, however the link between depression and RA is yet to be fully elucidated. Although there are several potential contributing factors to depression in RA, including physical symptoms like pain and fatigue (Dickens *et al.*, 2002, Sheehy *et al.*, 2006), functional disability and socioeconomic status (Margaretten *et al.*, 2011), neuroimmune pathways are repeatedly suggested to play a key role (Dantzer *et al.*, 2008b, Kojima *et al.*, 2009, Low *et al.*, 2009).

As mentioned in Chapter 1, pro-inflammatory cytokines (e.g. IL-6, TNF and IL-1) and C-reactive protein (CRP) are the most heavily investigated biomarkers of elevated inflammation in patients with depression (Dowlati *et al.*, 2010, Howren *et al.*, 2009). Biomarkers of systemic inflammation are also elevated in RA, and CRP levels have been shown to be positively associated with depressive symptoms in RA patients (Kojima *et al.*, 2009), although some evidence conflicts the association between CRP and depression in RA (Low *et al.*, 2009). Nonetheless, RA may provide a useful chronic inflammatory disease model in which to investigate the relationship between elevated pro-inflammatory cytokines and depressive symptoms (Liu *et al.*, 2012b), and for the purposes of this thesis the effects of chronic inflammation on emotional processing, which has not previously been studied in RA patients to our knowledge.

5.1.1 Aims

The aims of the current exploratory study were to examine differences in emotional processing measurements in relation to levels of inflammation in patients with a chronic inflammatory condition. Previous hypotheses in this thesis stated that pro-inflammatory states would cause negative biases in emotional processing, and suggested that when inflammation persisted chronically, deleterious effects on emotional processing could become more marked. The hypothesis of the present study, therefore, was that greater inflammation in patients with RA would correlate with more pronounced negative biases in emotional processing. Following enhanced disgust recognition effects induced by IFN-α administration, we further hypothesised that there would be a specific effect on affective processing of disgusted faces.

5.2 Methods

5.2.1 Participants and study design

40 patients (32 females, 8 males) were recruited through a National Health Service RA clinic at the Nuffield Orthopaedic Centre (Oxford, UK). This study was appended to an ongoing study at the Botnar Research Centre, within the Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, led by Professor Peter Taylor. Patients were therefore recruited and consented by rheumatology research nurses to take part in this sub study, which took place on the same day as the main study. This exploratory study was intended to be naturalistic, investigating associations between inflammation and emotional information processing, therefore strict inclusion criteria were not applied and patients with a history of depression were not excluded. Testing within the sub study was also kept to under one hour in duration, so as not to be too tiring for participants.

Patients had confirmed diagnoses of RA and were all receiving therapies as part of their routine clinical care. The following medications were taken by participants: disease-modifying antirheumatic drugs (DMARDs) methotrexate (*n*=11), hydroxychloroquine (*n*=13) and leflunomide (*n*=2); biologic therapies including anti-TNF drugs etanercept (*n*=1) and IL-6 receptor inhibitor tocilizumab (*n*=2); corticosteroid treatment prednisolone (*n*=1); and non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen (*n*=2), naproxen (*n*=2) and diclofenac (*n*=1). Other medications or supplements included folic acid (*n*=8), omeprazole (*n*=4), lansoprazole (*n*=1), cyclizine (*n*=1), codeine (*n*=1), paracetamol (*n*=2), tramadol (*n*=1), ramipril (*n*=2), mebeverine (*n*=1), bendroflumethiazide (*n*=1), thyroxine (*n*=1), fexofenadine (*n*=1) and salbutamol (*n*=1). Three participants had a history of clinical depression (>10 years ago) and were not currently receiving antidepressant treatment. Further information was collected including age, verbal IQ assessed using the National Adult Reading Test (NART) (Nelson, 1982) and duration of illness.

The study received full ethical approval by the NRES Committee South West - Frenchay and was overseen by the Oxford University Clinical Trials and Research Governance team and sponsored by the University of Oxford. All participants gave written informed consent prior to study procedures being performed.

Emotional processing in RA has not, to our knowledge, been assessed previously, therefore ethics approval was granted for recruitment of 40 patients based upon previous power calculations (Chapter 3). Unfortunately, of the 40 patients recruited into this study, 19 patients' data were removed from the current analyses which may mean that the current study was underpowered. Exclusions were for the following reasons: nine patients who were over 70 years old were excluded due to potential confounds of age effects on cognition; two patients were excluded due to current antidepressant use, which has been shown to modulate emotional processing (e.g. Harmer *et al.*, 2004); one patient was not computer

literate and found the computer tasks too difficult; one patient was visually impaired; two patients were unable to complete testing due to swollen and painful joints of the hands; two further patients were excluded as they were medically unwell during the testing period. This left only two male participants, thus the decision was made to include only female participants; as a result, 21 patients have been included in the present data, all of whom were female.

5.2.2 Subjective mood questionnaires

Participants completed subjective mood questionnaires employed within previous studies in this thesis; the Beck Depression Inventory (BDI) (Beck *et al.*, 1961); Positive and Negative Affective Schedules (PANAS) (Watson *et al.*, 1988); State Trait Anxiety Inventory (STAI) (Spielberger *et al.*, 1970) and visual analogue scales (VAS, measuring happy, sad, hostile, alert, anxious and calm).

5.2.3 Inflammatory marker (C-reactive protein)

Following completion of psychological tasks, blood samples were taken as part of the main study that were later analysed for C-reactive protein (CRP) for use in the sub study presented here. CRP is an acute phase reactant protein produced by hepatocytes that rises in response to inflammation (IL-6 and IL-1; Dantzer, 2012, Kojima *et al.*, 2009) and is thus a widely used general marker of inflammation (Lopresti *et al.*, 2014). As discussed in Chapter 1, CRP has been shown to be elevated in depressed patients compared to healthy controls (reviewed by Valkanova *et al.*, 2013). Indeed, CRP appears to have the largest body of evidence for associations with depression to date, including risk factors and treatment outcomes (Howren *et al.*, 2009, Raison *et al.*, 2013, Uher *et al.*, 2014, Wium-Andersen *et al.*, 2014). High CRP levels have also been associated with cognitive impairments in a range

of studies (Chang *et al.*, 2012, Dickerson *et al.*, 2007, Dickerson *et al.*, 2013, Roberts *et al.*, 2009).

For the purposes of this study, to examine differences in emotional processing in patients with low vs high inflammation, participants were split into two groups based on CRP levels; a low CRP group with CRP measurements < 3 mg/L and a high CRP group with CRP measurements > 3 mg/L. 3 mg/L CRP was chosen since this is a widely accepted cut-off point for high inflammation across a range of diseases, including depression (Miller *et al.*, 2013, Miller and Raison, 2015, Pearson *et al.*, 2003, Ridker, 2003, Uher *et al.*, 2014) . Raison *et al.* (2013) also reported that 45% of patients with non-response to conventional antidepressants displayed a CRP > 3 mg/L, and inflammation above this standard cut-off has been associated with altered reward circuitry in depression (Felger *et al.*, 2015b); this is discussed further in Chapter 7.

5.2.4 Emotional test battery

Following subjective mood questionnaires, participants completed the emotional test battery described in full in Chapter 2 – consisting of the facial expression recognition task (FERT), emotional categorisation, emotional memory and dot probe tasks, but not including the emotional recognition task due to time constraints.

5.2.5 Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics (version 22) using methodology outlined in previous chapters. Extreme outlying data (data lying at more than three times the participants' interquartile range above their third or below their first quartile) were removed from all psychological tasks. This resulted in minimal data (<2%) being lost as outliers on any task. CRP, demographic characteristics, mood measures and the emotional memory task were analysed using one-way ANOVAs. Data from the facial

expression recognition task, emotional categorisation task and dot probe task were analysed using repeated measures ANOVA, with inflammation group as the between-subject factor and valence as the within-subject factor. In the dot probe task, masking was added as an additional within-subject factor. Statistically significant interactions were followed up using simple main effects analyses. Where assumptions of equality of variances were broken, Greenhouse-Geisser procedure was used to correct the degrees of freedom. Data from each task were analysed separately, without correcting for multiple comparisons across tasks.

Correlational analysis was performed to assess the relationship between CRP and behavioural measures across all RA patients; Pearson's correlation coefficient was computed between CRP values (in mg/L) and neuropsychological task performance. Associations between CRP and subjective mood were also assessed in this way.

5.3 Results

5.3.1 Participant characteristics and subjective state

Participant demographics and subjective mood state are shown in Table 1. Two participants in the high CRP group had a history of clinical depression, compared to one participant in the low CRP group. The average duration of illness was 3.76 ± 1.59 years, with no significant differences between the two groups (p=0.11). As shown in Table 1, there were no significant between-group differences in depressed mood scores or pain score (p values >0.1). Furthermore, there were no correlations between CRP and BDI (r = 0.045, p = 0.85).

Medications that patients were receiving at the time of testing were detailed in the Methods section above, however it is useful to assess whether there were imbalances in RA treatments between the low and high inflammation groups. Table 2 shows DMARDs, biologic therapies, corticosteroid and NSAIDs taken by participants in each group. On the whole, the groups appear to be balanced, with the exception of biologic treatments as there were

three patients in the low inflammation group receiving biologic therapies, compared to no patients in the high inflammation group. However, this represents a minority of patients in both groups, so the difference in biologic treatments should not account for group differences in emotional processing tasks described below, although the possibility of differences in medications contributing in some way to group differences cannot be excluded.

Table 1. Demographic and subjective mood measures. Means (SE).

	Low CRP Group (*n*=12)	**High CRP Group (*n*=9)**	**Statistical significance**
Age	43.58 (5.01)	50.56 (6.93)	$p = 0.41$
Verbal IQ	121.73 (1.92)	116.36 (9.11)	$p = 0.15$
Cigarettes / day	2.27 (1.56)	3.00 (2.04)	$p = 0.78$
Alcohol units / week	9.27 (3.74)	2.12 (1.01)	$p = 0.13$
BDI	8.25 (1.47)	9.72 (2.11)	$p = 0.56$
Trait anxiety	34.92 (2.34)	38.63 (3.70)	$p = 0.38$
State anxiety	29.42 (2.43)	37.89 (3.62)	$p = 0.06$
PANAS: Positive	31.80 (2.36)	30.63 (4.17)	$p = 0.80$
PANAS: Negative	12.30 (1.37)	13.63 (2.51)	$p = 0.63$
VAS: Happy	63.68 (7.61)	66.33 (29.71)	$p = 0.83$
VAS: Sad	19.45 (9.44)	25.44 (12.01)	$p = 0.70$
VAS: Hostile	3.41 (1.60)	7.78 (5.88)	$p = 0.44$
VAS: Alert	72.36 (9.17)	55.00 (10.21)	$p = 0.22$
VAS: Anxious	17.59 (8.98)	13.44 (5.92)	$p = 0.72$
VAS: Calm	73.00 (10.26)	51.22 (13.51)	$p = 0.21$
Pain (VAS)	28.92 (7.39)	28.89 (7.85)	$p = 1.00$

BDI: Beck Depression Inventory; PANAS: Positive and Negative Affective Schedules; VAS: Visual Analogue Scales.

Table 2. RA medications taken by patients at the time of neuropsychological testing. Values represent numbers of patients taking each type of medication.

	Low CRP Group (*n*=12)	**High CRP Group (*n*=9)**
Methotrexate	6	5
Hydroxychloroquine	7	6
Leflunomide	2	0
Etanercept	1	0
Tocilizumab	2	0
Corticosteroids	1	0
NSAIDs	2	3

Disease-modifying antirheumatic drugs (DMARDs): methotrexate, hydroxychloroquine and leflunomide. Biologic treatments: etanercept and tocilizumab. NSAIDs: non-steroidal anti-inflammatory drugs.

5.3.2 C-reactive protein (CRP)

Patients were separated into a low CRP group (< 3 mg/L, *n*=12, mean CRP 0.70 ± 0.18 mg/L) and a high CRP group (> 3 mg/L, *n*=9, mean CRP 14.73 ± 6.05 mg/L) based on blood samples taken on the testing day. As can be seen, RA patients in the high CRP group have a much greater mean CRP level than the cut-off point, hence these values represent very high inflammation. The difference in terms of CRP levels between the two groups were significant ($F(1,20)=7.28$, $p=0.014$). The following emotional processing data are shown comparing these two groups, and correlations between CRP and emotional processing measures are reported.

5.3.3 Emotional test battery

5.3.3.1 *Facial expression recognition*

There was a main effect of inflammation group on accuracy in this task (see Figure 1; $F(1,17)=9.59$, $p=0.007$), whereby the high inflammation group were significantly less

accurate at recognising emotional facial expressions compared to the low inflammation group. However, there was no interaction between inflammation group and emotion (F(2.92, 49.62)=1.38, p=0.26). There was, as expected, a main effect of emotion (F(2.92, 49.62)=27.51, p<0.001). Post-hoc analysis revealed that accurate recognition of angry (F(1,20)=11.54, p=0.003) and sad (F(1,20)=6.31, p=0.021) faces were significantly reduced in the high inflammation group, and there were trends towards lower recognition of disgusted (F(1,19)=3.87, p=0.065) and fearful (F(1,19)=3.57, p=0.075) faces in this group. Whereas, no significant differences between groups were found for recognition of happy (F(1,20)=0.55, p=0.47) and surprised (F(1,20)=1.14, p=0.30) faces. This observation prompted the grouping of negative and positive emotions, as shown in Figure 2, which shows that there were no significant differences in positive emotion recognition (F(1,20)=1.51, p=0.23), yet there were significant differences in negative emotion recognition (F(1,20)=10.72, p=0.004). Discriminability index d' also showed a main effect of group (F(1,17)=9.15, p=0.008), but no emotion x group interaction (F(5,85)=1.14, p=0.35). Post-hoc analysis revealed that accurate recognition (d') of angry (F(1,20)=12.13, p=0.002), fearful (F(1,19)=5.60, p=0.029 and sad (F(1,20)=6.31, p=0.021) faces were significantly reduced in the high inflammation group, and there were trends towards lower recognition of disgusted (F(1,19)=4.09, p=0.058) faces in this group. No significant differences between groups were found for recognition of happy (F(1,20)=0.88, p=0.36) and surprised (F(1,20)=1.55, p=0.23) faces. Conservative beta analysis, in contrast, revealed no main effect of inflammation group (F(1,17)=0.81, p=0.38), nor an emotion x group interaction (F(2.88,49.03)=1.06, p=0.37), which indicates that the two groups did not demonstrate differences in conservative response styles for emotional faces in this task.

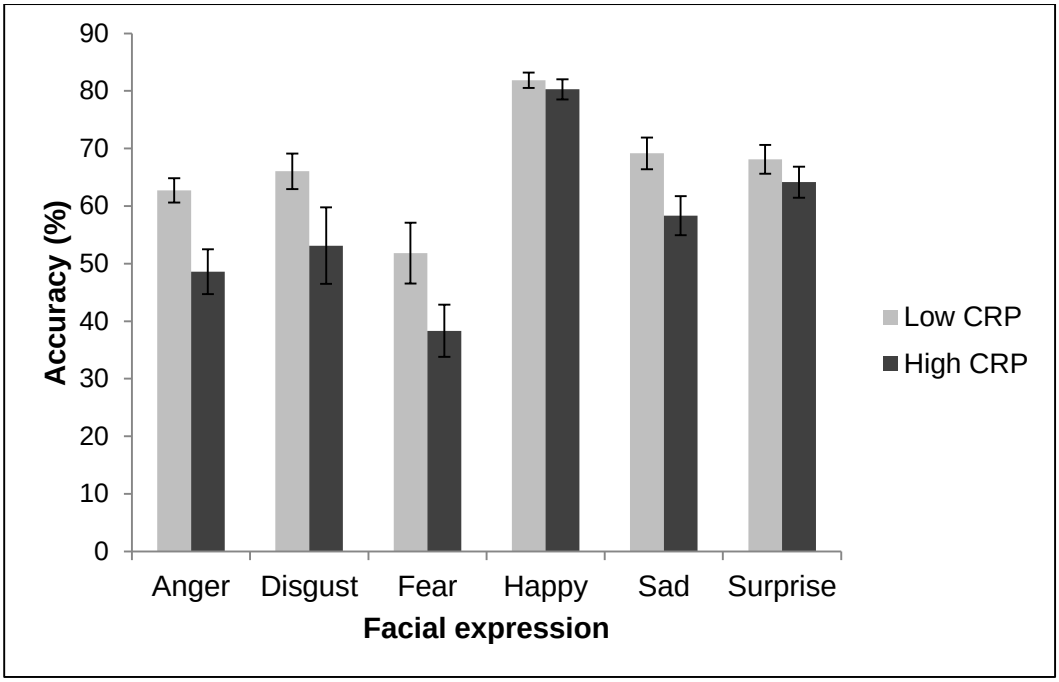


Figure 1. Performance in the facial expression recognition task in the low inflammation (light bars) and high inflammation (dark bars) groups. Values represent the mean percentage correct for each of the six basic emotions summed over the different intensity levels used in this task, with error bars representing standard error of the mean (SEM). Main effect of group: p=0.007.

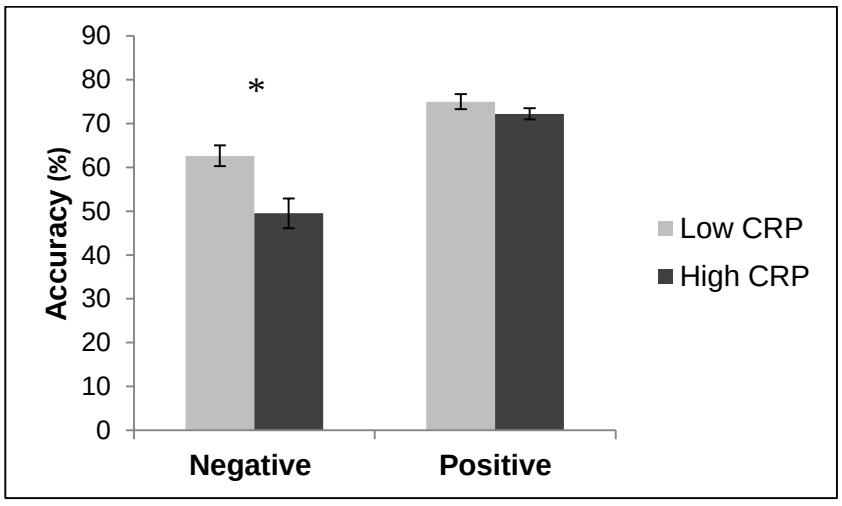


Figure 2. Performance in the facial expression recognition task in the low inflammation (light bars) and high inflammation (dark bars) groups for negative and positive emotions. Values represent the mean percentage correct summed for negative (anger, disgust, fear and sad) compared to positive (happy and surprise) valences over the different intensity levels used in this task, with error bars representing SEM. *p = 0.004.

Correlational analysis was conducted with CRP levels and accuracy in this task across all patients, which supported between-groups effects described above, as higher CRP levels were significantly associated with reduced recognition of all negative emotions (anger r = -0.57, p = 0.007; disgust r = -0.60, p = 0.005; fear r = -0.47, p = 0.035; sadness r = -0.60, p = 0.004; see Figure 3), but not positive emotions (happy r = -0.29, p = 0.2; surprise r = -0.38, p = 0.09) or neutral faces (r = 0.20, p = 0.39). Out of interest, VAS pain scores were also assessed for correlations with accuracy on this task using the same methodology, however there were no significant correlations (p values >0.1).

The threshold at which each emotion was accurately detected by the two groups was also analysed to explore this difference in recognition accuracy in more depth. There was a significant main effect of group (F(1,19)=7.84, p=0.011); as shown in Figure 4, the high CRP group appear to have generally higher thresholds for accurately recognising emotional faces. However, there was no emotion x group interaction (F(3.26,61.91)=0.94, p=0.43). There was a main effect of emotion (F(3.26,61.91)=21.94, p<0.001), as expected. Overall, these data support the notion that higher CRP was associated with reduced recognition of facial expressions, perhaps largely negative facial cues. Significant positive correlations were also found between CRP and anger (r = 0.58, p = 0.006), disgust (r = 0.43, p = 0.049) and sad (r = 0.66, p = 0.001) thresholds, but not for other emotions (p values >0.1).

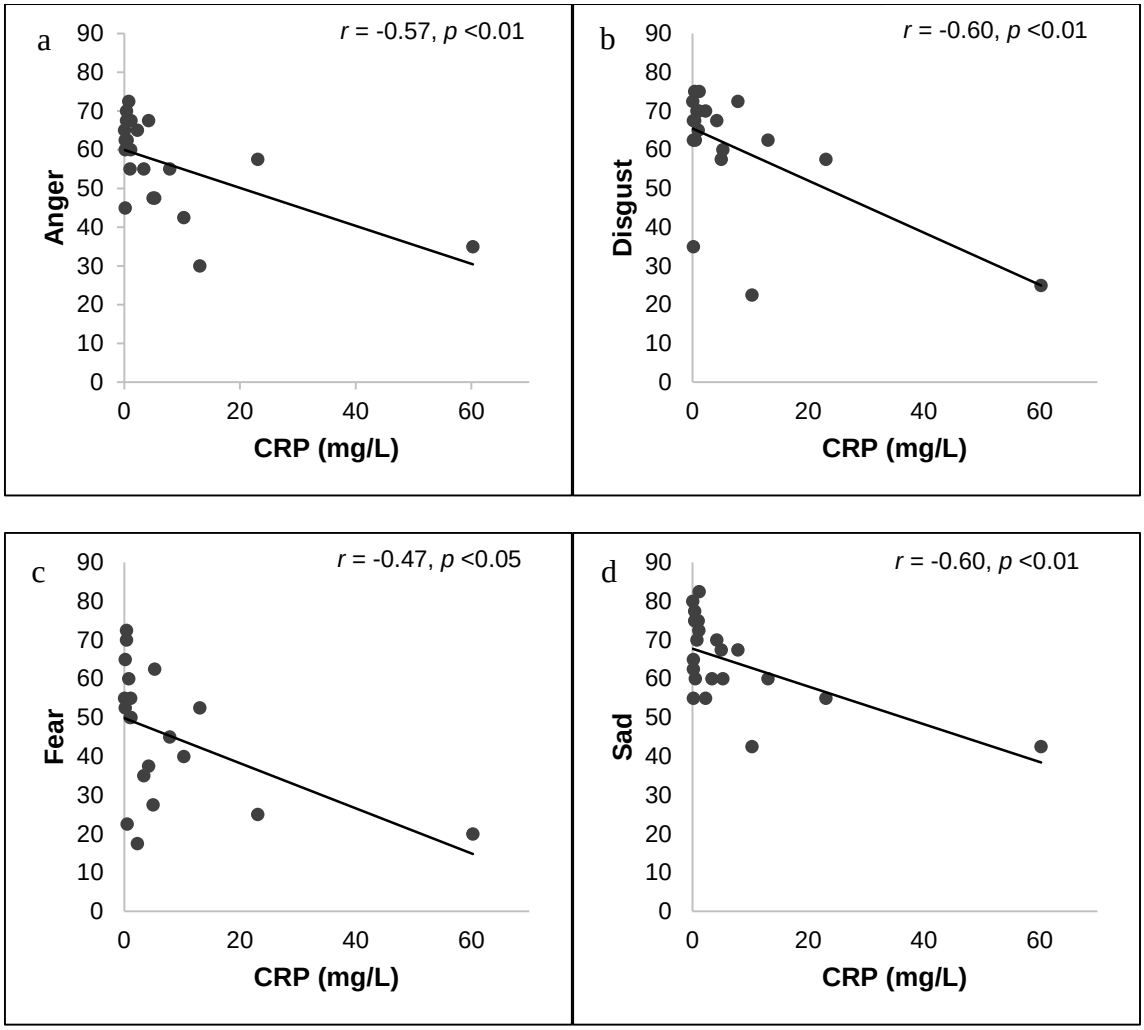


Figure 3. Correlation between C-reactive protein (CRP) levels and mean accuracy in the facial expression recognition task for negative emotional faces; a) anger, b) disgust, c) fear, d) sad.

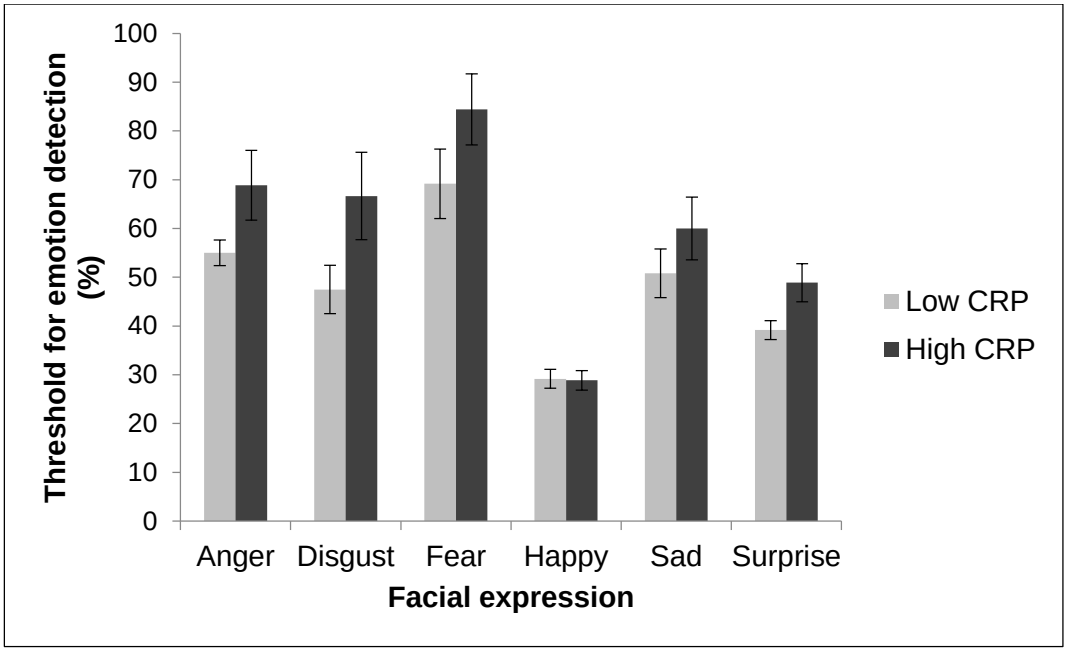


Figure 4. Threshold for accurate detection of facial expressions in the low inflammation (light bars) and high inflammation (dark bars) groups. Values represent mean percentage of thresholds, at which emotional expressions were accurately identified. Error bars show SEM. Main effect of group: $p = 0.011$.

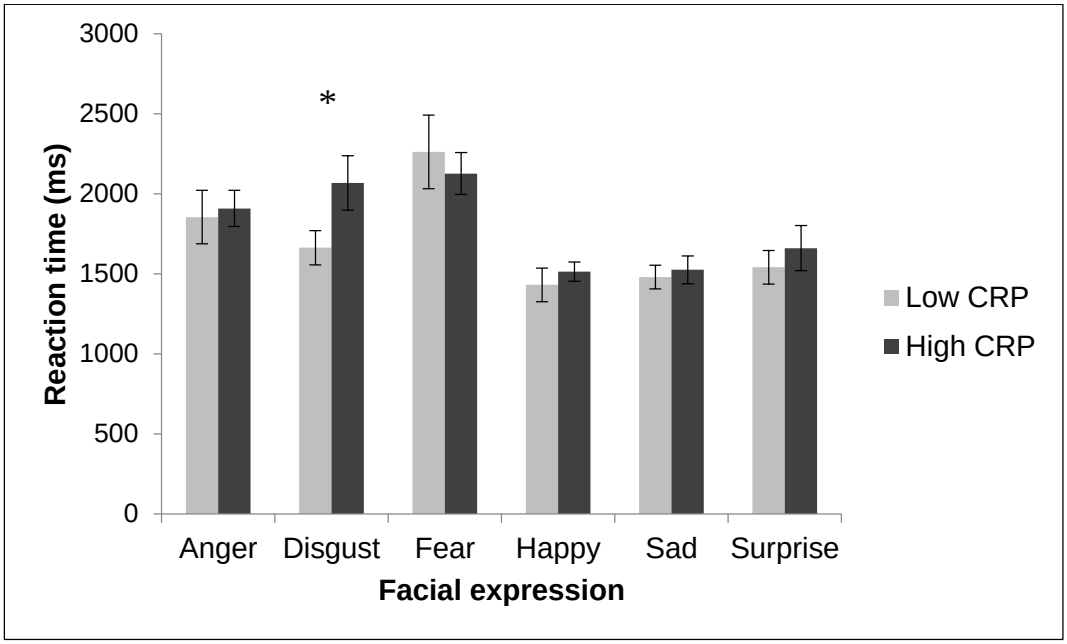


Figure 5. Reaction times for accurate detection of facial expressions in the low inflammation (light bars) and high inflammation (dark bars) groups. Values represent mean values, with error bars representing SEM. * $p = 0.05$.

There was no main effect of group in terms of reaction times in this task ($F(1,19)=0.41$, $p=0.53$) or an emotion x group interaction ($F(2.83,53.8)=1.58$, $p=0.21$). However, Figure 5 shows that reaction times for accurate recognition of disgusted faces appeared to be different. Indeed, post-hoc analysis revealed that the high inflammation group had significantly slower reaction times for this emotion compared to the low inflammation group ($F(1,20)=4.43$, $p=0.049$). No differences in reaction times for other emotions were found (all p values >0.4), thus this represents a specific effect on speed to identify disgusted facial expressions. Correlational analysis, however, found no significant association between CRP and reaction times to disgusted faces ($r = 0.30$, $p = 0.18$). CRP was positively associated with reaction times to surprised faces ($r = 0.54$, $p = 0.011$) and there was a trend association with sad faces ($r = 0.41$, $p = 0.064$). Reaction times to other emotional faces were not associated with CRP (p >0.1).

There was also no main effect of group on misclassifications in this task ($F(1,19)=0.25$, $p=0.63$) or an emotion x group interaction ($F(2.59,49.3)=0.55$, $p=0.62$). As expected, there was a main effect of emotion ($F(2.59,49.3)=7.17$, $p=0.001$). Correlational analysis found that CRP positively correlated with anger misclassifications ($r = 0.54$, $p = 0.012$), and there was a trend between higher CRP and more fear misclassifications ($r = 0.39$, $p = 0.077$), however there were no associations between CRP and any other emotion (p values >0.4).

5.3.3.2 *Emotional categorisation*

There were no between-group differences in accuracy to categorise self-referent personality characteristics in terms of an effect of group ($F(1,17)=0.12$, $p=0.73$; see Figure 6) or emotion x group interaction ($F(1,17)=0.19$, $p=0.67$). Similarly, there were no between-group differences in reaction times to categorise self-referent personality words (main effect of

group $F(1,19)=0.009$, $p=0.93$, emotion x group $F(1,19)=0.08$, $p=0.79$, main effect of emotion $F(1,19)=3.52$, $p=0.08$). No significant correlations were found between CRP and negative ($r = 0.19$, $p = 0.43$) or positive ($r = 0.21$, $p = 0.40$) accuracy in this task, nor reaction times (p values >0.1).

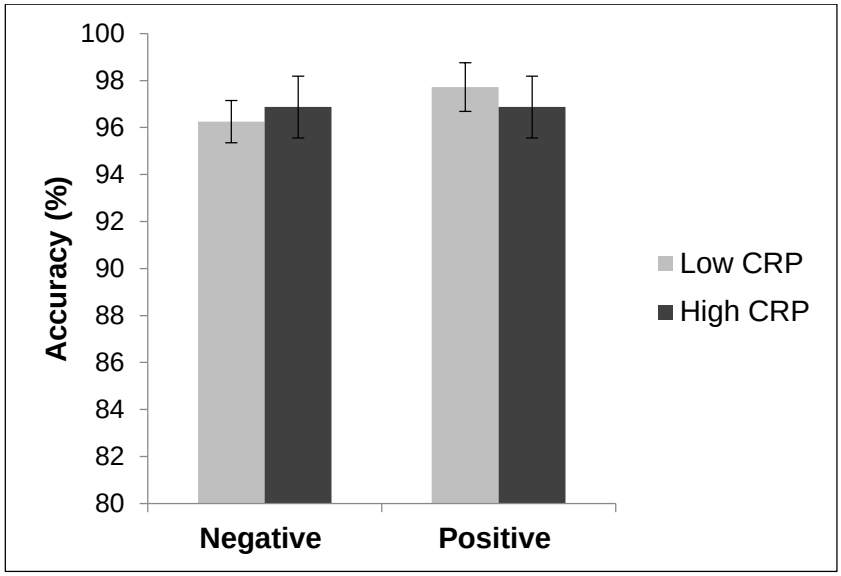


Figure 6. Accuracy in the emotional categorisation task in the low inflammation (light bars) and high inflammation (dark bars) groups. Values represent the mean percentage correct categorisation of positive and negative personality characteristic words used in this task, with error bars representing SEM.

5.3.3.3 *Emotional memory*

The total number of correct words recalled did not differ significantly between inflammation groups ($F(1,19)=1.61$, $p=0.22$), although there was a trend towards reduced total recall in the high inflammation group compared to the low inflammation group ($F(1,19)=4.0$, $p=0.06$; see Figure 7). There were no significant valence-specific differences in accurate recall of positive or negative valence words or false intrusions (p values >0.1).

Correlational analysis revealed significant negative associations between CRP and both total correct recall (r = -0.44, p = 0.049) and total recall (r = -0.51, p = 0.019), however CRP levels were not significantly associated with valence specific effects on recall or false alarms (p >0.1).

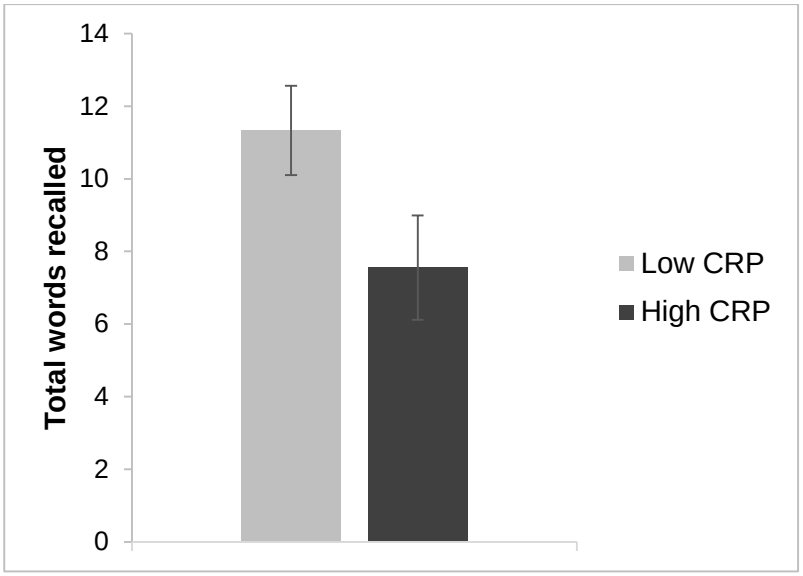


Figure 7. Total recall of personality characteristics in the low inflammation (light bars) and high inflammation (dark bars) groups. Values represent the mean number of trait words recalled, with error bars representing SEM.

5.3.3.4 *Dot probe attentional vigilance*

There was no mask x emotion x group interaction (F(1,19)=2.37, p=0.14), including mask as a within-subject factor. When masked and unmasked trials were considered separately, no emotion x group interaction was found in the unmasked condition (F(1,19)=0.37, p=0.54), though there was a trend emotion x group interaction in the masked condition (F(1,19)=3.97, p=0.061). Post-hoc analysis revealed that this trend was largely driven by a trend towards reduced vigilance to fearful faces in the high vs low CRP group (one-way ANOVA F(1,19)=3.22, p=0.089), which can be seen in Figure 8. Mean reaction times were

added as a covariate in an attempt to control for potential differences in motor function between the high and low inflammation groups, however this revealed no significant effects (all *p* values >0.1). Correlational analysis found a significant negative correlation between CRP and fear vigilance in the masked condition (r = -0.61, p = 0.004), yet there were no associations with fear vigilance in the unmasked condition, or happy vigilance across both conditions (*p* values >0.2).

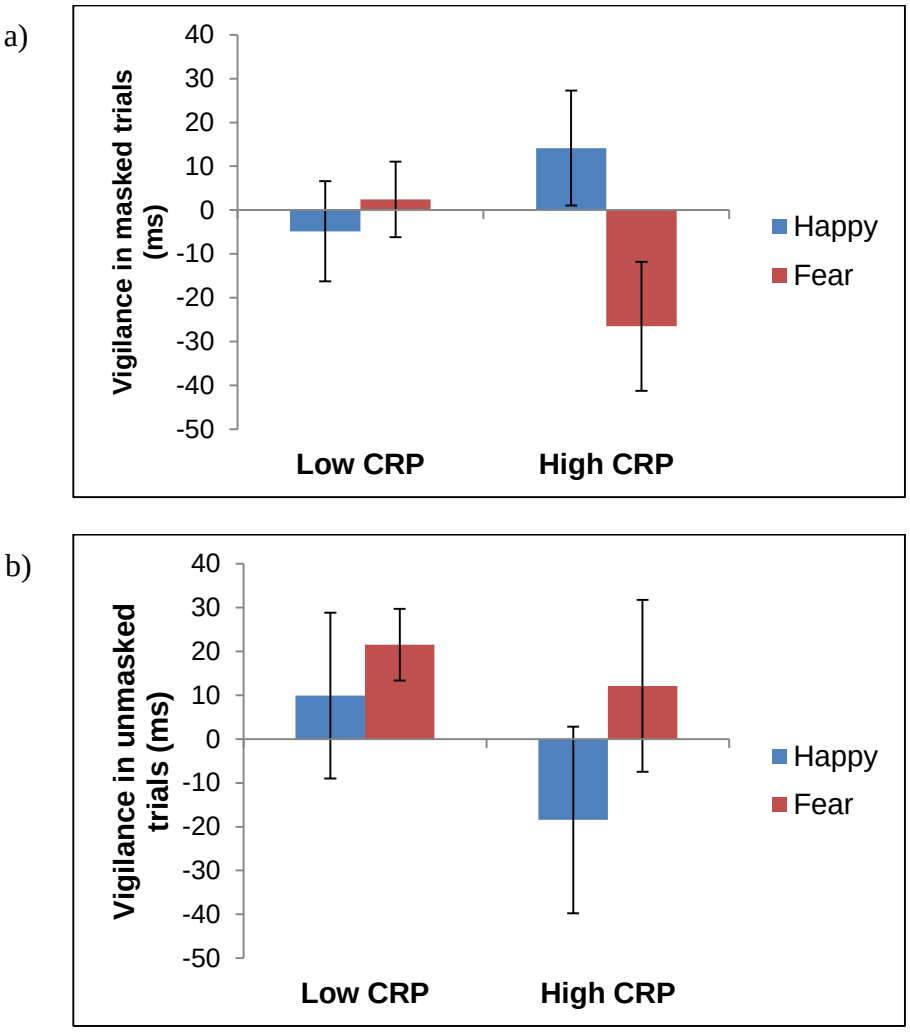


Figure 8. Effects of inflammation on attentional vigilance for happy and fearful facial expressions in the a) masked and b) unmasked conditions of the dot probe task. Attentional vigilance scores were calculated by subtracting the median reaction time from congruent trials (when the probe appeared in the same position as the emotional face) from incongruent trials (when the probe appeared in the opposite position to the emotional face, i.e. in the

position of the neutral face). A positive score indicates vigilance towards the emotional face, whereas a negative score reflects avoidance of the emotional face. Error bars show the SEM.

5.4 Discussion

The current study aimed to examine whether differential levels of inflammation in patients with a chronic inflammatory condition, RA, affected emotional processing, and to see whether effects could be likened to previous findings presented in Chapters 2 and 3. In the facial expression recognition task higher inflammation in the present study was associated with reduced accuracy in recognising emotional faces and post-hoc analysis revealed a valence specific effect of impaired recognition of negative, but not positive, emotional faces. In keeping with this observation, higher inflammation increased thresholds to detect emotional faces at a group level and CRP was associated with elevated thresholds to detect angry, disgusted and sad faces in particular. Reaction times appeared to be selectively slower for disgusted faces in the high inflammation group compared to the low inflammation group, however no significant correlation was found between this measure and CRP across all subjects. There were few effects on misclassifications in this task, except a correlation was found between elevated inflammation and increased misclassifications of angry faces. There were no effects of inflammation on emotional categorisation. Additionally, no valence specific effects were found in emotional memory, although there appeared to be an association between higher inflammation and reduced recall in general. Inflammation had limited effects on attentional vigilance in this patient cohort, as the only significant finding was an association between increased inflammation and reduced vigilance to fearful faces in the masked condition of the dot probe task. Effects observed in the present study are

therefore not in keeping with previous findings within this thesis in a number of ways, which will be discussed below.

Facial expression recognition findings here are contrary to effects found following IFN-α treatment, where inflammation was associated with enhanced disgust recognition, and they are also in opposition to enhanced false alarm responses to disgusted faces following typhoid vaccination. Together, the IFN-α and typhoid vaccine studies suggested enhanced sensitivity to disgusted faces resulted from immune system activation, however CRP in the current study was not associated with increased sensitivity to disgusted facial stimuli. The only specific effect with disgusted faces in the current study, without effects on other emotions, was the finding that the high inflammation group displayed slower reaction times to disgusted faces. This could be suggestive of an effect on processing of disgust, although CRP and latency to respond to disgusted faces were not significantly correlated, and this is one of many comparisons, therefore this was not a robust finding.

Contrary to specific effects on enhanced processing of disgusted faces, the current study found that high inflammation in RA patients was associated with impaired recognition of emotional faces, particularly stimuli with a negative valence. Whilst this is the first study to our knowledge to assess emotional processing in RA, deficits in facial expression recognition have previously been seen in depressed patients (Dalili *et al.*, 2015, Leppanen, 2006, Persad and Polivy, 1993, Rubinow and Post, 1992). Moreover, impairments in recognising facial emotions have been previously seen in patients with a range of chronic medical illnesses. For example, patients with multiple sclerosis have shown deficits in facial expression recognition compared to healthy controls (Berneiser *et al.*, 2014, Henry *et al.*, 2009, Phillips *et al.*, 2011). Berneiser *et al.* (2014) found that cognitive impairments were associated with depression and fatigue in this patient cohort (Berneiser *et al.*, 2014). They did not examine inflammatory markers, though multiple sclerosis is an immune mediated

disease of the central nervous system, characterised by chronic neuroinflammation (Patejdl *et al.*, 2016). Additionally, patients with chronic human immunodeficiency virus (HIV) infection have shown facial emotion recognition deficits, which correlated with more pronounced psychosocial impairments, and were suggested to result from disturbances in frontostriatal and cortico-limbic functioning (Clark *et al.*, 2010). HIV-related neuropsychological impairments have also been suggested to be linked to neuroinflammatory and neurodegenerative processes (Clark *et al.*, 2010). In addition, Clark *et al.* (2008) found facial expression impairments in patients with Parkinson's disease, and no differences in a control task between patients and healthy controls, suggesting specific effects on affective processing. Further studies have also found deficits in facial emotion recognition in Parkinson's disease (Dujardin *et al.*, 2004), including specific disgust impairments (Suzuki *et al.*, 2006), and deficits in recognising anger, fear and disgust in Huntington's disease (Sprengelmeyer *et al.*, 1996) – both of which are neurodegenerative conditions associated with neuroinflammation (Clark and Kodadek, 2016).

A central theme in such studies in chronic medical illness is the hypothesised link between reduced emotion recognition and decreased social functioning, as accurate recognition of facial expressions is an important aspect of social interaction (Berneiser *et al.*, 2014). Interestingly, inflammatory challenge with endotoxin (discussed in previous chapters) has been shown to increase feelings of social disconnection, which has been suggested to play a role in depressed mood (Eisenberger *et al.*, 2010b), and to impair social cognitive processing (Moieni *et al.*, 2015). Berneiser *et al*'s (2014) study in patients with multiple sclerosis supports the link between impaired facial expression recognition and social disconnection in these patients. Similarly, impaired emotional face recognition observed in patients with fibromyalgia syndrome, a chronic rheumatic condition characterised by chronic musculoskeletal pain, where depression, fatigue, sleep disturbance and reduced cognitive

performance are common symptoms, was suggested to contribute to reduced social functioning (Weiss *et al.*, 2013). Diminished facial affect recognition has also been associated with impaired psychosocial functioning in schizophrenia (Addington *et al.*, 2006, Kee *et al.*, 2003). Thus, it is plausible that the relationship between impaired facial expression recognition and social disconnection previously described in other chronic medical conditions may also be relevant to reduced social functioning reported in patients with RA (McInnes *et al.*, 2013, Taylor *et al.*, 2016). Based on observations in the current study, it may be reasonable to suggest that elevated inflammation may play a role in such social dysfunction.

Weiss *et al.* (2013) also discuss the importance of the amygdala and insula in facial expression recognition and interoception (discussed in Chapters 2 and 3) and suggest that since both regions are also involved in the affective processing of pain perception, presence of pain in fibromyalgia patients may reduce processing resources for tasks such as facial expression recognition. Pain scores in the current study did not correlate with accuracy on the facial expression recognition, however this hypothesis could be interesting to examine further as pain is a frequent symptom in RA (Taylor *et al.*, 2016).

Studies discussed above describe both general impairments in facial expression recognition and impairments in negative emotion recognition more specifically. The current study appeared to support a specific detrimental effect of inflammation on negative emotion recognition, however it could be argued that negative emotions in the facial expression recognition task are more cognitively demanding to identify than positive stimuli (e.g. happy faces), thus reduced recognition of negative stimuli may be indicative of more general cognitive impairments. Observations in the emotional memory task, where increased CRP was associated with reduced recall in a non-valence specific manner, support the notion of potential general cognitive impairments, which are thought to represent a significant burden

in RA (Appenzeller *et al.*, 2004, Bartolini *et al.*, 2002, Shin *et al.*, 2012). Further studies are required to replicate and expand findings in the present study before conclusions can be drawn, though the interpretation of current findings as evidence of an association between elevated inflammation and general cognitive impairments is conceivable when consideration is given to the body of literature linking inflammation and cognitive dysfunction, discussed below.

Cognitive impairments have been found to be associated with elevated inflammation in major depression (Carvalho *et al.*, 2014), as well as other psychiatric illnesses, such as bipolar disorder, in which several studies have shown that cognitive dysfunction is associated with greater levels of pro-inflammatory cytokines (Barbosa *et al.*, 2014, Bauer *et al.*, 2014, Rosenblat *et al.*, 2015). Cognitive decline in neurodegenerative diseases, such as Alzheimer's (Bettcher and Kramer, 2014) have also been linked to inflammation. In addition, cognitive impairments where inflammation is thought to act as a catalyst have been reported in relation to medical illnesses such as chronic hepatitis C virus (Monaco *et al.*, 2015), HIV (Hong and Banks, 2015) and Parkinson's disease (Rocha *et al.*, 2014), in addition to metabolic syndrome (Lasselin and Capuron, 2014), obesity (Miller and Spencer, 2014) and ageing (Capuron *et al.*, 2014). Potential mechanisms underlying this repeatedly hypothesised link include the ability of pro-inflammatory cytokines to alter monoamine levels, pathologically activate microglia, impair neuroplasticity and cause HPA axis dysregulation, which is thought to be synergistically capable of reducing cognitive performance (introduced in Chapter 1 and reviewed by Rosenblat *et al.*, 2015).

Often intertwined with cognitive dysfunction are symptoms of fatigue and sleep disturbance, which are both frequently reported in RA, as well as a range of other physical illnesses (Heesen *et al.*, 2006, Joaquim and Appenzeller, 2015, Lindqvist *et al.*, 2013). The presence of such symptoms could represent a further reason for data suggestive of cognitive

impairments in the present study. Furthermore, both fatigue and sleep problems have been linked to elevated inflammation (Dantzer *et al.*, 2014, Felger and Lotrich, 2013, Miller *et al.*, 2009), as discussed in the first and previous chapter.

Effects of inflammation on attentional vigilance, as measured by the dot probe task, were limited, showing only a significant correlation between increased CRP and increased avoidance of fearful faces in the masked condition. This effect is in opposition to increased vigilance to negative stimuli observed in depression (Bradley *et al.*, 1997, Fritzsche *et al.*, 2010, Gotlib *et al.*, 2004) and to increased avoidance of happy faces observed following IFN-α administration in Chapter 2. However, it is important to note here that this task utilised reaction times to examine altered vigilance; since reaction times may be altered by joint inflammation of the wrists and hands that occurs regularly in RA, using a reaction time task is not best suited to investigating effects of attentional vigilance in this patient cohort. Attempts were made to control for this factor using mean reaction times as a covariate in the analysis of this task, however this may not adequately control for reduced motor function. This point is discussed further in the limitations section below.

In the present study, CRP did not correlate with mood assessed by the BDI. This is similar to the lack of association found between mood scores and behavioural measures in the IFN-α study presented in Chapter 2. It is also in keeping with previous suggestions of a lack of correlation between CRP and negative mood symptoms of depression in RA (Low *et al.*, 2009, Sheehy *et al.*, 2006), although it is in contrast to a study by Kojima *et al.* (2009) that found a significant positive association between depressive symptoms and CRP in patients with RA. A possible reason for the lack of association in the present study may be that CRP levels are modified by anti-inflammatory medications, despite them appearing to be relatively well matched in the low and high inflammation groups. Additional limitations of CRP measurements in the current study are discussed later. Alternatively (or additionally)

mood scores in the context of RA may be confounded by somatic symptoms, rather than negative affective symptoms of depression (Low *et al.*, 2009). This might also account for the lack of robust effects in the emotional test battery. This raises an important point about the use of subjective mood rating questionnaires, such as the BDI, in chronic medical illnesses including RA; this point is further discussed below.

5.4.1 Limitations and further work

The current study employed a cross-sectional study design and recruited a heterogeneous RA patient population for this exploratory study; in doing so there are limitations that should be considered. Firstly, cross-sectional studies do not permit investigation of effects of inflammation, mood and emotional processing over time. RA patients recruited into this study reported marked fluctuations in mood over time, particularly during inflammatory 'flare-ups', however the current study was unable to capture potential temporal relationships; a limitation that similarly applies to published literature (Rathbun *et al.*, 2013). Not recruiting a homogenous sample also meant that factors such as duration of illness and anti-inflammatory medications taken may represent a significant confound of this study, particularly as the small sample size negated the possibility of reliably stratifying patients into groups depending on such factors. It is also possible that a bias may have been introduced into this study, as patients were not recruited consecutively from the RA clinic, and the patients who opted to take part may have been patients who were not suffering from clinical depression. Furthermore, a notable problem with the present study was that patients with marked inflammation and pain often did not wish to attend the study visit (there was a high incidence of missed visits), or when individuals did attend, swelling and pain in the joints of the hand on occasion meant that testing was halted. Even when testing was not terminated, results may be compromised by this factor, particularly in the dot probe task where reaction times are used to examine attentional vigilance. Motor function was not

controlled for in computer tasks. Neuropsychological and mood assessments were also limited to relatively short durations to avoid exhausting patients, particularly as this was not a standalone study. Hence cognitive tasks included in Chapter 3 were not used in this study. BDI was used to assess depressed mood for continuity with all studies in this thesis, however such mood questionnaires were perhaps crude for this patient population, collecting insufficient data on fatigue and sleep disturbances that may have affected behavioural measures. As aforementioned, BDI scores can be confounded by somatic symptoms; indeed 7 out of 21 BDI items assess somatic symptoms, thus caution should be taken when utilising this questionnaire in medical patients (Matcham *et al.*, 2013). The lack of 'gold standard' structured clinical interview to assess depressed mood therefore represents a limitation of this study. Further, assessment of feelings of social disconnection may have been useful in light of facial expression recognition effects observed.

Additionally, there was no control group in the current study; the reason for this was that for the purposes of this thesis, the main aim was to examine varying effects of chronic inflammation on emotional processing within a patient cohort, rather than between patients and healthy subjects. Had fewer patients been excluded from the sample of 40 subjects recruited, this may not have been such a limitation. Nevertheless, in hindsight an age-matched control group, in addition to low and high inflammation patient groups, could have aided the investigation of effects of chronic inflammation on emotional processing.

Although CRP is useful as a general marker of systemic inflammation that has been shown to correlate with pro-inflammatory cytokines including IL-6 and TNF-α (Cesari *et al.*, 2003), it is possible that psychological measures may have stronger relationships with more proximal inflammatory mediators (Low *et al.*, 2009). Therefore, further investigation would benefit from examination of a range of pro-inflammatory cytokines. Since HPA axis dysfunction may also be implicated in deleterious effects on cognition in chronic

inflammation (Heesen *et al.*, 2006, Rosenblat *et al.*, 2015), this would have been interesting to assess. Furthermore, better assessment of subjective pain may have helped the interpretation of the facial expression recognition findings, particularly in relation to suggestions that central nociceptive processing may diminish neural resources to process affective information (Weiss *et al.*, 2013).

It would be interesting for future work to recruit homogenous patient groups, for instance patients with and without co-morbid depression, or perhaps patients on biologic treatments such as TNF-α inhibitors, compared to those not receiving biologic therapies. The present study suggests that cognitive impairments are associated with elevated inflammation; thus future work could include a control task for emotional processing tasks, or a battery of cognitive tasks (similar to those in Chapter 3) to assess this in more detail. Further studies with a longitudinal design could also help to elucidate the relationship between chronic inflammation, emotional processing and cognition, including more robust assessments of depressed mood that are not so confounded by somatic symptoms present in many physical illnesses such as RA. This point is important, as it is difficult to disentangle whether depression associated with RA results from overlap of somatic symptomatology or neuroimmune pathways (Low *et al.*, 2009), and this remains to be clarified in the literature. Moreover, both immune activation and immune suppression occur in RA (Blume *et al.*, 2011), which makes it difficult to disentangle effects of pro-inflammatory cytokines on emotional processing in isolation in this patient cohort. Nonetheless, the current study suggests that increased CRP in patients with RA may have significant effects on affective processing that may contribute to mood symptoms or feelings of social disconnection, therefore it seems worthy of further, more careful, investigation. It would also be interesting to assess whether, in future, the emotional test battery could identify RA patients with a

depressive phenotype that is characterised by negative biases that could be remediated by antidepressant treatments.

5.5 Conclusions

Depressive symptoms in patients with RA are common and have significant detrimental effects on quality of life. There are many factors that may contribute to this association, which are likely to play differing roles in individual patients, yet a key hypothesis is that depression in RA may be associated with neuroinflammatory pathways. The present study, however, did not find an association between depressed mood scores and levels of inflammation measured by CRP. Nor did it provide support for our hypothesis that greater inflammation would be associated with more pronounced negative affective biases, therefore current findings are not aligned with previous observations in this thesis or negative emotional processing biases found in depression. Indeed, negative biases expected in facial expression recognition were not observed – on the contrary, impairments in emotional face recognition were found. There were similarly no negative biases apparent in emotional categorisation, emotional memory or attentional vigilance tasks. Instead, data were arguably more suggestive of cognitive impairment, which is a common feature in RA and a range of chronic medical illnesses. Previous studies have suggested that inflammatory pathways may play a key role in cognitive dysfunction, thus future studies could utilise a range of non-emotional cognitive tasks to investigate this relationship further. Longitudinal, larger scale studies are certainly required to clarify the temporal association between inflammation and both somatic and negative affective symptoms of depression in patients with chronic inflammatory conditions, which currently remains insufficiently understood.

Chapter 6

Tianeptine in an Experimental Medicine Model of Antidepressant Action

6.1 Introduction

The monoamine theory of depression has dominated the neuropharmacology of depression for many years; as a result the vast majority of antidepressants in clinical use potentiate the activity of serotonin (5-HT) and/or noradrenaline (NA) by inhibiting neurotransmitter re-uptake into pre-synaptic nerve terminals. As discussed in Chapter 1, the efficacy of these conventional antidepressant agents is established, though it is estimated that one third of depressed patients fail to respond to standard medication (Rush, 2007), and many patients are left with residual symptoms (Kennedy and Paykel, 2004). Together, the pressing clinical need for more effective antidepressants and the growing appreciation that the pathophysiology of depression is more complex than the monoamine deficit alone have driven work to elucidate novel neurobiological targets.

Tianeptine is an atypical antidepressant with an interesting pharmacological action that remains debated. It is licensed as an antidepressant in much of Europe, Latin America and Asia, with studies showing a broadly equivalent efficacy to more commonly used agents such as selective serotonin re-uptake inhibitors (SSRIs) (Kasper and Olie, 2002) and acceptable tolerability (Guelfi *et al.*, 1992). Initial studies classified tianeptine paradoxically as a selective serotonin re-uptake enhancer (SSRE) (Fattaccini *et al.*, 1990, Kato and Weitsch, 1988, Lejeune *et al.*, 1988, Mennini *et al.*, 1987), rather than re-uptake inhibitor (SSRI), largely because it appeared to enhance the re-uptake of serotonin in certain areas of the brain and attenuate an induced serotonin-syndrome in animal studies (De Simoni *et al.*, 1992). Later work disputed early findings, instead arguing that the efficacy of tianeptine is

attributable to events downstream of the monoamine synapse (McEwen *et al.*, 2010), though in healthy subjects Lechin *et al.* (2006) demonstrated an acute decrease in plasma serotonin and increase in platelet serotonin following a single oral dose of tianeptine, consistent with an effect to enhance serotonin uptake. Nevertheless, research has focused on several other potential mechanisms of action of tianeptine, such as normalising deleterious effects of stress on glutamate transmission, restoring normal neuroplasticity in limbic brain regions (reviewed by McEwan *et al.*, 2010) and decreasing the HPA axis response to stress (Castanon *et al.*, 2003, Delbende *et al.*, 1994). Intriguingly, evidence also suggests tianeptine has protective properties against the pathophysiological effects of inflammatory cytokines (introduced in Chapter 1) induced by peripheral administration of lipopolysaccharide (LPS; the active component of endotoxin) and IL-1β (Castanon *et al.*, 2001, Castanon *et al.*, 2003, Castanon *et al.*, 2004, Plaisant *et al.*, 2003).

Early, non-conscious amelioration of negative affective biases discussed previously in this thesis has been proposed as a final common pathway for the effects of a range of clinically effective antidepressant agents (Pringle *et al.*, 2011). Indeed, the neurocognitive model of antidepressant drug action posits that over time, a shift towards a positive bias could provide a more positive social environment in which re-learning of emotional associations might contribute to subsequent improvements in subjective mood (Cowen and Browning, 2015, Pringle *et al.*, 2011). Such changes in emotional processing are evident following acute dosing of antidepressants (Harmer, 2010, Harmer *et al.*, 2009a, Harmer *et al.*, 2003a) using the neuropsychological tasks that have been used throughout this thesis, and occur independently of changes in mood (Harmer *et al.*, 2009b).

6.1.1 Aims

Given the intriguing actions of tianeptine, this study aimed to utilise the same established battery of neuropsychological tasks used throughout this thesis, to assess the acute impact on emotional processing of a single oral dose of tianeptine (12.5mg) in 40 healthy volunteers in a double-blind between-groups design, with a view to improving understanding of its action at a neuropsychological level. Measures of working and verbal memory (as used in Chapter 3) were also included given that tianeptine has been shown to block stress-induced memory impairments in rodents (Conrad *et al.*, 1996, Zoladz *et al.*, 2008) and improve neurocognitive function in depressed patients (Jeon *et al.*, 2014). We hypothesised that tianeptine would produce positive biases in the processing of emotional information, based upon previous studies that have shown such effects with a range of clinically effective antidepressant treatments in healthy subjects (Harmer and Cowen, 2013).

6.2 Methods

6.2.1 Participants and study design

This was a double-blind, placebo-controlled parallel group study with 1:1 randomisation. We studied 40 healthy volunteers (20 male; mean age 22; SD 2.5; range 18-30 years) recruited through local and online advertising. Participants were screened using the Structured Clinical Interview for DSM-IV (SCID) (Spitzer *et al.*, 1995) to ensure no current or past history of any Axis I psychiatric disorder. Eligible subjects were psychotropic naïve and physically fit to participate as ascertained by a physical examination and recording of an electrocardiogram. Non-pregnancy was confirmed on the morning of testing. Inclusion criteria also included fluency in English (due to the nature of the tasks), age 18-40 and BMI 19-30. The study was approved by the University of Oxford Medical Sciences Inter Divisional Research Ethics Committee, and all participants gave written informed consent.

Participants undertook the National Adult Reading Test (NART) (Nelson, 1982) and Eysenck Personality Questionnaire (EPQ) (Eysenck and Eysenck, 1975) at screening to characterise the sample.

Subjects were randomly assigned to receive either a single dose of 12.5mg of tianeptine or matched placebo in a double-blind manner. The standard treatment dose of tianeptine is 12.5mg three times daily due to its short half-life of 2.5 hours (Salvadori *et al.*, 1990). The randomisation schedule was drawn up by a member of the group not otherwise involved in the study to maintain allocation concealment. Block randomisation by sex was used to ensure equal distribution. For the testing visit, participants attended the department between 08.30am and 09.15am having fasted following an early light breakfast.

6.2.2 Subjective mood questionnaires

Participants completed the following questionnaires at baseline, prior to receiving the single dose of tianeptine or placebo: Beck Depression Inventory (BDI) (Beck *et al.*, 1961); Positive and Negative Affective Schedules (PANAS) (Watson *et al.*, 1988); State Trait Anxiety Inventory (STAI) (Spielberger *et al.*, 1970); Befindlichkeits Scale (BFS) (Von Zerssen *et al.*, 1974) and visual analogue scales (VAS, measuring happy, sad, hostile, alert, anxious and calm). After 50 minutes, during which time participants remained in the department, the subjective questionnaires were repeated.

6.2.3 Emotional test battery

The tasks constituting the emotional test battery (facial expression recognition, emotional categorisation, emotional memory and dot probe) are described in full in Chapter 2. The same versions of these tasks were used in this study, and in Chapters 2, 3 and 5, to allow comparison of effects between studies. Testing was commenced 60 minutes after dose administration to coincide with peak plasma concentration (Salvadori *et al.*, 1990).

6.2.4 Cognitive tasks

The *n*-back task and Rey Auditory Verbal Learning Test (AVLT) (Rey, 1964) utilised in this study have also previously been described in Chapter 3. Once more, the same versions of these tasks were used in the present study.

6.2.5 Statistical analysis

Extreme outlying data (data lying at more than three times the participants' interquartile range above their third or below their first quartile) were removed from all psychological tasks. This resulted in less than 2% of data being lost as outliers on any task. Demographic measures, subjective state rating scores and emotional memory were analysed using one-way ANOVA. Mood and energy scales completed before and after drug administration were compared between the two groups using repeated measures ANOVA, with treatment as the between-subject factor and time as the within-subject factor. Data from the facial expression recognition task, emotional categorisation task, emotional recognition task and dot probe task were analysed using repeated measures ANOVA, with treatment group as the between-subject factor and valence as the within-subject factor. In the dot probe task, masking was added as an additional within-subject factor. *N*-back data were also analysed using repeated measures ANOVA, with treatment as the between-subject factor and complexity as the within-subject factor. Statistically significant interactions were followed up using simple main effects analyses. Where assumptions of equality of variances were broken, Greenhouse-Geisser procedure was used to correct the degrees of freedom.

6.3 Results

6.3.1 Group matching

The drug and placebo groups were well matched in terms of demographics, including verbal IQ as measured by the NART and personality traits measured by the EPQ (see Table 1).

Table 1. Demographic, mood and personality measures at baseline. Means (standard deviations).

	Placebo (*n* = 20)	**Tianeptine 12.5 mg (*n* = 20)**	**Statistical significance**
Age	22.1 (2.7)	21.9 (2.2)	$p = 0.77$
Gender	10M:10F	10M:10F	N/A
BMI	23.0 (2.1)	23.3 (1.8)	$p = 0.60$
Verbal IQ	114.6 (5.4)	114.8 (5.3)	$p = 0.93$
Trait Anxiety	29.8 (6.0)	31.2 (7.0)	$p = 0.49$
BDI	1.1 (1.7)	2.0 (2.7)	$p = 0.21$
EPQ: E	13.0 (1.6)	12.3 (1.6)	$p = 0.14$
EPQ: N	14.5 (1.9)	14.8 (2.6)	$p = 0.67$
EPQ: P	19.6 (1.9)	19.6 (1.7)	$p = 0.93$
EPQ: L	14.7 (2.0)	13.8 (1.9)	$p = 0.13$

BMI: body mass index; BDI: Beck Depression Inventory; EPQ: Eysenck Personality Questionnaire, where E: extroversion, N: neuroticism, P: psychoticism, L: lie.

6.3.2 Subjective state

The following subjective state measures were, as expected, unaffected by tianeptine administration: BDI ratings, state anxiety, BFS scores, PANAS, visual analogue scales of happiness, sadness, hostility, alertness, anxiety and calmness (all p values >0.1, see Table 2).

Table 2. Subjective state changes over time. Values are ratings at 50 minutes after treatment minus baseline ratings. Means (standard deviations).

	Placebo (*n* = 20)	**Tianeptine 12.5 mg (*n* = 20)**	**Statistical significance**
BDI	-0.3 (0.7)	-0.3 (1.4)	$p = 1.0$
State Anxiety	-1.0 (3.2)	-0.2 (3.1)	$p = 0.40$
BFS: Mood	1.4 (4.0)	0.2 (4.4)	$p = 0.37$
BFS: Energy	1.3 (3.8)	1.3 (4.0)	$p = 0.97$
BFS: Total	2.7 (6.8)	1.4 (7.0)	$p = 0.56$
PANAS: Positive	-1.7 (3.9)	-1.5 (4.6)	$p = 0.85$
PANAS: Negative	-0.4 (0.8)	-0.4 (1.2)	$p = 1.0$
VAS: Happy	0.6 (6.2)	-0.7 (8.3)	$p = 0.59$
VAS: Sad	-0.7 (3.1)	-2.5 (7.8)	$p = 0.34$
VAS: Hostile	-0.6 (4.0)	-1.3 (3.0)	$p = 0.53$
VAS: Alert	-1.8 (13.4)	-1.4 (24.0)	$p = 0.94$
VAS: Anxious	-3.4 (6.2)	-4.8 (12.3)	$p = 0.65$
VAS: Calm	2.2 (8.9)	-4.4 (16.4)	$p = 0.12$

BDI: Beck Depression Inventory; BFS: Befindlichkeits Scale; PANAS: Positive and Negative Affective Schedules; VAS: Visual Analogue Scales.

6.3.3 Emotional test battery

6.3.3.1 *Facial expression recognition*

There was a main effect of treatment group on accuracy in this task (see Figure 1: $F(1,38)=4.67$, $p=0.04$), with tianeptine-treated participants being generally less accurate; however there was no interaction between intervention group and emotion ($F(5, 190)=0.11$, $p=0.99$). Accuracy was analysed over the different morphed intensity levels of emotional expressions that were presented in random order in this task, however this failed to identify any significant differences between the two groups (all p values > 0.1).

There were no effects of tianeptine on this task in terms of misclassifications or reaction times (all *p* values >0.4).

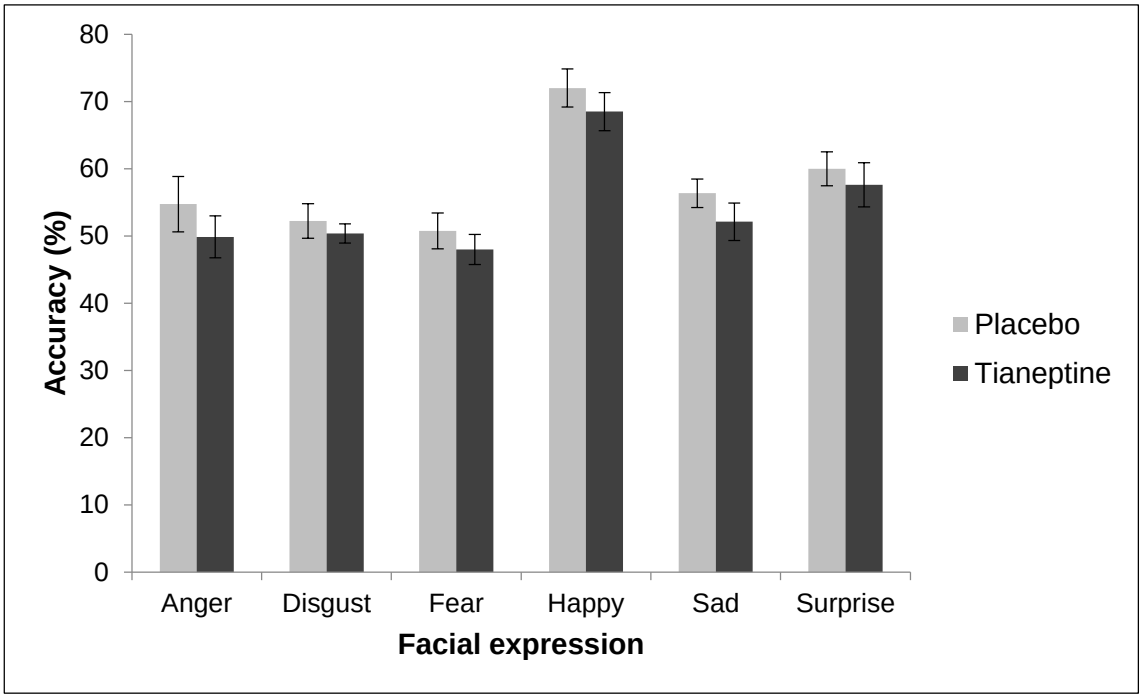


Figure 1. Performance in the facial expression recognition task following placebo (light bars) or tianeptine (dark bars). Values represent the mean percentage correct for each of the six basic emotions summed over the different intensity levels used in this task, with error bars representing standard error of the mean (SEM). A main effect of treatment group (p <0.05) was found, however this was not valence specific and there was no emotion x group interaction (p >0.05).

6.3.3.2 *Emotional categorisation task*

There were no between-group differences in reaction times to categorise self-referent personality characteristics in terms of an effect of group ($F(1,38)=0.24$, $p=0.63$) or emotion x group interaction ($F(1,38)=0.47$, $p=0.50$).

6.3.3.3 *Emotional memory*

The total number of correct words recalled did not differ significantly between the groups and there were no differences in false intrusions (all *p* values >0.1). However, there was a significant between-group difference in the percentage of positive words correctly recalled in this task; the tianeptine group recalled a significantly lower proportion of positive words compared to the placebo group (one-way ANOVA $F(1,38)=4.97$, $p=0.03$; see Figure 2).

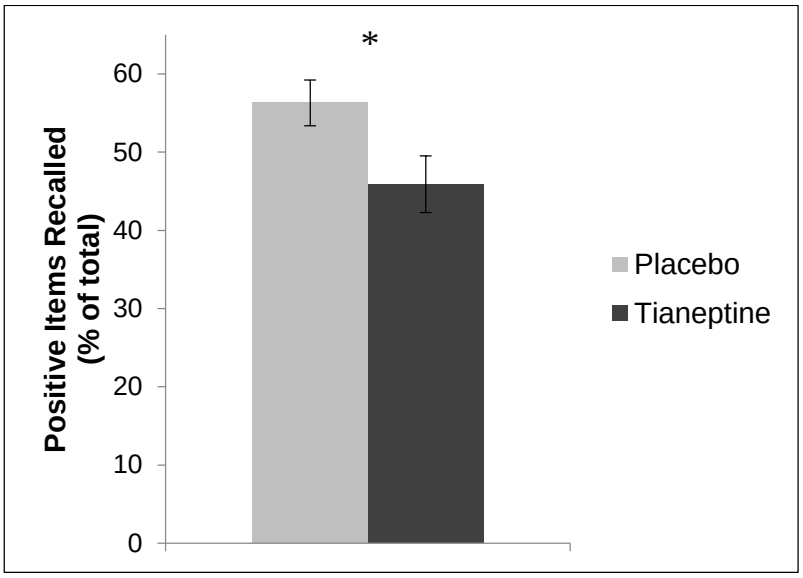


Figure 2. Recall of positive personality characteristics following a single dose of placebo (light bar) or tianeptine (dark bar). Values represent the mean percentage of positive words recalled from the total (positive and negative characteristic words). Error bars show SEM. * p <0.05.

There was a marginal main effect of treatment group on accuracy in the emotional recognition memory task as deduced by the discriminability index *d′*, whereby the tianeptine group correctly recognised more positive and negative items overall compared to the placebo group (see Figure 3: $F(1,38)=4.69$, $p=0.04$), although this effect was not valence specific (group x valence interaction ($F(1,38)=0.30$, $p=0.59$). No difference was found between groups when positive words (one-way ANOVA $F(1,38)=1.56$, $p=0.22$) and negative words

(one-way ANOVA $F(1,38)=2.97$, $p=0.09$) were considered separately. Tianeptine did not affect performance in terms of reaction times or false alarms in this task (all p values >0.1).

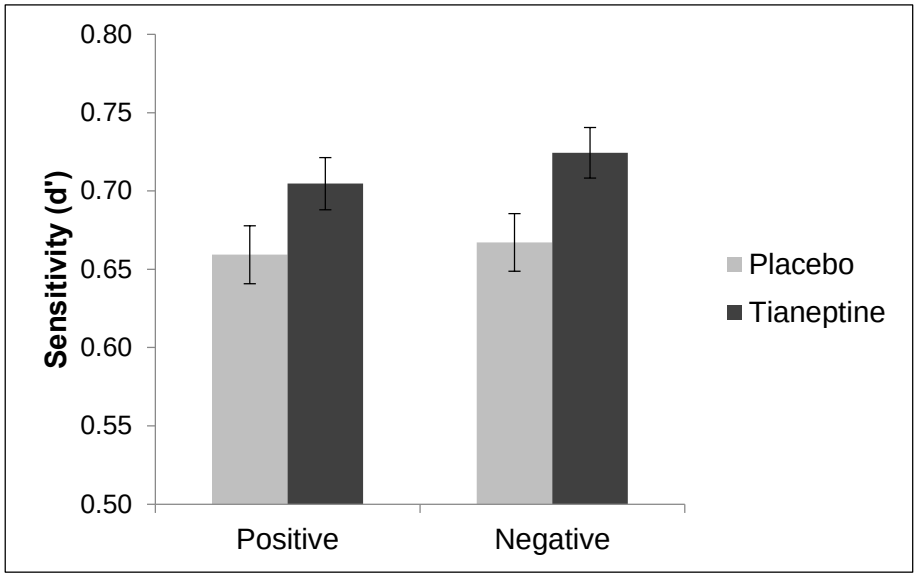


Figure 3. Discriminatability index (d') for accuracy in the emotional recognition task. d' ranges from 0 to 1, with higher numbers indicating higher accuracy. This figure shows accuracy for correctly recognised positive and negative personality characteristic words for placebo (light bars) and tianeptine (dark bars) treated groups. The tianeptine group correctly recognised more positive and negative items overall compared to the placebo group (p <0.05). Error bars show SEM.

6.3.3.4 *Dot probe attentional vigilance*

There was an interaction between emotion and treatment group ($F(1,38)=5.34$, $p=0.03$), including mask as a within-subject factor. When masked and unmasked trials were considered separately, there was a trend towards an emotion x group interaction in the unmasked ($F(1,38)=3.89$, $p=0.056$; see Figure 4), but not masked ($F(1,38)=0.09$, $p=0.77$) condition. Simple main effects analysis revealed that this was driven by a significant reduction in vigilance scores for happy faces in the tianeptine-treated group compared to the placebo group (one-way ANOVA $F(1,38)=4.12$, $p=0.05$; Figure 4).

Figure 4. Effect of tianeptine on attentional vigilance for happy and fearful facial expressions in the unmasked condition of the dot probe task. Values are attentional vigilance scores in the placebo (light bars) and tianeptine (dark bars) treated groups. Attentional vigilance scores were calculated by subtracting the median reaction time from congruent trials (when the probe appeared in the same position as the emotional face) from incongruent trials (when the probe appeared in the opposite position to the emotional face, i.e. in the position of the neutral face). A positive score indicates vigilance towards the emotional face, whereas a negative score reflects avoidance of the emotional face. * p=0.05. Error bars show SEM.

6.3.4 Cognitive tasks

6.3.4.1 *N-back*

There were no between-group differences in accuracy (F(1,37)=0.30, p=0.59) or latency (F(1,37)=0.33, p=0.57) on the control task. One volunteer's data belonging to the placebo group was removed from the zero-back dataset due to anomalous responses that met criteria for an extreme outlier. As expected, there was a significant main effect of complexity (F(2,

76)=37.50, $p<0.001$) on accuracy, however there was no main effect of treatment group, or a treatment group x complexity interaction for either accuracy or latency (all p values >0.1).

6.3.4.2 *AVLT*

Tianeptine did not affect declarative verbal memory performance as assessed using the AVLT (all p values >0.2).

6.4 Discussion

The current findings indicate that a single dose of tianeptine produced less consistent, or even opposite, effects on emotional processing compared to previous findings with clinically effective antidepressant treatments, including citalopram (Harmer *et al.*, 2004), reboxetine (Harmer *et al.*, 2003a), duloxetine (Harmer *et al.*, 2008) and mirtazapine (Arnone *et al.*, 2009). This is despite the ability of the task battery to detect changes with both conventional antidepressant treatments, and more novel antidepressant strategies, such as agomelatine (Harmer *et al.*, 2011b), vagus nerve stimulation (Critchley *et al.*, 2007) and negative ion administration (Harmer *et al.*, 2012, Malcolm *et al.*, 2009). Tianeptine reduced memory for positive self-referent personality characteristics and reduced attentional vigilance for happy faces in the dot probe task, in the absence of changes in subjective mood. Tianeptine also reduced accuracy in the recognition of facial expressions of emotion; however this was a non-selective effect that lacked specificity to any of the six basic emotions featured in the task. Correct recognition of positive and negative words was increased by tianeptine, but again this did not represent a valence specific effect. There were no effects of the drug on emotional categorisation, or other non-emotional outcome measures of working memory or declarative memory.

Although tianeptine is regarded as a clinically effective antidepressant (Kasper and Olie, 2002), the effects in the present study were more in keeping with negative affective biases

seen in depressed patients discussed previously in this thesis than with positive biases induced by a range of antidepressants in healthy volunteers (reviewed by Harmer, 2013). Of relevance, the emotional test battery used has been shown to be sensitive to manipulation of serotonergic transmission both by monoaminergic antidepressants and through tryptophan depletion. As mentioned in Chapter 2, acute tryptophan depletion has been shown to decrease the recognition of facial expressions of fear in healthy female volunteers (Harmer *et al.*, 2003b), and specifically reduce the recognition of happy faces in recovered depressed patients (Hayward *et al.*, 2005). In remitted depressed patients, tryptophan depletion led to decreased memory for positive words in a self-referential adjectives task, though only in those exhibiting a depressive response (Booij *et al.*, 2005). In healthy subjects, tryptophan depletion has also been found to impair recall of neutral and positive words, but not of negative words (Klaassen *et al.*, 2002), and the current study demonstrated decreased recall of positive words following tianeptine administration. The current data therefore suggest that the acute effects of tianeptine may have some similarities to those of reduced serotonin function in these tasks, though it is important to note that we found a general reduction in accuracy of facial expression recognition rather than a valence specific effect. In addition, tryptophan depletion is known to impair memory consolidation (Mendelsohn *et al.*, 2009), however the tianeptine group were marginally more accurate on the emotional recognition task for both positive and negative stimuli, and no effect of tianeptine on the AVLT was found.

The present findings in the attentional dot probe task suggest that tianeptine increased attentional avoidance of happy facial expressions, an intriguing finding that cannot be fully explained based on previous observations in the literature, but that appears to follow the trend towards acute tianeptine promoting a more negative bias. As explained in Chapters 1 and 2, depression has been associated with increased attentional vigilance towards negative

words in the attentional dot probe task (Bradley *et al.*, 1997, Mogg *et al.*, 1995). On the contrary, tryptophan supplementation has been found to decrease attentional vigilance towards negative words in healthy female volunteers (Murphy *et al.*, 2006). Again, findings in the present study could be indicative of a reduction in available serotonin. Attentional vigilance effects in the happy condition of this paradigm have previously been suggested to be relevant to threat-related processing biases (Cooper and Langton, 2006), whereby the neutral face represents a more threatening face relative to the happy expression; attention biased towards the neutral face produces a relative 'avoidance' of the happy face. If this interpretation is applied to the present data, the implication is that tianeptine increased attentional vigilance to mildly threatening or ambiguous neutral faces. Although not significant, this is perhaps also reflected by the tianeptine group displaying increased vigilance towards fearful stimuli in the unmasked condition compared to placebo group. As noted in Chapter 2, the IFN-α study found similar effects to those observed following acute tianeptine; this will be discussed further in Chapter 7.

This is the first study to analyse the effects of acute tianeptine administration on emotional processing to our knowledge. The finding of a negative early effect is intriguing in the context of tianeptine's possible mechanism of action - a consensus for which has remained elusive. Investigation of the possible role of serotonin re-uptake enhancement has produced contradictory results. Tianeptine had been shown to increase serotonin re-uptake in *ex vivo* rat platelets (Kato and Weitsch, 1988), produce opposite effects to sertraline on serotonin metabolite levels, and reduce symptoms of an induced serotonin syndrome (De Simoni *et al.*, 1992). Further investigation by Malagie *et al.* (2000) challenged this, showing no marked alterations in extracellular serotonin following tianeptine administration in conscious rats. Technical limitations of early microdialysis techniques such as the presence in solution of citalopram may have elicited some autoreceptor effects to explain this

discrepancy. Additional findings such as the lack of effect on the firing rates of serotonergic neurons have cast further doubt on the role of serotonin in tianeptine's mechanism of action (reviewed by McEwen *et al.*, 2010). Of note, tianeptine shows no affinity for known neurotransmitter receptors (Kato and Weitsch, 1988). In a single dose study of healthy volunteers similar to the present study, however, tianeptine induced significant acute effects on serotonin levels (Lechin *et al.*, 2006). Following a single 12.5mg oral dose of tianeptine, plasma free serotonin levels were reduced when compared to placebo administration; significantly so from 45 minutes following the dose. There was a parallel significant increase in platelet serotonin, suggested therefore to represent enhanced platelet uptake of serotonin.

The present results are in keeping with current understanding of tianeptine's actions in that it appears distinct from traditional monoaminergic antidepressants. Specifically, given the alignment of dosing and timing of this study with that of Lechin *et al.* (2006), the negative emotional biases seen here appear to be in keeping with an acute serotonin re-uptake enhancement; a mechanism which though controversial has been repeatedly proposed for tianeptine. Any serotonin re-uptake enhancement effect is of course counter-intuitive to tianeptine's purported antidepressant effects. However, it is consistent with differences between acute and repeated doses of SSRI treatment on threat processing. For example, a single dose of citalopram (alongside increasing attentional bias to positive words) increased the recognition of fearful faces (Browning *et al.*, 2007), however seven days of treatment with citalopram has been shown to reverse this effect (Harmer *et al.*, 2004). This is in line with the clinical phenomenon of an initial anxiogenic effect on commencing SSRIs which subsides with chronic treatment (Bauer, 2015). It is therefore possible that repeated dosing of tianeptine would remediate negative affective biases observed in the present study.

Much work has diverged from serotonergic manipulation as being central to tianeptine's action. For example, downstream effects on neuroplasticity have been implicated with tianeptine preventing or reversing stress-induced changes such as in hippocampal volume and cell proliferation in tree-shrews (Czeh *et al.*, 2001). Such effects may be mediated via modulation of glutamatergic neurotransmission; tianeptine reduced stress-induced amygdala glutamate efflux in rats (Piroli *et al.*, 2013) and affects signalling cascades such as potentiating AMPA receptors via phosphorylation of the GluA1 subunit (Szegedi *et al.*, 2011). Tianeptine's modulation of the glutamatergic system has also been suggested to occur indirectly via activation of opioid receptor signalling (Gassaway *et al.*, 2014). Interestingly, tianeptine is additionally thought to be able to protect against the deleterious effects of pro-inflammatory cytokines activated under conditions of chronic stress in animals (Castanon *et al.*, 2003, Castanon *et al.*, 2004, McEwen *et al.*, 2010, McEwen and Olie, 2005, Plaisant *et al.*, 2003) and to attenuate sickness behaviour *in vivo* following peripheral LPS or IL-1β administration (Castanon *et al.*, 2001), which the authors suggested may indicate a peripheral site of action of tianeptine. Further discussion of work that could explore this proposed mechanism of action follows.

6.4.1 Limitations and further work

This is an interesting study that demonstrated for the first time that acute effects of tianeptine are dissimilar to effects observed previously following single doses of a range of clinically effective antidepressant therapies, thereby supporting suggestions that tianeptine has a distinct mechanism of action. However, there are various limitations of the present study that warrant consideration. Firstly, a clear limitation of the current study is the lack of serotonin assays to replicate the previous findings of Lechin *et al.* (2006) and to support the hypothesis that negative biases observed in this study may be a result of reduced serotonin availability. As in Chapters 3 and 4, healthy participants were used in this study, permitting

exploration of effects without confounds of fluctuations in clinical symptoms and in the absence of subjective mood changes. This suggests that emotional processing effects observed should represent direct effects of tianeptine on emotional processing, rather than a secondary effect of mood improvement. However, it should be noted that effects from this experimental medicine model, in which a relatively homogeneous sample of mainly young, high functioning students who were all in good health were studied, may not be generalizable.

To fully characterise the effects of tianeptine on emotional processing, additional studies using repeated dosing regimens are required. This is of particular importance considering the ambiguous mechanism of action of tianeptine; whatever the initial action of tianeptine, it seems likely that the mechanisms through which it exerts its therapeutic effects lie in downstream adaptive changes (McEwan *et al*., 2010), which are likely to become apparent over time. Further work is therefore needed to elucidate effects at time points further along the treatment pathway if greater consensus as to the nature of the therapeutic mechanism of tianeptine is to be reached. Of particular relevance to the present thesis, the protective properties of tianeptine that have been reported in animal models of peripheral LPS and IL-1β administration appear to occur with chronic, but not acute administration of this drug (Castanon *et al.*, 2003, McEwen and Olie, 2005). Chronic administration of tianeptine, rather than the single dose used in the present study, would allow such protective effects of tianeptine to be examined. Indeed, it would be interesting to investigate tianeptine in the models of innate immune system activation discussed throughout this thesis to see whether tianeptine shows any protective properties against inflammatory cytokines in humans, including inflammatory activity associated with chronic stress. Should the hypotheses surrounding protective effects of tianeptine be supported in human studies, it would be interesting to explore neuropsychological effects of tianeptine in subsets of depressed

patients that exhibit elevated inflammation, particularly patients who have failed to respond to conventional antidepressant treatments, or patients with IFN-α-induced depression.

6.5 Conclusions

To summarise, tianeptine produced intriguing effects on emotional processing that were unexpected in view of previous findings with a range of antidepressant therapies; rather than reducing negative biases as expected, a single dose of tianeptine showed a tendency to increase negative biases in emotional memory and attention. These effects have been proposed to reflect reduced serotonin function, and as previously mentioned in Chapter 2, they are similar to effects observed following IFN-α administration – the potential meaning of this observation is discussed further in the following chapter. A repeated dosing study is necessary to assess whether early negative effects on emotional processing found in this study following a single dose of tianeptine would be reversed with chronic tianeptine treatment. Furthermore, since animal studies suggest that tianeptine may have potential protective properties against inflammatory cytokines, it would be intriguing to examine chronic administration of tianeptine in individuals with elevated inflammation to investigate whether this drug has potential use as an antidepressant treatment for subtypes of depressed patients with raised inflammatory cytokines.

Chapter 7

General Discussion

7.1 Summary of findings

Multiple lines of evidence have contributed to the hypothesis that chronic, low-grade inflammation may cause more persistent alterations in neuropsychological function that might be instrumental in a range of psychiatric disorders, including major depression (detailed in Chapter 1; Anisman, 2009, Miller *et al.*, 2009). However, the mechanisms for potential modulation of mood and cognitive function are insufficiently understood. The current thesis therefore aimed to enhance understanding of the neuropsychological underpinnings of the link between inflammation and depression. Since negative biases in processing emotional information are thought to play a central role in depression pathophysiology, this work primarily investigated whether inflammation and pro-inflammatory cytokines can modulate emotional processing. In order to do this a series of studies were conducted using human models of immune system activation that are well-recognised in the literature, in which prior investigations have largely focused upon changes in neural activity and mood, but not neuropsychological function.

The first study (Chapter 2) investigated the effects of chronic IFN-α treatment in patients with hepatitis C virus on emotional processing. The findings suggest that IFN-α, which activates the endogenous pro-inflammatory cytokine network (Capuron and Miller, 2004), produced negative biases in emotional processing. In the facial expression recognition task, IFN-α had a highly specific effect of enhancing recognition of disgusted faces, and reaction times to respond to negative emotional stimuli were significantly reduced. Negative biases were also demonstrated by increased accuracy in negative self-referent categorisation of

emotional trait words, and a trend towards enhanced negative emotional memory. Negative attentional biases in the dot probe task were also found following IFN-α.

In order to examine the effects of inflammation on emotional processing further, and without potential confounds of physical illness symptoms, the second study (Chapter 3) utilised an experimental model of inflammatory challenge with typhoid vaccination in healthy subjects, which significantly increased peripheral IL-6 levels. A specific effect of false discrimination of disgust was found, whereby the typhoid vaccine group misclassified more faces as disgusted in the facial expression recognition task, and displayed a significantly less conservative response style for disgusted faces compared to the placebo group. Negative biases in emotional categorisation, emotional memory or attentional vigilance, however, were not found. In non-emotional cognitive tasks, typhoid vaccine had no effects on sustained attention, no marked effects on working memory, mild effects on improving learning in an auditory verbal learning task, as well as improved task performance in a probabilistic reward learning task – an opposite effect to a recent study using the same experimental immune model and a similar reward task (discussed in Chapter 3; Harrison *et al.*, 2015c).

The third study investigated effects of inflammation on sleep using the typhoid vaccination model. Inflammatory challenge was shown to disrupt several sleep continuity parameters (Chapter 4), including decreased sleep efficiency and increased wake after sleep onset, thereby supporting evidence that suggests innate immune cytokine activation can disrupt sleep continuity in ways that may be pertinent to depression (Kupfer and Ehlers, 1989, Raison *et al.*, 2010c).

The following chapter (Chapter 5), examined the effects of chronic inflammation in patients with rheumatoid arthritis (RA) on emotional processing. Negative biases expected in facial

expression recognition were not observed – on the contrary, impairments in emotional face recognition were found in patients with high inflammation (measured by CRP). Furthermore, negative biases were not apparent in emotional categorisation, emotional memory or attentional vigilance tasks. Instead, data were arguably more suggestive of cognitive impairment, which is discussed later.

Chapter 6 investigated the effects of the atypical antidepressant tianeptine on emotional processing, which may be of interest when consideration is given to its purported acute serotonergic re-uptake enhancing properties and the effects of inflammatory cytokines on serotonin metabolism (detailed in Chapter 1). Tianeptine was shown to reduce positive emotional memory and significantly enhance avoidance of happy faces in a similar manner to effects seen with IFN-α in Chapter 2, as discussed below.

7.2 Inflammation and emotional processing

The main hypothesis in this thesis posited that inflammation would negatively bias emotional information processing, which would be comparable to negative affective biases found in depressed patients, and that negative biases would become more pronounced with chronic exposure to inflammation. Chronic IFN-α administration, whereby patients are exposed to prolonged elevation of pro-inflammatory cytokines, showed evidence that firmly supported this hypothesis. Interestingly, effects on emotional memory and attentional biases in this study appeared to be suggestive of reduced serotonin function. The battery of emotional processing tasks used in this thesis have been shown to be sensitive to serotonin modulation via SSRIs and tryptophan depletion (Pringle and Harmer, 2015); indeed, results in the IFN-α study can be likened to effects previously observed with tryptophan depletion and following tianeptine administration (Booij *et al.*, 2005, Cooper *et al.*, 2015, Hayward *et al.*, 2005, Klaassen *et al.*, 2002), thereby suggesting that effects might be associated with

reduced serotonin availability. As previously described (Chapters 1 and 2), evidence provides a compelling argument for inflammatory cytokines reducing serotonin function (Bonaccorso *et al.*, 2002, Capuron *et al.*, 2002b, Raison *et al.*, 2009, Raison *et al.*, 2010b).

It should be noted that IFN-α also induced significant increases in depressive symptom severity, in line with multiple previous studies (Capuron *et al.*, 2009). Although we cannot exclude the possibility that negative affective biases resulted from depressed mood (despite the lack of correlations between behavioural task performance and mood scores), rather than direct modulation by inflammatory cytokines, results from this study are still interesting in that they suggest for the first time that negative affective biases may also be a factor in inflammation-associated depression.

Inflammatory challenge with typhoid vaccination, however, did not provide strong support for this hypothesis, despite previous evidence of typhoid vaccination and endotoxin administration modulating neural circuitry thought to be involved in emotional information processing and depression (Disner *et al.*, 2011, Elliott *et al.*, 2011), such as the subgenual anterior cingulate cortex (Harrison *et al*., 2009a), insula (Harrison *et al*., 2009b), orbitofrontal cortex (Kullmann *et al*., 2013) and amygdala (Inagaki *et al.*, 2012). As discussed in Chapter 3, a plausible explanation for the lack of expected behavioural effects may be that neural activity measurements are more sensitive to inflammatory stimuli than behavioural task measurements, possibly due to the recruitment of additional neural resources to maintain task performance. Alternatively, the time point chosen for neuropsychological assessment could have been before changes in emotional processing manifest, as insufficient data are available on the timeline of potential neuropsychological modulation. It is also possible that acute immune system activation in this model is insufficient to induce measureable behavioural changes, and that such changes may only be

evident with prolonged exposure to pro-inflammatory cytokines, such as chronic IFN-α administration.

Observations in patients with a chronic inflammatory condition did not support our hypothesis, however, as negative biases were not apparent in the RA study. It was suggested in Chapter 5 that amygdala and insula involvement in affective processing of pain perception may interfere with processing resources for cognitive tasks (Weiss *et al.*, 2013), hence this could perhaps provide an explanation for the observation of impaired facial expression recognition task performance. A further suggestion for the lack of negative affective biases in the emotional test battery could be absence of core depressive symptoms in the patient cohort; patients recruited into this study did not have clinical depression, thus immune-related differences in neuropsychological function between RA patients with and without depression could not be examined. In contrast, depressed mood was significantly elevated at a group level in the IFN-α study, suggesting that this provided a more relevant model of potential immune mechanisms in depression. Additionally, it is probable that the small sample size was underpowered to detect negative biases, particularly in such a heterogeneous patient cohort tested in the RA study. Furthermore, due to the cross-sectional study design there was no information on the time course of CRP levels, without which we cannot reliably state that those patients with high inflammation on the day of testing had consistently elevated inflammation, or vice versa for the low inflammation group; this is touched upon further under 'Methodological considerations'.

7.2.1 Inflammatory cytokines and processing of facial expressions of disgust

The IFN-α study provided the first evidence of enhanced disgust recognition following inflammatory cytokine therapy. This was a highly specific effect, demonstrated by an enhanced ability to discriminate disgusted faces, which occurred in the absence of alterations

in facial expression recognition of other emotions. The typhoid vaccination study also showed intriguing effects of increased false discrimination of disgusted faces, which although different to the IFN-α effect of enhanced accurate discrimination, still suggest that immune system activation might be implicated in enhanced sensitivity to disgust. In the RA study, the only valence specific effect in the facial expression recognition task was increased reaction times to disgusted faces in the high inflammation group, which might be suggestive of prolonged evaluation of disgusted facial stimuli, although inflammation was not associated with increased accurate or false discrimination of disgusted faces. Taken together, inflammation may be involved in enhanced processing of social signals of disgust; a novel suggestion that has not previously been hypothesised in the literature to our knowledge.

Disgust processing has previously been linked with reduced serotonin function. For example, tryptophan depletion has been shown to enhance recognition of disgusted faces in a recovered depressed sample (Hayward *et al.*, 2005) and speeded recognition of disgusted faces in remitted depressed subjects (Merens *et al.*, 2008), whereas tryptophan supplementation reduced recognition of disgusted faces in healthy volunteers (Murphy *et al.*, 2006). Inflammatory cytokines, including IFN-α treatment, have been shown to reduce serotonin availability (Miller *et al.*, 2013), which could account for similarities between IFN-α findings in this thesis and previous tryptophan depletion studies.

Furthermore, the insula cortex has been implicated in disgust processing. Indeed, disgusted faces have been shown to activate the insula in depressed patients (Surguladze *et al.*, 2010), whereas lesion to the insula has been associated with impaired ability to recognise disgusted faces (Calder *et al.*, 2000) and direct electrical stimulation of the insula has been shown to interfere with disgust processing (Papagno *et al.*, 2016). A previous study with typhoid vaccination found that activity in the insula predicted IL-6 associated mood changes (notably

fatigue) three hours post-vaccination (Harrison *et al.*, 2009b). The typhoid vaccination study in the present thesis found a significant increase in peripheral IL-6 levels at a similar time point, thus although our study did not include brain imaging it could be suggested that typhoid vaccination increased insula activity and that this might have contributed to increased mislabelling of disgusted emotional faces.

Taken together, it could be argued that this preliminary work, combined with previous work that has separately demonstrated the ability of inflammatory cytokines to both reduce serotonin function and increase insula activity, suggests a link between elevated inflammation, reduced serotonin availability and enhanced emotional processing of disgusted stimuli that has not previously been described. As mentioned in Chapter 2, this could provide evidence of a link between hypotheses that posit an evolutionary purpose of disgust recognition to avoid threats of infectious disease that is related to insula cortex activity (Curtis *et al.*, 2004) and evolutionary theories of behavioural modification induced by inflammatory mechanisms to promote healing, while also maintaining vigilance to further pathogen exposure, injury or attack (Miller *et al.*, 2013). In modern times such interactions may play a role in the development of depression (Miller and Raison, 2015), or at least some core symptoms of depression (Sprengelmeyer *et al.*, 2011). Though this is a thought-provoking hypothesis, further work is certainly required to examine such a claim.

7.3 Inflammation and non-emotional cognition

Inflammatory cytokines, including IFN-α therapy and the cytokine-inducer endotoxin, have been implicated in cognitive impairment, which may be relevant to depression (Capuron and Miller, 2011, Carvalho *et al.*, 2014, Dantzer *et al.*, 2008b). However, findings in this thesis appear to be inconsistent between studies. In the IFN-α study, non-affective cognitive testing was not conducted, though evidence of cognitive impairment or psychomotor

slowing in the emotional test battery was not found – on the contrary, reaction times to negative stimuli were decreased. The typhoid vaccination study included an additional battery of cognitive tasks, in which impaired cognitive function was not evident; working memory was not altered, which is in keeping with a previous study that showed no effects of endotoxin on accuracy in this task (Grigoleit *et al.*, 2011), nor was sustained attention. In a verbal learning task typhoid vaccine paradoxically slightly improved non-emotional memory performance, which is in contrast to reduced memory performance found following endotoxin administration (though different tasks were used here; Reichenberg *et al.*, 2001). These observations prompted discussion in Chapter 3 of the complex relationship between inflammatory cytokines and cognition, as cytokines may have protective properties under normal circumstances, potentially promoting learning and memory (Yirmiya and Goshen, 2011). However, when inflammation persists chronically, this highly co-ordinated system may become disrupted and lead to detrimental effects on neural plasticity and neurogenesis that could contribute to impaired cognitive function (via mechanisms discussed in Chapter 1 and by Miller and Raison, 2015, Yirmiya and Goshen, 2011). This requires further investigation, yet it might have important implications for extrapolating relevant meaning from findings in healthy volunteer models to patients with chronic inflammation.

Findings in the RA study, notably reduced recognition of negative facial emotions and a non-valence specific association between increased CRP and reduced recall, were indeed suggestive of a relationship between increased inflammation and impaired cognitive function. Cognitive dysfunction is thought to represent a significant burden in RA (Appenzeller *et al*., 2004, Bartolini *et al*., 2002, Shin *et al.*, 2012), and impairments in recognising facial emotions have been previously reported in a range of medical illnesses that are characterised by chronic neuroinflammation (see Chapter 5). Thus, it is possible that more persistently raised pro-inflammatory states are associated with reduced cognitive

function. Additional research is needed to examine whether this could be via direct effects of inflammatory cytokines, or indirect effects such as fatigue and sleep disturbance, which have both been linked to increased inflammation (Dantzer *et al.*, 2014, Felger and Lotrich, 2013). Further work could also benefit from assessing whether there are differences in both affective and non-affective cognition in these patients.

7.4 Inflammation and sleep continuity

Inflammatory mechanisms have been proposed to play a pathophysiologic role in sleep disruption in depression (Felger and Lotrich, 2013, Imeri and Opp, 2009, Motivala *et al.*, 2005). This thesis investigated the effects of typhoid vaccination on sleep polysomnography to reveal significant disruption of sleep continuity parameters, which have been suggested to be a state dependent measure in depression (Kupfer and Ehlers, 1989, Weinberger *et al.*, 2015). As described in Chapter 4, similar sleep continuity changes have been seen following IFN-α and endotoxin administration (Mullington *et al.*, 2000, Raison *et al.*, 2010c). Intriguingly, evidence also suggests that anti-inflammatory treatment can improve sleep continuity in patients with treatment resistant depression and evidence of high inflammation (Weinberger *et al.*, 2015), and in patients with RA (Zamarron *et al.*, 2004). This study has therefore supplemented compelling suggestions in the literature that inflammatory cytokines are capable of disrupting sleep continuity in ways that might be relevant to some depressed patients, and that anti-inflammatory treatments may have therapeutic application in counteracting these effects. It should be noted, however, that whilst inflammation-induced sleep continuity changes in the literature and the present thesis appear to be relatively consistent, effects of inflammatory cytokines on slow wave or REM sleep are less clear and perhaps require further investigation (discussed in Chapter 4).

Interestingly, sleep deprivation studies have shown that disturbed sleep can impair processing of emotional facial expressions (Cote *et al.*, 2014, van der Helm *et al.*, 2010). RA is commonly associated with marked sleep disruption (Abad *et al.*, 2008), and examination of emotional processing in RA patients in the current thesis showed that high inflammation was associated with impaired facial expression recognition, rather than negative affective biases that were hypothesised. It would therefore be interesting to assess whether impairments observed in the RA study could be associated with sleep disruption.

7.5 Methodological considerations

7.5.1 Healthy volunteer models of inflammation-associated depression

Limitations of each study were discussed in relevant chapters, many of which were specific to those studies, hence they will not be covered further here. However, there are some wider methodological considerations that should be given further consideration. Firstly, although models of inflammatory challenge in healthy subjects are interesting from a mechanistic point of view and provide a paradigm without confounding factors such as physical illness and psychological stress, findings from these studies may not reliably extrapolate into a clinical setting where a range of factors may interact with immune pathways. Furthermore, although symptoms of sickness behaviour overlap with depressive symptoms and provide evidence of the ability of inflammatory mechanisms to modulate behaviour (discussed in Chapter 1), there is still limited understanding of how these mechanisms may change over time in a manner that may lead to clinical depression. In addition, it is generally thought that inflammatory mechanisms induced by experimental inflammatory challenge are relevant to the deleterious effects of psychological stress, however this remains to be fully determined (Krishnadas and Harrison, 2016); this is an important question for future research to address. It should also be noted that participants in typhoid vaccination studies

in this thesis were largely young, healthy, high functioning students, and thus data from these participants may not accurately reflect all clinical populations.

Further work may also be needed to examine whether healthy volunteer models of inflammation truly elicit a depression-like syndrome in healthy subjects. Typhoid vaccination in the current thesis did not alter subjective mood, which is in keeping with a previous typhoid vaccine study (Paine *et al.*, 2013), and contrary to previous reports of significant effects on mood in the same model (Harrison *et al.*, 2009a, Wright *et al.*, 2005). Healthy volunteer models (including both typhoid vaccination and endotoxin) also do not appear to adequately mimic depressive symptoms related to negative views of self, past, present and future, or feelings such as worthlessness, guilt, hopelessness and suicidal ideation (DellaGioia and Hannestad, 2010). It may be that these paradigms have better application as models of individual symptoms that are potentially relevant to subgroups of depressed patients instead, for example examination of emotional processing biases or sleep continuity disruptions as in the present thesis.

7.5.1.1 *Further suggestions for healthy volunteer models*

Psychological stress is implicated in promoting central inflammatory responses that may be involved in depression aetiology (Anisman, 2009, Capuron and Miller, 2011, Pariante and Lightman, 2008, Slavich and Irwin, 2014). Previous healthy subject studies have shown that psychological stressors can significantly increase inflammatory cytokines (Muscatell *et al.*, 2014, Steptoe *et al.*, 2007). As mentioned in Chapter 3, there is also evidence that psychological stress combined with typhoid vaccination can synergistically upregulate cytokine responses and negative mood (Brydon *et al.*, 2009, Strike *et al.*, 2004). Thus, it would be interesting to see whether combining typhoid vaccination with a psychological stressor would affect neuropsychological function, as this could represent a healthy

volunteer model that simulates some mechanisms thought to be involved in depression slightly more closely. As mentioned in Chapter 6, it would also be interesting to investigate purported cytoprotective effects of tianeptine against inflammatory challenge and chronic stress in a human model, since much of the work with tianeptine has been conducted in animal models.

A further 'two-hit' model of depression in healthy volunteers was suggested in Chapter 4 that postulated combining typhoid vaccination with sleep polysomnography to assess whether the downstream effects of cytokine-induced sleep continuity changes may alter neuropsychological function. Sleep and inflammatory cytokines appear to have a bidirectional relationship, whereby sleep disruption can increase circulating pro-inflammatory cytokines and increased inflammatory cytokines can disrupt sleep (as seen in the present thesis) (Imeri and Opp, 2009). Sleep disruption has also been shown to impair facial expression recognition (discussed above), thus it would be interesting to examine this relationship further in this model.

7.5.2 IFN-α as a model of inflammation-associated depression

IFN-α is notorious for inducing neuropsychiatric symptoms, which display marked overlap with idiopathic major depressive symptoms in otherwise healthy individuals (Capuron *et al.*, 2009), and thus this model of inflammation-associated depression has allowed valuable insights into depression pathophysiology (Hoyo-Becerra *et al.*, 2014). The present thesis found significant effects of this treatment on depression severity ratings, state anxiety, anhedonia and fatigue, and provided novel evidence that suggests this treatment can negatively bias emotional information processing in a manner that is comparable to negative affective biases observed in depression (Harmer *et al.*, 2011a). This model of inflammation-associated depression provides some of the most robust evidence of the involvement of pro-

inflammatory cytokines in depression, as discussed throughout this thesis and supported by the study in Chapter 2. It remains a challenge, however, to identify which patients are vulnerable to the development of depression since not all patients experience mood disorder (Capuron and Miller, 2004). The sample size in the IFN-α study in this thesis was too small to examine whether baseline neuropsychological function was predictive of later negative affective biases and depressive symptomatology, which could be a key avenue for further larger scale studies to investigate.

There has been debate over the similarities and differences between IFN-α-induced depression and idiopathic major depression (Capuron *et al.*, 2009, Dahl *et al.*, 2015). The IFN-α study in this thesis suggests both similarities to major depression, in that negative biases appeared to be comparable to those observed in depression, and a potentially distinct inflammatory mechanism of enhanced disgust processing. It seems important for future work to study subtypes of depressed patients in which there is evidence of elevated inflammation, rather than a heterogeneous depressed sample, if we are to better understand the involvement of inflammation in certain depressed patients, and to ascertain the extent to which pathways involved in IFN-α-associated depression are relevant to major depression.

Furthermore, there were no peripheral or CSF markers of inflammation or the kynurenine pathway measured in the IFN-α study, which would have helped to test the hypothesis presented in this thesis that negative affective biases observed following IFN-α administration could be due to cytokine-induced reductions in central serotonin availability. It should also be noted that although discussion regarding IFN-α effects on neuropsychological function has largely focussed on monoaminergic function, as introduced in Chapter 1 there are a number of pathways through which IFN-α could cause behavioural changes that are also thought to be relevant to patients with major depression (reviewed by Hoyo-Becerra *et al.*, 2014), including HPA axis dysfunction. There were no cortisol

measures in this study, thus HPA axis function was not assessed; as mentioned in Chapter 3 the diurnal cortisol slope has been shown to be altered in depression (Jarcho *et al.*, 2013) and there is evidence in IFN-α-treated patients of flattened diurnal cortisol slope and reduced glucocorticoid receptor sensitivity, which may in turn further increase inflammation (Felger *et al.*, 2015a, Raison *et al.*, 2010a). Interestingly, it has been suggested that individuals with melancholic depression have a higher diurnal cortisol slope compared with individuals with atypical depression, whereas atypical depression has been associated with significantly higher levels of inflammatory markers than melancholic depression (Lamers *et al.*, 2013) - suggesting that these two subtypes of depression may have different biological correlates. Future studies could use an array of markers to try to ascertain which immune-related pathways may be relevant to negative biases in emotional information processing in various subtypes of depression.

7.5.3 Chronic physical illness with inflammation

As discussed in previous chapters, depression is more prevalent in a range of chronic medical illnesses compared to the general population (Haddad, 2009). The present thesis studied emotional processing in patients with RA as a model of chronic inflammation, however it should be noted that similar to depressed patients, RA patients display heterogeneity in their disease phenotype and the potential causes of depression in RA are multifactorial (Margaretten *et al.*, 2011). In order to identify medically ill patients in which neuroimmune mechanisms may play a crucial role in mood, future investigations should more carefully recruit homogeneous patient groups, particularly those patients with and without depression with a similar duration of illness, to better understand the role of inflammation in depression pathophysiology in these patients. This could better control for physical symptoms of RA and the psychological burden of a chronic physical illness. The

RA study within this thesis did not do this and thus the relevance of findings may be compromised.

Another important point to raise when discussing depression in chronic physical illness is the use of subjective mood rating scales. In the RA study, the Beck Depression Inventory (BDI) was used as a measure of depressed mood for continuity throughout this thesis. However, mood questionnaires can be heavily confounded by somatic symptoms that commonly present in chronic physical illnesses (Matcham *et al.*, 2013). When administering this questionnaire to patients in the RA study, physical symptoms of RA did appear to impact mood scores. For example, some patients described reduced functional ability and pain associated with their illness contributing to loss of pleasure from various activities they used to enjoy. Patients also reported feelings of guilt in relation to constraints their illness placed upon family and social lives, and some patients felt pessimistic about the future due to fears of disease progression. Loss of energy and fatigue were a common complaint, along with changes in sleep patterns, and some patients reported loss of interest in sex secondary to their physical condition. This means that depressed mood scores measured by subjective questionnaires like the BDI can be increased without patients experiencing sadness and depressed mood *per se*. Using subjective measures of depressed mood may therefore be inadequate for identifying medically ill patients with depression. It is also possible that different methods of mood assessment contribute to large variations in the reported prevalence of depression in RA. Based on negative affective biases that were evident following immune system activation with IFN-α, it would be interesting to investigate cognitive biases in RA further to see whether it may be possible to identify medically ill depressed patients according to the presence of negative affective biases.

7.5.3.1 *Cross-sectional study design*

As highlighted in Chapter 5, cross-sectional studies do not permit temporal investigations of inflammation, mood and neuropsychological changes over time. Correlation of an inflammatory marker with mood and behavioural changes may be too simplistic, as factors such as the duration of exposure to elevated inflammation, fluctuations in inflammation over the course of the illness, and treatment with anti-inflammatory medications are not captured.

Correlational analyses also do not address whether inflammation plays a causal role in depression; that is, they do not indicate whether elevated inflammatory cytokine levels can cause depression, or whether they are somehow secondary to the disorder, for example the product of sleep disturbance or physical disease (Slavich and Irwin, 2014). This argument lends support to other human models of immune depression used in this thesis, which aim to investigate causality. Furthermore, to date there are very few studies that conduct statistical tests of mediation (Byrne *et al.*, 2016), and as mentioned previously this was also not conducted in the present thesis. This type of analysis could be particularly beneficial to longitudinal research in the future to investigate potential causal relationships between inflammation and the onset of depression. In summary, future longitudinal studies, rather than cross-sectional studies, in chronic physical illness will be important to scrutinise the potential role of neuroimmune mechanisms in depression aetiology in subsets of patients.

7.6 Inflammatory markers and depression

There is a large body of correlational evidence indicating that patients with depression have elevations in circulating pro-inflammatory cytokines (Dowlati *et al.*, 2010, Hodes *et al.*, 2015). However, it should be noted that few studies have measured central inflammatory markers (Byrne *et al.*, 2016) and thus there is still insufficient evidence to confirm the extent to which peripheral circulating inflammatory markers mirror central levels (Krishnadas and

Harrison, 2016). Therefore, the relationship between central inflammatory markers and depression symptomatology remains to be fully elucidated.

The present thesis also highlighted some discrepancies regarding the association between depressed mood and peripheral inflammatory cytokine levels. In the typhoid vaccine studies (Chapters 3 and 4), no correlations were found between IL-6 and subjective mood state, which remained unaltered despite significant increases in IL-6. This finding was in line with some studies (e.g. Harrison *et al.*, 2009a), and contrary to others (e.g. Wright *et al.*, 2005), though questionnaires used to assess mood differed between studies presented here and previous studies in this model. Nevertheless, in the literature the typhoid vaccine model is discussed as inducing negative mood, which may need further work to confirm.

The RA study also found no correlation between CRP and depressed mood score, though it is possible that confounds of somatic symptoms in RA discussed above may have disguised potential associations. This effect is in keeping with previous suggestions of a lack of correlation between CRP or serum TNF-α and negative mood symptoms of depression in RA (Low *et al.*, 2009, Sheehy *et al.*, 2006, Uguz *et al.*, 2015), but contrary to other reports of a significant positive association between depressive symptoms and CRP in patients with RA (Kojima *et al.*, 2009). Discrepancies therefore exist in the literature that require further investigation.

Unfortunately in the IFN-α study, no inflammatory markers were examined. Previous studies have reported significant positive correlations between increased depression scores and elevated pro-inflammatory cytokines (Bonaccorso *et al.*, 2001, Wichers *et al.*, 2007), yet another study reported significant associations between increased TNF-α, but not IL-6, and development of depressive symptoms (Raison *et al.*, 2010a). In this model, there is intriguing evidence to suggest that reduced serotonin function may be related to increased

pro-inflammatory cytokine activity, which may in turn be linked to the emergence of depression in some patients. The findings in the IFN-α study in this thesis also provide new evidence that the emergence of negative biases may be linked to depression onset, which could also be related to reduced serotonin function following therapy. Perhaps this suggests that future work should look beyond simply measuring peripheral inflammatory markers and also measure biomarkers such as tryptophan, kynurenine metabolites and emotional processing. In addition, there is growing evidence that genetic factors may denote enhanced risk of IFN-α-induced depression (Bull *et al.*, 2009, Felger and Lotrich, 2013, Smith *et al.*, 2012). Interestingly, a recent study identified patients with a biological sensitivity to IFN-α treatment using peripheral blood transcriptomics for the first time and found significant differences in the expression of several inflammatory, oxidative stress and neuroplasticity pathways, which have been suggested to provide potential biomarkers for the identification of individuals at risk of developing inflammation-associated depression (Hepgul *et al.*, 2016). This work provides further impetus to extend investigation beyond simple correlational analysis between state inflammatory markers and depression.

Another important point to raise is the limited measurement of inflammatory markers in the present thesis. The typhoid vaccine study only measured IL-6 (previous studies have not shown significant elevations in TNF-α or IL-1, e.g. Harrison *et al.*, 2009a), and the RA study measured CRP as a general marker of inflammation only. As mentioned previously in this thesis, the complex and highly orchestrated nature of the inflammatory cytokine network means that measuring limited numbers of cytokines may not provide a full picture of the intricate interplay between pro- and anti- inflammatory cytokines. This highlights a general issue in this research field relating to which inflammatory markers should be measured (Krishnadas and Harrison, 2016) and suggests that wider arrays of cytokines should be measured until greater understanding is reached. Furthermore, cytokines are thought to be

important in preserving healthy brain function (Chapter 3 discussed the possibility that acutely elevated pro-inflammatory cytokines may promote learning and memory) and regulating normal sleep (Imeri and Opp, 2009). Thus, it seems too simplistic to assume that surplus levels of pro-inflammatory cytokines are wholly detrimental to the brain. Further studies are therefore required to refine which inflammatory markers should be measured and how the effects on the brain change with differing exposure to inflammation, including both beneficial and harmful effects. It is possible that more sophisticated modelling techniques may be required do this (for an example see Baker *et al.*, 2013), as it remains a challenge to capture the associations between this complex network of inflammatory markers and behavioural and cognitive changes that may be relevant to depression aetiology (Krishnadas and Harrison, 2016).

7.7 Final remarks

There is compelling evidence that inflammatory cytokines have an important role in subtypes of depressed patients, yet the neuropsychological underpinnings of this relationship are unknown. A large body of evidence suggests that negative affective biases are involved in depression onset and maintenance, and results from this thesis provide intriguing preliminary evidence that suggests innate immune system activation may modulate negative affective biases in ways which could be pertinent to depression pathophysiology. In particular, this thesis found that chronic IFN-α therapy can induce negative biases in emotional information processing and findings in the present thesis have led to the hypothesis that enhanced disgust processing may represent a distinct effect of inflammation on emotional processing. Additionally, the ability of typhoid vaccination to impair sleep continuity was shown for the first time, which could signify a further inflammatory pathway that is relevant to depression aetiology.

Remediation of negative affective biases is widely suggested to represent a final common pathway for antidepressant response, thus it would be fascinating to examine depressed patients with elevated inflammation to determine whether negative biases are displayed in these patients, and further whether they can be ameliorated using targeted therapies, including anti-inflammatory treatments or atypical antidepressants such as tianeptine. By combining models of cognitive neuroscience and immune system activation it may be possible to build an integrated paradigm of mechanisms implicated in inflammation-associated depression, which could be extended in future to aid the pressing clinical need to develop novel treatment strategies for subtypes of depressed patients.

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