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Delineating Molecular Mechanisms of Inflammation-Induced Delirium Using a Novel Mouse Model



by

Donal Skelly

Thesis submitted for the degree of Doctor of Philosophy at the University of Dublin, Trinity College

Submitted September 2012

School of Biochemistry and Immunology and

Trinity College Institute of Neuroscience

Trinity College, Dublin 2

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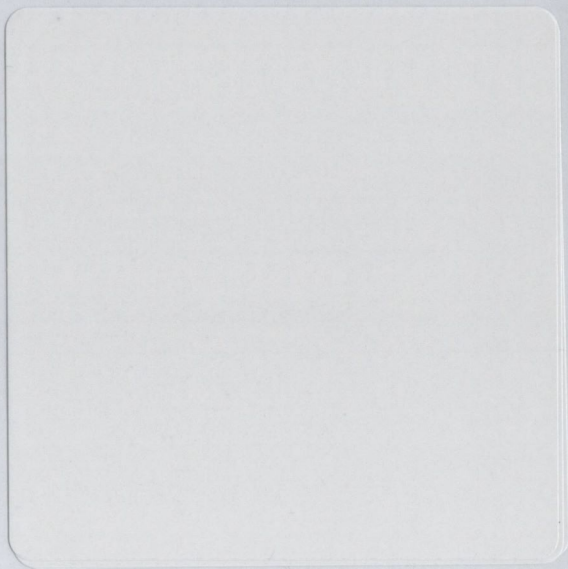

II Summary

Delirium is a syndrome of acute and transient confusion with disruption of attention and cognition; it is a common but underdiagnosed neuropsychiatric condition, which is predictive of increased functional decline, dementia and mortality. Despite its high incidence and cost, the molecular mechanisms underlying delirium are poorly understood. Prior cognitive impairment is the primary risk factor for delirium and systemic inflammation is a frequent trigger. The objective of the current thesis was to investigate the CNS response to systemic inflammation, particularly where there is underlying neurodegeneration, using a novel animal model of delirium during dementia.

Alteration in CNS function, mediated by systemic lipopolysaccharide-(LPS) induced inflammation, in the form of sickness behaviour, has been widely studies and described. However, detailed comparisons of systemic challenges with proinflammatory cytokines IL-1β, TNF-α and IL-6 across compartments are lacking. The aim of the first study in this thesis was to compare the systemic and CNS effects of LPS and the proinflammatory cytokines which are commonly implicated in system-brain interactions. Female C57BL/6 mice were challenged intraperitoneally with LPS (100 μg/kg), IL-1β (15 or 50 μg/kg), TNF-α (50 or 250 μg/kg) or IL-6 (50 or 125 μg/kg). We investigated effects on core body temperature, open field activity, plasma levels of inflammatory markers at 2 hours post injection. We also examined levels of hepatic, hypothalamic and hippocampal inflammatory cytokine transcripts. Hippocampal mRNA of markers of endothelial and microglial activation were also measured. As expected, LPS elicited the widest inflammatory response. Systemic IL-1β- and TNF-α- challenges induced robust systemic and central inflammation with some interesting differences, namely CXCL1, IL-6 and uPAR. IL-6 was very limited in its ability to induce systemic inflammatory responses and CNS transcripts but did induce components of the hepatic acute phase response. We also injected further animals with systemic IL-1β (15μg/kg) to analyse responses across 1, 2, 4, 8 and 24 hours. These results form a useful reference source of information on peripheral and central immune responses to peripheral LPS and classic proinflammatory cytokines, and offer information on appropriate doses with which to interrogate the role of peripheral cytokines in delirium-like episodes.

Previous work from our group modelled aspects of delirium during dementia by exploiting progressive neurodegeneration in the ME7 mouse model of prion disease and superimposing systemic inflammation induced by injection of LPS. In the second study of this thesis this model of delirium during dementia was used to demonstrate that the progression of neurodegenerative disease increases the incidence, severity and duration of acute episodes of

cognitive impairments and this increasing susceptibility is associated with decreasing density of hippocampal pre-synaptic terminals. There was also evidence of increased expression of cyclooxygenases and prostaglandin synthases and receptors, which is indicative of a disease-related amplification of the CNS prostaglandin system. Accordingly, hippocampal PGE2 was observed to be elevated by disease. Pre-treatment with the COX-1 inhibitors piroxicam and SC-560, but not the COX-2 inhibitor nimesulide, provided significant protection against LPS-induced working memory deficits in diseased animals. Piroxicam blocked systemic and central PGE2 synthesis but did not affect proinflammatory cytokine levels in the blood or brain. These data are consistent with the idea that prior pathology is the major predisposing factor for acute cognitive impairments induced by systemic inflammation and that prostaglandins synthesized by COX-1 have a role in this predisposition. The hypothesis that systemic cytokines play a role in the exacerbation of CNS inflammation in ME7 disease was also investigated. It was demonstrated, using the synthetic glucocorticoid dexamethasone-21-phosphate to inhibit systemic cytokine synthesis, that in LPS (500 μg/kg) –challenged animals, systemic cytokines are required to induce hypothermia but not sickness behaviour or acute neurodegeneration. Furthermore, it was shown that intraperitoneally administered LPS is detectable in plasma. These results indicate that LPS itself, binding at the cerebral vasculature, can transduce systemic inflammatory signal into the CNS, which interacts with, and exacerbates, ongoing pathology.

While systemic infection is a widely accepted risk factor for delirium, sterile systemic inflammation, resulting from trauma or surgery, can also interact with chronic neurodegeneration to induce delirium. IL-1β is a potent mediator of both aseptic and septic systemic inflammation and was shown in the first chapter to induce a robust CNS immune response. In the third study of this thesis, we investigated the role of systemic IL-1β in inflammation-induced acute cognitive dysfunction in animals with neurodegeneration. LPS (100 μg/kg)-induced acute cognitive dysfunction in ME7 animals was accompanied by equivalent systemic IL-1β levels but elevated hippocampal IL-1β mRNA levels in comparison with normal animals. Simultaneous treatment of ME7 animals with IL-1RA (10 mg/kg) and LPS offered significant protection from the LPS-induced acute cognitive impairment. Systemic injection of IL-1β (15 μg/kg) was sufficient to induce acute and transient cognitive impairment, and exaggerated hippocampal IL-1β mRNA transcription, in ME7 but not normal animals. These results suggest that systemic IL-1β can mediate and elicit inflammation-induced acute episodes of cognitive dysfunction in animals with prior pathology, and have implications for understanding the mechanisms by which both septic and aseptic inflammation can interact with and exacerbate dementia.

This thesis identifies one major predisposing factor, presence of COX-1-dependent prostaglandins, and one major triggering factor, systemic IL-1β, for acute disruption of cognition during neurodegenerative disease. Taken together these data have relevance for our understanding of how systemic inflammation and ongoing neurodegeneration interact to produce episodes of delirium. Further elucidation of these mechanisms could provide novel therapeutic targets for this complex deleterious syndrome and would also have implications for understanding of how systemic inflammation contributes to progression of dementia.

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V List of Abbreviations

6-ODHA	6-hydroxydopamine
ABC	Avidin-biotin complex
AChE	Acetylcholinesterase
ACTH	Adrenocorticoctropic hormone
AD	Alzheimer's disease
ADAM	A disintegrin and metalloproteinase
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
APP	Amyloid precursor protein
Aβ	Amyloid Beta
BBB	Blood-brain barrier
BSA	Bovine serum albumin
CA1	Cornu ammonis 1
Ca2+	Calcium
CA3	Cornu ammonis 3
CAM	Confusion assessment method
CD11b	Cluster of differentiation molecule 11 b
CD14	Cluster of differentiation molecule 14
CD206	Cluster of differentiation molecule 206
cDNA	complementary Deoxyribonucleic acid
CFC	Contextual fear conditioning
CLP	Caecel ligation and puncture
CNS	Central Nervous System
COX	Cyclooxygenase
cPGES	Cytosolic prostaglandin E-synthase
CRF	Corticotrophin-releasing factor
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Cycle threshold
CVO	Circumventricular organ
DAB	Diaminobenzidine
DAMP	Damage-associated molecular pattern
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DPX	Di-N-Butyle Phthalate in xylene
DSM-IV	Diagnostic and statistical manual of mental disorders -IV
E. coli	Escherichia coli
EAE	Experimental allergic encephalomyelitis
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalography
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay

EPSP	Excitatory postsynaptic potential
GABA	Gamma aminobutyric acid
GAPDH	Glyceraldehyde-3-phosphate dehyrdrogenase
GC	Glucocorticoid
gDNA	genomic Deoxyribonucleic acid
GR	Glucocorticoid receptor
gp130	Glycoprotein 130
H_2O_2	Hydrogen peroxide
H_2S0_4	Sulphuric acid
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
HMGB1	High mobility group protein B1
HPA	Hypothalamic-pituitary-adrenal
HPGDS	Haematopoietic prostaglandin D synthase
HPLC	High-performance liquid chromatography
HRP	Horseradish peroxidase
IBA-1	Ionized calcium binding adaptor molecule-1
i.c.	Intracerebral
i.c.v.	Intracerebroventricular
i.p.	Intraperitoneal
ICD-10	International classification of diseases-10
ICU	Intensive care unit
IFN	Interferon
IFN-α	Interferon-alpha
IFN-β	Interferon-beta
IL-1	Interleukin-1
IL-1R1	Interleukin-1 receptor type 1
IL-1R1-/-	Interleukin-1 receptor type 1 genetic knock-out
IL-1R2	Interleukin-1 receptor type 2
IL-1RA	Interleukin-1 receptor antagonist
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-6-/-	Interleukin-6 genetic knockout
IL-6R	Interleukin-6 receptor
IL-8	Interleukin-8
iNOS	inducible nitric oxide synthase
IP-10	Interferon-gamma-induced protein-10
IRF	Interferon-regulatory factor
IκB	Inhibitor of kappa B
JAK	Janus kinase
kDA	kiloDalton
LPGDS	Lipocalin type prostaglandin D synthase
LPS	Lipopolysaccharide
LTP	Long-term potentiation

MAP	Mitogen-activated protein
MCI	Mild cognitive impairment
MCP-1	Monocyte chemoattractant protein-1
mPGES-1	Microsomal prostaglandin E synthase-1
mPGES-2	Microsomal prostaglandin E synthase-2
MPO	Median preoptic nucleus
mRNA	messenger Ribonucleic acid
MS	Multiple sclerosis
MWCO	Molecular weight cut-off
MWM	Morris water maze
MyD88	Myeloid differentiation primary response gene (88)
Na2HPO4	Sodium phosphate dibasic
NaCl	Sodium Chloride
NaH2PO4	Sodium phosphate monobasic
NBH	Normal brain homogenate
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLRP3	NOD-like receptor family, prying domain containing 3
NO	Nitric oxide
NSAID	Non-steroidal anti-inflammatory drug
NTS	Nucleus tractus solitarius
PAI-1	Plasminogen activator inhibitor-1
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PD	Parkinson's disease
PET	Positron emission tomography
PGA2	Prostaglandin A2
PGD2	Prostaglandin D2
PGE2	Prostaglandin E2
PGEM	Prostaglandin E metabolite
PGF2	Prostaglandin F2
PGFS	Prostaglandin F synthase
PGG2	Prostaglandin G2
PGH2	Prostaglandin H2
PGI2	Prostaglandin I2
PGIS	Prostaglandin I synthase
pNPP	para-Nitrophenylphosphate
POCD	Postoperative cognitive dysfunction
PPARγ	Peroxisome proliferator-activated receptor-gamma
ppb	parts-per-billion
PRR	Pattern recognition receptor
PTX3	Pentraxin 3
PVM	Perivascular macrophage
Q-PCR	Quantitative-polymerase chain reaction
RIP	Receptor-interacting protein

RNA	Ribonucleic acid
RT	Reverse transcription
SAA2	Serum amyloid A protein
SEM	Standard error of the mean
SIE	Systemic inflammatory event
SOCS3	Suppressor of cytokine signalling 3
SSRI	Selective serotonin reuptake inhibitors
STAT	Signal transducers and activators of transcription
TACE	Tumor necrosis factor-alpha cleaving enzyme
TdT	Terminal deoxynucleotidyl transferase
TLR4	Toll-like receptor-4
TMB	Tetramethylbenzidine
TNF-R1	Tumor necrosis factor-alpha receptor 1
TNF-R2	Tumor necrosis factor-alpha receptor 2
TNF-α	Tumor necrosis factor-alpha
TNF-αIP2	Tumor necrosis factor-alpha-induced protein 2
TRADD	Tumor necrosis factor-alpha-receptor-associated death domain protein
TRAF	Tumor necrosis factor-alpha receptor factor
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
uPAR	Urokinase Plasminogen-activator receptor
VCAM	Vascular cell adhesion molecule
VD	Vascular dementia
β-ME	Beta-mercaptoethanol

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Chapter 1: Introduction

1.1 Delirium

Delirium is a complex, heterogeneous and serious neuropsychiatric state, with core elements of inattention and fluctuating course and related features of altered arousal, disorganized thinking, psychosis, and perceptual and sleep-wake cycle disturbances (Lipowski, 1987, Desforges and Lipowski, 1989, Trzepacz, 1999, Cole, 2004, Inouye, 2006). A diverse etiology and spectrum of severity of symptoms, in addition to its fluctuating course complicates formal identification of the syndrome. However, in elderly populations it is highly prevalent and life threatening; compared to patients of similar age, those admitted to hospital with delirium have a considerably worse prognosis (George et al., 1997, Ely et al., 2004).

Many patients recover from a delirious episode once the precipitating factor has been addressed and, until recently, delirium was seen as a transient and unremarkable, if serious, complication of many diseases. However, the past two decades have brought recognition of its deleterious consequences. The syndrome is potentially lethal and associated with subsequent loss of independence, early institutionalisation and persistent functional and cognitive decline (Inouye et al., 1998, Rockwood et al., 1999, MacLullich et al., 2009). Older people admitted to an acute care hospital with delirium who survive, are significantly more likely to be dead within in one year (McAvay et al., 2006), or within six months if they are admitted to the emergency department (Han et al., 2010). It has recently been reported that the increased risk of institutionalisation, dementia and death is independent of many socio-demographic (including gender, age, marital status and living arrangements) and clinical (presence of dementia, co-morbidity and illness severity) factors (Witlox et al., 2010). In addition, delirium is highly distressing for patients, carers and family (Breitbart et al., 2002), particularly in the end stage of life (Lawlor et al., 2000, Morita et al., 2004).

There are also large economic and social burdens associated with the syndrome. 49% of all hospital days are spent caring for delirious patients, accounting for $6.9 billion of US hospital's annual expenditure (Inouye, 2006). 1-year healthcare costs of a patient who has had a delirious episode are more than 3 times the cost of a patient who has not developed delirium. Thus, it is estimated that delirium costs the US healthcare system up to $152 billion per year (Leslie et al., 2008). While direct comparisons to other conditions of ageing are hampered by differences in methodology, the fact that diabetes mellitus is estimated to cost $92 billion per year illustrates the enormous, and largely unrecognised costs of delirium (Leslie and Inouye, 2011).

1.2 Prevalence and diagnosis

The prevalence of delirium in the community is only 1-2% (Folstein et al., 1991, Rahkonen et al., 2001) but prevalence increases with age; a community study showed that 10% of non-demented people aged 85+ had a delirious episode over a three year period (Rahkonen et al., 2000). In a general hospital, roughly one in five patients are thought to be delirious at any one time and amongst the elderly and patients in intensive care units and post-operative wards, the prevalence can be greater than 50% (Pisani et al., 2003, Dyer et al., 1995). Detection and diagnosis rates of delirium are low (Clegg et al., 2011), thus, up to 80% of delirious patients are not adequately managed (Michaud et al., 2007). If delirious patients are correctly identified, there are limited specific therapeutic options available and currently there are no licensed treatments (Skrobik, 2010).

There are many alternative terms for the clinical presentation of this condition: acute brain disorder, acute confusion and acute encephalopathy amongst others. Furthermore, there are numerous medical definitions of delirium, the widely used diagnostic and statistical manual of mental disorders (DSM-IV) (APA, 1994) and international classification of diseases (ICD-10) (WHO, 1992) definitions have core features of a disturbance in consciousness (reduced awareness along with an inability to focus, maintain or shift attention), altered perception and cognition (memory and problem-solving impairment not better accounted for by dementia) and acute onset or fluctuating course. Other common features are hallucinations, delusions, inappropriate affect and motor alterations. Diagnosis using these criteria is based on clinical history, behavioural observation and cognitive assessment. The time course which delirium takes can vary hugely. It is conventionally conceptualised as a transient disorder (Lipowski, 1983), indeed, many individuals may only display symptoms over a number of hours. Recent studies, however, show that up to 20% of individuals may experience persistent delirium. Such patients can have episodes lasting 3-6 months after initial onset (Cole et al., 2009, Meagher et al., 2012).

Without careful assessment delirium is often confused with other psychiatric conditions, namely psychosis, depression and dementia. Standardised clinical assessments, informed by formal examination of mental status by experienced clinicians, have been recommended over non-standardised clinical observations and interviews (Hogan et al., 2006, O'Mahony et al., 2011). However, there is much debate about the shortcomings of even the most widely used assessment, the Confusion Assessment Method (CAM) (Inouye et al., 1990). The contentious reception given by researchers (Bellelli and Trabucchi, 2010, Radtke et al., 2010, Young and Arseven, 2010) to a recent study, which recommended continued use of the CAM (Wong et al.,

2010), reflects a widespread sense that the condition is currently not satisfactorily diagnosed, let alone treated.

Delirious patients can be divided into 3 motoric subtypes: hypoactive, hyperactive and mixed. Hypoactive or 'negative' features can include apathy, withdrawal, lethargy, confusion and inability to follow commands or communicate. Hyperactive or 'positive' manifestations include agitation, aggression, psychosis (hallucinations and delusions) and/or hyper-reaction, while patients with mixed delirium can display both hypoactive and hyperactive traits. Some evidence suggests differential outcomes dependent on motoric subtype; the prognosis for hypoactive patients may be worse (Yang et al., 2009) but there is disagreement in the literature on this issue (Peterson et al., 2006, Meagher, 2009). The Cognitive Test for Delirium (Hart et al., 1996, Hart et al., 1997) was originally designed to diagnose and rate the severity of cognitive impairments during delirium. It also allows for relatively detailed assessment of multiple neuropsychological domains. As such, it is a useful tool to aid investigation of alterations in facets of cognition, which allows researchers to detect subsyndromal delirium (the presence of some core criteria for the syndrome which are not sufficient to reach diagnostic threshold) and to study links with risk factors and triggers.

1.3 Risk factors

A large body of work has demonstrated that ageing, prior incipient or acquired cognitive impairment and dementia are the major risk factors for delirium (Inouye and Charpentier, 1996, Elie et al., 1998, Fick et al., 2002). Dementia is a decline in cognition, usually occurring in those over the age of 65 years, which interferes with daily functioning. An individual usually displays signs of impairments in cognition for at least 6 months before diagnosis is made and the decline in functions of daily living is predominantly irreversible. A number of central nervous system (CNS) conditions, such as traumatic brain injury, vascular dementia, frontotemporal dementia and dementia with Lewy bodies, can result in the symptoms of dementia. However, the large majority of dementia is caused by Alzheimer's disease (AD). Individuals diagnosed with this condition usually present with insidious progressive memory loss and develop impairments in other areas of cognition such as executive function and language (McKhann et al., 1984, Dubois et al., 2010). Mild cognitive impairment (MCI) is a related, relatively newly defined condition that refers to a transitional zone between normal cognition and dementia (Gauthier et al., 2006). Its diagnosis generally requires subjective and objective memory or cognitive impairment in individuals whose activities of daily living are unaffected. The prevalence is 10% in those aged between 70 and 79 years and 25% in those

between 80 and 89 years (Roberts et al., 2008). About 80% of MCI patients develop dementia within 6 years. MCI patients, particularly those with memory impairments (amnestic MCI), are about seven times more likely to progress to AD than healthy elderly individuals (Boyle et al., 2006).

The fact that individuals with prior cognitive decline and dementia are those most likely to develop delirium probably relates to the fact that milder stimuli or disturbances trigger delirium in these populations, in contrast to healthy individuals who require more severe physiological alterations (Inouye and Charpentier, 1996). However, the biological basis for their increased susceptibility to episodes of delirium is not understood. Cognitive reserve conceptualises how the mind or cognition is resistant to the effects of pathology or functional loss, by way of greater physical size, efficiency of function or alternative processes (Stern, 2009). Reserve factors such as education, career and brain size or complexity (synaptic density and metabolic activity) are associated with lower dementia rates and may explain disparities between disease pathology and dementia symptoms (Valenzuela and Sachdev, 2006, Fotenos et al., 2008). A small number of studies have examined the relationship between delirium and cognitive reserve but this approach is in its infancy (Jones et al., 2010). A review of neuroimaging studies has reported that increased white matter disease (thus, lowering neuronal connectivity) confers higher risk of developing delirium (Soiza et al., 2008). Educational attainment in hospitalised elderly patients has also been shown to decrease the risk of onset of delirium (Jones et al., 2006). Thus, there is evidence that reduced cognitive reserve through ageing or pathology might explain the particular vulnerability of the elderly and demented to delirium following physiological alterations.

While these arguments appear plausible, they have not been adequately assessed. Indeed many questions regarding how and why underlying brain pathology is the major risk factor for delirium have remained largely untouched, despite the pressing nature of the problem. Delirium resolves more slowly when superimposed on dementia (Boettger et al., 2011): this is important because resolution within 2 weeks is associated with better functional recovery (Kiely et al., 2006). Furthermore, episodes of delirium worsen disease trajectory in dementia patients. An analysis of a large prospective cohort in Massachusetts demonstrated that episodes of delirium accelerate the trajectory of cognitive decline in patients diagnosed with Alzheimer's disease (Fong et al., 2009). Delirium also predicts further loss of independence and death in dementia patients (Fick et al., 2002). There are additional factors which necessitate the study of the relationship between delirium and dementia. A recent systematic meta-analysis showed that delirium increases the likelihood of subsequently developing dementia, even in elderly patients without previous functional or cognitive impairment (Witlox et al., 2010). Thus, it is clear that

delirium can initiate a downward spiral of acquired disability and decline in previously healthy elderly individuals; such negative outcomes have important societal implications.

The population in Ireland, like that in many countries, is ageing. In the next ten years there will be a 44% increase in the number of people over the ages of 65, with the greatest increase in the so-called oldest old – those over 80 years old. Between 2011 and 2041, the number of people aged 80 and over will increase by 250% to 457,962 people (Barrett et al., 2011). Chronic cognitive impairment in such an aged population is hugely costly, both on an economic and social level. It is estimated that the cost of care for dementia patients in the UK exceeds that of cancer, stroke and heart disease patients combined (Luengo-Fernandez et al., 2010). Most patients suffering from dementia are cared for in the home, often by their spouse and this caregiving is associated with significant psychological and physical morbidity (Dunkin and Anderson-Hanley, 1998).

In summary, there is considerable evidence that delirium can initiate *de novo* impairment in aged populations, or hasten progression of incipient dementia. Consequently, understanding its triggers and mechanisms of action is of genuine importance.

1.4 Precipitating factors

Many diverse factors can immediately precede and trigger delirium. Common causes include peripheral and systemic infections; drug intoxication, interaction or withdrawal; metabolic abnormalities, such as hypoglycaemia, hypoxia, hyponatremia and acute hepatic encephalopathy; psychological stress; pain; acute CNS injury; trauma and surgery. Based on these triggers, researchers have developed several theories of the pathophysiological processes ongoing during episodes of delirium. It should be stated that there has largely been a lack of biological evidence to support these theories. That is not to say that such approaches are erroneous, rather a lack of etiologically relevant animal models has hampered efforts to move beyond hypotheses based on clinical observations.

Neurotransmitter theories, which implicate disruption of fundamental neurotransmitters, mainly acetylcholine depletion or dopamine elevation, are the most widely cited approaches to understanding delirium (Trzepacz, 2000). The cholinergic theory holds that a deficiency in acetylcholine signalling elicits episodes of delirium (Hshieh et al., 2008). An early animal model of delirium used the muscarinic antagonist atropine to elicit cognitive dysfunction and electroencephalography (EEG) changes commensurate with changes seen in delirium patients

(Trzepacz et al., 1992) The cholinergic hypofunction theory is attractive given the high prevalence of delirium in AD, which is characterised by degeneration of the basal forebrain cholinergic system (Davies and Maloney, 1976). It is also supported by evidence of delirium in patients treated with anticholinergic medication (Tune et al., 1981) and resolution of symptoms in patients treated with cholinesterase inhibitors (Overshott et al., 2008). However, the failure of a recent trial with the acetylcholinesterase inhibitor rivastigmine for intensive care unit (ICU) delirium has emphasized that delirium is complex and heterogeneous and is unlikely to be corrected by the maintenance of levels of one neurotransmitter. That clinical trial was stopped early after increased mortality was observed in the treatment group (van Eijk et al., 2010). In fact, the rivastigmine-treated patients also displayed delirium of greater severity and duration. The dopamine theory proposes that an excess of this neurotransmitter, particularly in the prefrontal cortex can lead to delirium (Trzepacz, 2000). This theory is supported by evidence of delirium in patients treated with dopamine agonists and observations that delirious episodes can be abrogated with dopamine receptor antagonists (Sommer et al., 2002).

Another approach to understanding delirium, which has garnered considerable interest in recent years, is the inflammatory hypothesis (Cunningham, 2011, van Gool et al., 2010). There is now a large body of evidence to show that inflammation can elicit delirium (Cunningham, 2011, Cerejeira et al., 2010, van Gool et al., 2010). A number of clinical studies have demonstrated that systemic infections are highly associated with delirium diagnosis, (George et al., 1997, Elie et al., 1998, Chang et al., 2008). Additionally, sterile systemic inflammation, resulting from tissue trauma, such as hip fracture (Kat et al., 2008, van Munster et al., 2008), burns or surgery, especially cardiac surgery (Rudolph et al., 2009), can also induce delirium. While direct CNS infection can cause delirium in many individuals (McCullers et al., 1999), even routine inflammatory events appear to precipitate delirium in the demented (Inouye and Charpentier, 1996). Relatively mild urinary tract infections elicit trivial cognitive effects in young healthy individuals but can trigger delirium in elderly (Eriksson et al., 2011) and demented individuals (Manepalli et al., 1990). The clearest empirical evidence for a combination of systemic inflammation and prior pathology inducing acute cognitive dysfunction comes from animal work from our laboratory. Murray et al., (2012) showed that systemic inflammation superimposed on neurodegenerative disease in mice elicits acute and reversible impairment of working memory and attention, which mirrors aspects of impairment fundamental to delirium.

Proinflammatory cytokines have been implicated in development of sickness behaviour (Section 1.8). Recent work from our laboratory and others has suggested that an exaggerated central response to inflammation may be mechanistically important for eliciting delirious episodes. This implies that delirium may be somewhat akin to an extreme sickness behaviour

type response to systemic inflammation (Cunningham and Maclullich, In Press). This research area will form the focus of this thesis; as such the evidence for this theory will be examined below. The approach is closely related to theories which suggest that dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and cortisol can precipitate delirium (Maclullich et al., 2008). Activation of the HPA axis is an important component of the systemic response to inflammation (see Section 1.7) and there is evidence that altered activity of the HPA axis is a factor in delirium (McIntosh et al., 1985, Robertsson et al., 2001, Pearson et al., 2010).

It seems likely that the neurotransmitter, inflammatory and HPA axis hypotheses of delirium are not mutually exclusive and that each approach will contribute to understanding the complex underlying pathophysiology. Other theories of delirium concentrate on disruption of intraneuronal signal transduction (Van Der Mast, 1998) or sleep-cycles (Lewis and Barnett, 2004) but basic work exploring such approaches is lacking. The diversity of theories probably reflects the large number of precipitating factors which can elicit the syndrome of delirium.

The manifold triggers have also been categorised into direct brain insults and aberrant stress responses (Maclullich et al., 2008, Cunningham and Maclullich, In Press). The advantage of this conceptual framework, which can integrate the above theories, is how it divides the precipitating factors in to ones which, when experienced, appear to elicit delirium in all people, and ones which inordinately affect vulnerable populations. The particular interest of our laboratory is how susceptibility and stimuli interact to trigger delirium and the current approach to conceptualising the triggers helps compare and study such interactions. *Direct brain insults* comprise of trigger injuries: systemic, pharmacological or metabolic changes which profoundly alter CNS function. Examples of such insults are energy-depriving states (e.g. hypoxia), metabolic derangement (e.g. hyponatremia), CNS trauma (e.g. haemorrhage) major infection (e.g. sepsis or primary CNS) and drug toxicity (e.g. cholinergic antagonists). The *aberrant stress response* category comprises factors which do not directly affect CNS function and can be psychological (e.g. fear), sympathetic nervous system disturbances (e.g. acetylcholine) or inflammatory (e.g. cytokines). Those with a compromised CNS (quite possibly lower cognitive reserve) can develop delirium from seemingly inconsequential levels of these factors, while healthier persons require a more potent insult. Systemic inflammation, in particular, is an example of stimuli which, as previously stated, can differentially impart cognitive dysfunction on individuals.

In summary, systemic inflammation can trigger delirium. Routine systemic inflammation has more pernicious effects on demented individuals, eliciting delirium and hastening progression of the neurodegenerative disease. A body of basic and clinical work offers valuable insight into

neuroimmune interactions in health and disease and can potentially be utilised to identify mediators of inflammation-induced delirium in dementia.

1.5 The innate inflammatory response

The innate immune system drives the non-specific response to pathogens and forms the body's first line of defence, without conferring long-lasting immunity. The innate immune system activates a subsequent adaptive immune response, which, in a manner that demonstrates the intertwined nature of the immune system, can ultimately recruit the innate immune response to eliminate injurious material (Janeway et al., 2001).

Cells of the innate immune system include granulocytes (mast cells, basophils and eosinophils) and phagocytes (monocytes and neutrophils). Such circulating cells translocate into tissue in response to signals from resident innate immune cells, such as dendritic cells and macrophages. These tissue-specific cells express pattern recognition receptors (PRRs), which identify pathogen-associated molecular patterns (PAMPs). PAMPs are evolutionarily conserved microbe-associated molecules such as lipopolysaccharide (LPS) and double-stranded ribonucleic acid (RNA). In addition, PRRs can recognise damage-associated molecular patterns (DAMPs). DAMPs are conserved endogenous cell-associated molecules, such as high mobility group protein B1 (HMGB1) (Gordon, 2002), which are released by stressed cells in response to sterile tissue trauma.

In response to PRR identification of sterile or septic factors associated with trauma or infection, innate-immune cells trigger a complex set of interactions known as the inflammatory response (Nathan, 2002). Cells of the vasculature, such as endothelial cells, are also capable of triggering an inflammatory response. The process involves initial local production of histamine, bradykinin, cytokines, chemokines and prostaglandins which sensitise pain receptors, elicit vasodilatation and control neutrophil recruitment. Upon arrival, neutrophils recruit other leukocytes such as monocytes, which undergo changes once they enter tissue to become macrophages. These cells, and other innate immune cells, release cytokines, which in turn maintain, propagate and modulate acute inflammation.

1.6 A primer of relevant inflammatory mediators

Cytokines are small soluble secreted protein molecules that are used in intercellular signalling, particularly during inflammation (Feghali and Wright, 1997). Prostaglandins are complex and

diverse lipid compounds, which are enzymatically derived from the lipid bilayer of the cell membrane. They form part of the prostanoid class of fatty acid derivatives and have important physiological and inflammatory functions (Hata and Breyer, 2004). Studies have implicated proinflammatory cytokines, namely interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Koj, 1996, Dinarello, 2000), and prostaglandins, especially the E2 subtype (PGE2) (Tilley et al., 2001), as key molecules for mediating systemic inflammation and transduction of signals into the CNS (Konsman et al., 2002). In addition, central induction of the same proinflammatory cytokines and prostaglandins appear to be fundamental in the central response to systemic inflammation (Dantzer, 2004). These inflammatory mediators, which will be studied and discussed in detail in the course of this thesis, are briefly characterised below, in terms of synthesis, signalling and role in neuro-immune interactions.

1.6.1 LPS

LPS is a component of the cell wall of gram negative bacteria, and acts as a potent PAMP and activator of an inflammatory response. It is a widely used prototypical proinflammatory stimulus, which acts as a robust activator of systemic inflammation, and induces production of a wide spectrum of cytokines and other inflammatory mediators (Abbas et al., 2004). LPS binds with the PRR, Toll-like receptor (TLR) 4 and the co-receptor cluster of differentiation molecule 14 (CD14) (Hoshino et al., 1999). Activation of TLR4 leads to activation of two independent intracellular pathways; the myeloid differentiation primary response gene (88) (MyD88)-dependent pathway and –independent pathways. The MyD88-dependent pathway, which is also downstream of IL-1 ligand-receptor activity, triggers production of proinflammatory cytokines, via translocation of the NFκB transcription factor and initiation of the mitogen-activated protein (MAP) kinase biochemical cascade. The MyD88-independent pathway produces type 1 interferons and interferon-stimulated regulatory elements such as interferon-gamma (IFN-γ)-induced protein 10 (IP-10) by activating the interferon-regulatory factor (IRF) 3 (Lu et al., 2008).

1.6.2 Cytokines

IL-1β

This member of the IL-1 cytokine family was one the first cytokines to be identified and characterised. It is a potent cytokine, elevated in both septic and aseptic inflammation (Dinarello, 1996, Chen and Nuñez, 2010). In the periphery, IL-1β is generally not produced by an unstimulated cell (Dinarello, 1996). In the course of this thesis, IL-1β will be the primary focus of IL-1 signalling during inflammation. Studies which use IL-β antibodies and suppression of cleavage of IL-1β to its active form indicate that IL-1β signalling is the major

contributor to IL-1 signalling during inflammation (Dinarello, 2005, Rothwell et al., 1997). However interleukin-1 alpha (IL-1α), another IL-1 protein also appears to have an important role in the systemic and central response to inflammation. The IL-1α protein has an significant role in sterile inflammation but IL-1β appears to be more rapidly expressed and extracellularly active than the predominantly intracellular and locally acting IL-1α (Chen et al., 2007). Recent evidence has demonstrated critical sequential roles for IL-1α and IL-1β in the inflammatory response to necrotic cell death (Chen et al., 2007, Rider et al., 2011). IL-1α, from dying cells, initiates an inflammatory response and triggers neutrophil recruitment, while later IL-1β is essential for macrophage recruitment and propagation of the inflammatory response (Rider et al., 2011). IL-1α has also been implicated in driving movement of white blood cells into the CNS during ischemia and multiple sclerosis (Thornton et al., 2010).

The IL-1 gene is NFκB-responsive and Il-1β is produced in response to local tissue damage (Ortiz et al., 2007), surgery (Haeffner-Cavaillon et al., 1989) and infection (Sansonetti et al., 2000). Post-translation, immature and inactive pro-IL-1β (31 kDa) is cleaved by a specific converting protease, caspase-1 (Thornberry and Lazebnik, 1998), to active IL-1β (17.7 kDa). Two specific receptors for IL-1β (and α) have been characterised (Dinarello, 2009). Its biologic activity is mediated by activation of the type 1 IL-1 receptor (IL-1R1), an 80 kDa protein. The expression of this protein has been demonstrated in the CNS. The type II IL-1 receptor (IL-1R2), a 68 kDa protein, does not seem to elicit any biologic effects upon binding with IL-1β; thus, it is thought to be a decoy receptor. Via IL-1R1, IL-1β regulates gene transcription, largely by activation of NFκB. It can also elicit activation of the MAP kinase biochemical cascades. The IL-1 receptor antagonist (IL-1RA) is a competitive receptor antagonist, upregulated by IL-1 signalling, which binds IL-1R1 but does not elicit intracellular signalling. Administration of exogenous IL-1RA has been shown to be suppress many aspects of the inflammatory response both in the periphery and the CNS (Dinarello, 2005, Loddick et al., 1997).

A clear body of work describes the potency of IL-1β in neuro-immune interactions (Gosselin and Rivest, 2007, Gosselin and Rivest, 2008). In the CNS, its basal expression is low but it is seemingly physiologically active. Expression is rapidly upregulated following ischemia, trauma, hypoxia and neurotoxic or inflammatory stimuli (Allan et al., 2005, Simi et al., 2007). There is also considerable basic and clinical evidence of a role for IL-1β in neurodegeneration. Neuronal death is increased if IL-1β is administered centrally or peripherally after acute brain injury (Lawrence et al., 1998, McColl et al., 2007). In addition, IL-1RA administration has demonstrable neuroprotective effects in acute brain injury (Lawrence et al., 1998, Clark et al., 2007). It has proven difficult to implicate IL-1β directly in clinical studies of

neurodegeneration, this may be largely due to the fact that locally acting IL-1β exerts the deleterious effects (Simi et al., 2007). Additionally, only small amounts of IL-1β (which are often undetectable) can drive clinical diseases (Dinarello, 1996). Presence of the cytokine has been reported on glial cells surrounding β-amyloid plaques (Griffin et al., 1995) and in the cerebrospinal fluid (CSF) of AD patients (Blum-Degena et al., 1995, Cacabelos et al., 1994, Cacabelos et al., 1991). It has also been shown that circulating levels of peripheral cytokines, which are believed to be downstream of IL-1β (namely, IL-6 and acute phase proteins) correlate with infarct volume in the week after stroke, and with clinical outcomes after 12 months (Smith et al., 2004).

IL-6

This cytokine is a complex and particularly pleiotropic inflammatory mediator (Heinrich et al., 2003). The 26 kDa cytokine is released in response to LPS, IL-1β and TNF-α and signals through the IL-6 receptor (IL-6R). This receptor comprises of two subunits, the non-signalling extracellular α binding protein and the signal-transducing receptor glycoprotein 130 (GP130). A soluble IL-6R can also bind IL-6, but this inactive complex must bind with cell-surface GP130 to elicit signal transduction (Taga et al., 1989). The intracellular tyrosine kinases of the Janus kinase family (JAK) are activated via formation of the IL-6/IL-6R complex. Activated JAKs can subsequently phosphorylate transcription factors of the signal transducers and activators of transcription (STAT) family, and STAT3 in particular. STAT proteins translocate to the nucleus and induce transcription of IL-6 responsive genes. The MAP kinase pathway can also be activated by IL-6 signalling.

IL-6 actions are intricate, context-dependent and can be construed as pro- or anti-inflammatory (Xing et al., 1998, Jones et al., 2005, Greenhill et al., 2011). IL-6 administration, elicits robust elevation of hepatic SAA2 protein, an acute phase protein, (Marinkovic et al., 1989, Wang and Dunn, 1998) and the cytokine is known to have a role in a number of autoimmune diseases (Ishihara and Hirano, 2002). However, it has been reported to induce anti-inflammatory mediators IL-1RA and soluble TNF receptor p55 expression (Hopkins, 2007). Furthermore, JAK-STAT signalling may contribute to inflammation resolution, via suppressor of cytokine signalling 3 (SOCS3) activation (Baker et al., 2009, Croker et al., 2012). However, the physiological role of SOCS3 remains controversial and it has also been reported that IL-6 can exert anti-inflammatory effects in a SOCS3-independent manner (Yasukawa et al., 2003).

While central administration of IL-6 can induce a fever response (Lenczowski et al., 1999) and animals given a peripheral administration of IL-6 antibodies fail to develop a normal fever response after inflammatory challenge (Harden et al., 2006), there is little compelling evidence

that systemic administration of IL-6 alone can induce a CNS proinflammatory cytokine response. Wang and Dunn, have demonstrated an acute and short-lived HPA activation after peripheral IL-6 administration (1998). However, peripheral IL-6 challenge fails to elicit endothelial cell NFκB signalling or cyclooxygenase (COX)-2 transcription, which are seen after peripheral IL-1β and TNF-α challenge (Lacroix and Rivest, 1998, Laflamme and Rivest, 1999).

Absence of the induction of its important soluble and membrane-anchored receptor, and GP130 by other pro-inflammatory cytokines, may offer a mechanistic explanation for its lack of effect (Hopkins, 2007, Rivest, 2001). It has been argued that the induction of IL-6R and GP130 by other inflammatory mediators, such as LPS, TNF-α or IL-1β, is necessary for IL-6 signalling at the brain microvasculature (Rivest, 2001), since IL-6 itself does not appear to induce their production. Evidence now suggests that it has a key role in modulating the activity of other inflammatory mediators (Nilsberth et al., 2009). Overexpression of cerebral IL-6 elicits neurologic disease symptoms, such as neurodegeneration and acute-phase-protein production (Campbell et al., 1993). Central IL-6 elevation has been reported in the hippocampus and cortex of AD patients (Wood et al., 1993, Bauer et al., 1991).

TNF-α

This cytokine was first identified over three decades ago as a product of innate immune cells, which can cause lysis and necrosis in various cell types, particularly tumour cells (Carswell et al., 1975). It is a potent inflammatory signaller, which can induce the production of IL-1β and IL-6 (Fong et al., 1989) and has been implicated in rheumatoid arthritis and other autoimmune diseases (Feldmann and Maini, 2003).

Post-translation, TNF-α is a 26 kDa membrane-bound protein, which is cleaved to its soluble form (17 kDa) by TNF-α cleaving enzyme (TACE or A disintegrin and metalloproteinase {ADAM}-17) (Black et al., 1997). Both forms are biologically active and signalling is mediated via two receptors, TNF- receptor 1 (TNF-R1; p55) and TNF receptor 2 (TNF-R2; p75) (Tartaglia and Goeddel, 1992). Ligand binding to p55 elicits complex downstream signalling pathways, via recruitment of cytoplasmic adaptor proteins, the TNF receptor factors (TRAFs) and TNF-receptor-associated death domain proteins (TRADD). The association of FAS-associated death domain and Receptor-interacting protein (RIP) kinase with TRADD elicits activation of caspases and ultimately apoptosis. TRAF recruitment and signalling elicits activation of the NFκB and MAP kinase pathways (Chen and Goeddel, 2002). p75 signalling is largely dominated by TRAF signalling and downstream activation of NFκB and MAP kinase. It can also contribute to apoptosis (Bamji et al., 1998), especially when working in concert with p55 (Varfolomeev and Ashkenazi, 2004).

Similar to IL-1β, TNF-α has been shown to be a mediator of neuro-immune interactions (Turrin and Rivest, 2004, Gosselin and Rivest, 2007). While TNF-α has physiological roles in the CNS, there is also evidence of dysregulation of TNF-α signalling in neurodegeneration. Elevation of CNS TNF has been found in ischemic stroke, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) (McCoy and Tansey, 2008). TNF-α expression is observed around amyloid plaques upon post-mortem examination of AD brains (Dickson et al., 1993). Polymorphisms in the TNF gene promoter region also have been shown to increase risk of AD in an in a number of studies (Laws et al., 2005, Perry et al., 2001).

1.6.3 Prostaglandins

Synthesis

Prostaglandins are a large family of lipophilic, physiological and inflammatory mediators, which are generated from arachidonic acid by activity of cyclooxygenase (COX), the essential and rate-limiting enzyme. Prostaglandin G2 (PGG2) is initially synthesized via the bis-oxygenase activity of COX, followed by generation of prostaglandin H2 (PGH2) via peroxidase activity. This is the starting point for a number of other synthase enzymes to synthesize a wide spectrum of prostanoids, the production of all of which is affected by COX inhibition. Systemic prostaglandins may have an important role in the systemic inflammatory response, which ultimately activates the CNS, but they have a short half life and have generally been assumed not to play a fundamental role in signalling into the brain. However, prostaglandins are lipophilic and can diffuse across the cerebrovasculature (Eguchi et al., 1992, Davidson et al., 2001). Thus, there is a necessity for further investigation of the role of systemic prostaglandins in CNS inflammation.

There is considerable evidence from epidemiological studies, and meta-analyses thereof, that inhibition of COX enzymes with non-steroidal anti-inflammatory drugs (NSAIDs) significantly protects against subsequent development of AD (McGeer et al., 1996, in 't Veld et al., 2001, Etminan et al., 2003, Vlad et al., 2008). However, clinical trials have consistently failed to demonstrate beneficial effects in terms of AD treatment or prevention (Aisen et al., 2003, Lyketsos et al., 2007, Martin et al., 2008). Whether the protection offered by COX inhibition results from depletion of prostaglandins (be that central or peripheral), or by some other mechanism remains unclear. COX inhibition leads to depletion of a wide spectrum of prostaglandins, some of which can ultimately have neurotoxic outcomes, while others can trigger neuroprotective effects (Cunningham and Skelly, 2011). Furthermore, there is some evidence that 'off-target' effects of NSAIDs can affect inflammatory and amyloidgenic pathways. In particular, some NSAIDs (indomethacin, ibuprofen and diclofenac) act as agonists

of peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor transcription factor, which can inhibit NFκB activation and may play an a role in the neuroprotective effects of NSAIDS (Heneka et al., 2010). However, the NSAIDs employed in this thesis are not thought to have these off-target effects and are utilised for their ability to inhibit prostaglandin production.

Two major isoforms of COX have been described, COX-1 and COX-2 (both 72 kDa), which appear to differentially affect inflammation, blood flow, cell death and fever in the periphery and CNS (Bosetti, 2007). Traditionally, it has been understood that COX-1 is the 'housekeeping' enzyme which is constitutively and widely expressed in peripheral tissue and contributes prostaglandins that are important in physiological function (Miller, 2006). COX-1-derived prostaglandins offer protection to mucosal epithelial cells of the stomach and intestine, and their absence leads to gastrointestinal damage, haemorrhage and ulceration. There is also evidence that COX-1-dervived prostaglandins are important in kidney and platelet functioning, along with gestation (Vane et al., 1998). COX-2 is not thought to be expressed under normal conditions in the periphery but is quickly synthesised upon immune stimulation (Crofford, 1997). Thus, COX-2 has been understood to be the primary regulator of inflammation-induced prostaglandins. Selective COX-2 inhibitors have proven very successful in treating some chronic inflammatory conditions (Wood et al., 2001). However, it now seems that both COX isozymes have a role in mediating prostaglandin production in response to inflammatory signalling. COX-1 may be responsible for prostaglandin synthesis in the early stage of inflammation, prior to transcription and synthesis of COX-2 (Park et al., 2009). Moreover, it appears that at high concentrations of arachidonic acid, brought about by acute inflammation, cell injury or exogenous application of arachidonic acid, COX-1-mediated prostaglandin production is greater than COX-2 (Shitashige et al., 1998). While different subcellular locations of the COX isozymes has been reported (Morita et al., 1995), another study, using immunoelectron microscopy, showed that both COX-1 and COX-2 seem to be localised to the endothelial reticulum and nuclear envelope (Spencer et al., 1998).

In the CNS, COX-1 is primarily expressed in microglia and some neuronal populations (Yermakova et al., 1999, Hoozemans et al., 2001) and has recently been shown in perivascular macrophages (PVMs) in rodents (Garcia-Bueno et al., 2009). COX-2, unlike in much of the periphery, is expressed basally in the CNS and is detected in neurons in many brain regions, both in humans and in rodents (Yasojima et al., 1999, Hoozemans et al., 2001). The role of COX-2 in neurodegenerative diseases has been beset by contradictory findings (Choi et al., 2009). However, COX-1, while generally regarded as constitutively active and not induced by inflammation, is increased in a number of neurodegenerative diseases, including AD

(Yermakova et al., 1999, Hoozemans et al., 2001), prion disease (Deininger et al., 2003) and HIV dementia (Griffin et al., 1994). Furthermore, the hypothesis that COX-1 is central to mediating the neuroinflammatory response, given its predominant localisation in microglia, has been gaining support in the literature (Choi et al., 2009).

Prostaglandin E2

Prostaglandin E2 (PGE2) has a number of roles in the periphery, particularly in inflammation, vascular homeostasis and renal function (Miller, 2006). It is the most commonly studied prostaglandin in terms of neuroinflammation and seems to be fundamental for fever, hyperalgesia and activation of the HPA axis. Elevated PGE2 in AD patients, with respect to controls, has been reported (Montine et al., 1999). However, one longitudinal study reported early elevated PGE2, followed by declining levels as memory declined (Combrinck et al., 2006), indicating dynamic alterations in the prostaglandin system (possibly specific to PGE2) during neurodegenerative disease.

There are three PGE2 synthases which convert PGH to PGE2: microsomal prostaglandin E synthase-1 (mPGES-1), microsomal prostaglandin E synthase-2 (mPGES-2) and cytosolic prostaglandin E-synthase (cPGES). mPGES-1 was the first PGE2 terminal synthase identified (Jakobsson et al., 1999) and was shown to be induced in response to IL-1β (Forsberg et al., 2000). The synthase preferentially couples with COX-2-derived PGH2, however, it can work with COX-1 (Radilova et al., 2009) and can be induced in the absence of COX-2 expression (Choi et al., 2008). Although widely understood to be inducible, mPGES-1 has been reported to be constitutively expressed in neurons, microglia, astrocytes and endothelial cells and upregulated in AD (Chaudhry et al., 2008). mPGES-2 has been shown to be capable of coupling with both COX-1 and COX-2 (Murakami et al., 2003, Zhang et al., 2006), although it may show a slight preference for interacting with COX-2 (Murakami et al., 2003). Recently, mPGES-2 protein has been shown in neurons, activated microglia and endothelial cells in normal human brains, and it may be upregulated in neurons in AD (Chaudhry et al., 2010). Others have reported that it is constitutively expressed in quiescent microglia in healthy brains (Ikeda-Matsuo et al., 2005). cPGES is also constitutively expressed in the CNS but its transcription is upregulated in the rat brain in response to LPS challenge (Tanioka et al., 2000). There is also evidence that upon immune stimulation, cPGES can translocate from the cytosol to the nuclear membrane to form PGE2 more rapidly during inflammation. COX-1 and cPGES preferentially couple but as with the other PGE synthases, the relationship does not seem to be necessarily exclusive. It appears that there are situations where coupling of either of the COX isozymes and any of the synthases can occur (Park et al., 2006). cPGES positive staining has

been shown in microglia, neurons and endothelial cells in healthy brains but it is reported to be decreased in the neurons of AD patients (Chaudhry and Dore, 2009).

The role of any local pool of PGE2 appears to depend on both its source (COX and terminal synthase) and its receptor (subtype and location – see below). It seems that the diversity of the receptor actions and expression patterns enables this one prostaglandin subtype to exert a vast spectrum of biological effects. PGE2 signals through the EP receptors 1-4 (Andreasson, 2010) and investigation of discrete roles for each receptor is currently an area of considerable research activity.

Physiologically, EP1 receptors have an important role in activation of the HPA axis (Matsuoka et al., 2003) and also have a role in striatal dopamine signalling (Kitaoka et al., 2007, Matsuoka et al., 2005). However, the EP1 receptor is also thought to mediate neurotoxic effects in ischemia/excitotoxicity, as its inhibition or deletion has been shown to offer neuroprotection via neuronal specific mechanisms, such as improved Ca^{2+} homeostasis (Kawano et al., 2006) and vasoconstriction (Ahmad et al., 2006a). EP2, and possibly EP4 receptors in hippocampal neurons have a role in synaptic plasticity, whereby post-synaptic COX-2/ mPGES1 metabolise PGE2. This PGE2 is released during neuronal activity to act on presynaptic EP2/ EP4 receptors, increasing excitability (Sang et al., 2005). Consistent with these findings, EP2 appears to be largely neuroprotective in stroke/excitotoxicity (Ahmad et al., 2006b, Liu et al., 2005, McCullough et al., 2004). However, others report that microglial EP2 receptors can contribute to amyloid load and oxidative damage in the APP/PS1 AD model (Liang et al., 2005). Moreover, EP2 receptors can hamper microglial phagocytosis and contribute to neurotoxicity (Shie et al., 2005a). Conversely, the EP4 receptor has recently been reported to have an anti-inflammatory role, as it limits proinflammatory cytokine synthesis following LPS challenge (Shi et al., 2010). The EP3 receptor appears to be responsible for temperature regulation and the generation of fever (Ushikubi et al., 1998, Lazarus et al., 2007) and is reported to be the most highly expressed prostaglandin receptor in the brain (Sugimoto and Narumiya, 2007). It has also been hypothesized to be involved in acute cognitive impairments via decreased BDNF transcription (Hein and O'Banion, 2009), although this concept is yet to be explored.

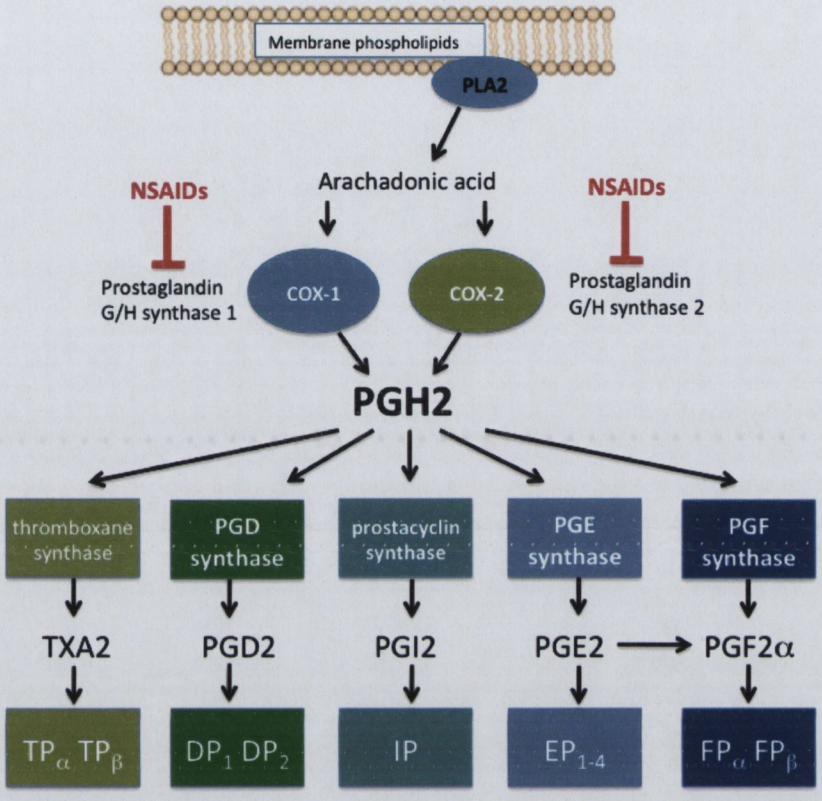

Figure 1.1 *Prostanoid synthesis and action.*
The prostanoids are derived from the release of arachidonic acid from membrane phospholipids by phospholipase A2. Arachidonic acid is subjected to bis-oxygenase and peroxidase activities of the cyclooxygenases (or prostaglandin G/H synthases) to form PGG2 and then PGH2. PGH2 is the substrate for the synthases PGE2 synthase, PGD2 synthase, prostacyclin synthase, PGF2α synthase (PGF2α can also be synthesized directly from PGE2) and thromboxane synthase to synthesize the individual classes of prostaglandins. These classes all have discrete receptor subtypes, through which they initiate their actions. The cyclooxygenases, 1 and 2, are the primary targets of NSAIDs, which may be specific for one isoform or another, selective, or non-selective. Blocking the production of PGH2 via COX inhibition uniformly reduces the levels of all downstream prostanoids. Taken from Cunningham and Skelly (2011).

Table 1.1 *CNS physiological and pathological roles of EP receptors*
Taken from Cunningham and Skelly (2011).

Receptor	Putative Functions	References
EP1	Mediates LPS-induced HPA axis activation	(Matsuoka et al., 2003)
	Promotes dopaminergic signalling in the striatum	(Matsuoka et al., 2005) (Kitaoka et al., 2007)
	Participates in control of impulsive behaviour	(Matsuoka et al., 2005)
	Contributes to neurotoxic effects following excitotoxic insult and ischemic brain damage (via improved Ca^{2+} signalling and vasoconstriction)	(Kawano et al., 2006) (Ahmad et al., 2006a)
EP2	Promotes synaptic plasticity via increases in the amplitude of hippocampal stimulus-evoked EPSPs	(Sang et al., 2005)
	Neuroprotective in models of stroke/excitotoxicity	(McCullough et al., 2004) (Liu et al., 2005) (Ahmad et al., 2006b)
	Contributes to amyloid load and oxidative damage in APP/PS1	(Liang et al., 2005)
	Hampers phagocytosis and mediates neurotoxicity	(Shie et al., 2005b)
EP3	Mediates temperature response to PGE2, LPS and IL-1β	(Ushikubi et al., 1998, Lazarus et al., 2007)
	Mediates LPS-induced HPA axis activation;	(Matsuoka et al., 2003)
EP4	Promotes synaptic plasticity via increases in the amplitude of hippocampal stimulus-evoked EPSPs	(Sang et al., 2005)
	Mediates cerebral vasoconstriction	(Maubach et al., 2009)
	Limits LPS-induced proinflammatory cytokine synthesis	(Shi et al., 2010)
	Mediates PGE2–induced wakefulness via activation of the histaminergic system	(Huang et al., 2003)

Prostaglandin D2

Prostaglandin D2 (PGD2) has been implicated in allergic reactions such as asthma (Matsuoka et al., 2000) and is also known to be the primary mediator of vasodilatation and permeability (Cheng et al., 2006). There is a dearth of research on PGD2 in the brain. It is known to be the most common prostaglandin in the CNS and has been shown to play an important role in sleep (Hayaishi, 2000).

It acts on 2 receptors, DP1 and DP2 (Nagata et al., 1991) and is synthesised by haematopoietic prostaglandin D synthase (HPGDS) and lipocalin-type prostaglandin D synthase (LPGDS) (Urade and Hayaishi, 2000). HPGDS is argued to be the most important enzyme for

inflammation-induced PGD2. It has been shown to have an important role in microglial activation and neuroinflammation (Mohri et al., 2006). In this study, microglial HPGDS and astrocytic DP1 were found to be upregulated in a genetic demyelination animal model. Elevated PGD2, produced by activated microglia was shown to induce astrogliosis and oligodendrocyte apoptosis. Genetic knockout and pharmacological blockade experiments demonstrated that these occurred in a HPGDS- and DP1-dependent manner. It has also been shown to be expressed on microglia surrounding senile plaques in models of Alzheimer's disease and in AD itself, and is thought to couple with COX-1 (Mohri et al., 2007). LPGDS (or β-trace) is one of the most commonly expressed proteins in CSF (Hoffmann et al., 1993) and is expressed on perivascular and microglial cells (Nagasaka et al., 2004, Lee et al., 2012). While it is known to be induced by IL-1β elevation (Muraki et al., 2004), its coupling with the COX isozymes is as yet unclear (Ueno et al., 2005, Mathurin et al., 2011).

Prostaglandin F2 and prostacyclin/ prostaglandin I2 have been studied considerably less, in terms of CNS function and pathology. While CNS transcription of their respective synthases is examined in this thesis, they are not focussed upon to any great extent.

1.7 Neuro-immune interactions

Intricate interactions between the CNS and immune system have been established over the past 25 years. Peripherally produced inflammatory mediators are unlikely to simply diffuse into cerebral tissue to exert effects. It is now widely understood that PAMPS, systemic pro-inflammatory cytokines and prostaglandins can transduce systemic inflammation into the CNS via a number of routes. These routes of communication can be of fast neural basis or a slower humoral basis (Konsman et al., 2002, Dantzer et al., 2008).

Afferent cranial nerves, namely the vagus and trigeminus, activated by locally synthesised cytokines, signal from the visceral organs to the brainstem, to elicit hypothalamic and limbic activation. Electrical stimulation of the vagal nerve induces CNS inflammation, in the form of IL-1β mRNA and HPA activation (Hosoi et al., 2000). The sensory fibres of neurons in the vagus nerve express IL-1R1 and prostaglandin E2 receptor EP3 and local IL-1 produced in tissue stimulates vagal activity, via prostaglandin-dependent and –independent mechanisms (Ek et al., 1998). COX-1-like but not COX-2-like immunoreactivity has been shown on vagal afferents at central terminals and cell bodies, thus this isozyme may play a role in vagal transmission of inflammatory signal (Matsumura et al., 2000). LPS itself is also capable of directly activating the vagal nerve (Hosoi et al., 2005). It has also been argued that complement

factor C5 elicits PGE2 production in the liver, which, in turn, activates vagal afferents in the liver, thus eliciting a CNS response (Blatteis et al., 2004).

Cells along the vasculature of the CNS, such as cerebral endothelial cells and perivascular macrophages (PVMs), can be activated by circulating PAMPs and peripherally generated-pro-inflammatory mediators, such as IL-1β and TNF-α. Recent studies have shown how LPS is capable of directly activating the brain-barrier components PVMs and endothelial cells (Chakravarty and Herkenham, 2005, Gosselin and Rivest, 2008, Serrats et al., 2010). Both endothelial cells and PVMs seem to play particularly important roles in the elevation of brain prostaglandins, PGE2 has been the most widely studied during systemic inflammatory states (Ek et al., 2001). The interplay between the two cell types, in transducing systemic signals into the CNS, is wholly intricate and there is considerable debate regarding their individual contributions (Saper, 2010). At present, it is apparent that there is complex cross-talk between the cells under inflammatory conditions (Serrats et al., 2010), which results in the production of mediators that are fundamental to neuro-immune interactions.

Circulating inflammatory mediators can directly signal into the parenchyma at circumventricular organs (CVOs). These specialised CNS areas, including the area postrema, median eminence, pineal body and choroid plexus, lack the classical tight junctions of the blood-brain barrier (BBB) (Dantzer et al., 2008). At these sites, neurons can respond directly to circulating inflammatory mediators, the axons of these neurons then project into the protected parenchyma through a glial barrier (Flier et al., 1994).

Finally, circulating inflammatory cytokines can also be transported across the BBB by soluble saturable transport receptors (Banks and Kastin, 1991, Gutierrez et al., 1993, Gutierrez et al., 1994). There is also some evidence that LPS and other immune stimuli, can damage the BBB via matrix metalloproteinase action (Rosenberg, 2002). In addition, it is important to state that in ageing and dementia there is evidence of increased BBB breakdown (Farrall and Wardlaw, 2009). Thus, this offers another route of for neuro-immune interactions, in what are already vulnerable populations.

The relative importance of each of these routes remains a subject of debate and undoubtedly the contribution of each can depend on the nature and strength of the immune signal (Dantzer and Kelley, 2007). The pre-eminence that was given to the CVO route (Flier et al., 1994) has been somewhat supplanted in recent times (Saper, 2010). No prostaglandin receptors are expressed in this area, yet many of the brain responses to systemic inflammation (see Section 1.8) appear to depend on prostaglandin synthesis and signalling in the CNS (Oka et al., 2000). Hence, focus has switched to other routes which can generate central pools of prostaglandins. The complex

interaction between non-neuronal cells of the cerebral vasculature, PVMs and endothelial cells has been increasingly implicated in CNS prostaglandin production (Serrats et al., 2010). These cells are capable of activating many responses and can produce prostaglandins downstream of both COX-1 and COX-2 (Garcia-Bueno et al., 2009). While focus initially centred on COX-2, there is now a growing body of evidence showing that prostaglandins derived from both isozymes play an important role in the CNS response to inflammation (Choi et al., 2006, Choi et al., 2009).

The brains resident macrophages, microglia are activated by, and produce inflammatory mediators. Microglia account for about 15% of CNS cells and represent the first, and most important line of the CNS defence (Francesca, 2001). Of myeloid lineage, microglia are mononuclear phagocytes, which, recent evidence suggests, all stem from a common myeloid progenitor cell from early in embryogenesis (Ginhoux et al., 2010). This work implies adult microglial cells are renewed by mitosis in the CNS, rather than infiltration of myeloid cells derived from peripheral haematopoietic stem cells. Old binary conceptualisations of quiescent and activated states have been succeeded by an awareness of the constant motility and dynamic nature of these cells. Neurotoxic stimuli, disturbance of the CNS environment and inflammatory mediators induce rapid alteration in their morphology, cell receptors and secretory profile. As the main constituent of the CNS innate immune system, microglia play a key role in neuroinflammatory responses, both acute and chronic (Streit et al., 2004, Ransohoff and Perry, 2009).

Excessive systemic inflammation can be deleterious for tissue, be that in the periphery or the CNS. There is now strong evidence to suggest that CNS circuitry modulates and balances systemic immune functioning. One inflammatory pathway mediating such a response has been termed the cholinergic anti-inflammatory pathway, as acetylcholine release, both central and peripheral, appears to be a key modulator of systemic inflammation (Tracey, 2002, Tracey, 2009). The CNS, utilising efferent vagal innervations as a conduit, tonically modulates systemic inflammation, via the α7 subtype of nicotinic cholinergic receptor (Parrish et al., 2008). It is obvious that dysregulation of such a system could produce an aberrant systemic inflammatory response. In light of evidence for degeneration of basal forebrain cholinergic neurons (Davies and Maloney, 1976) and decreased autonomic functioning in the cognitively impaired (Collins et al., 2011), an interesting but as yet unexplored issue is whether there is an altered systemic response to inflammation during AD, due to brainstem neurodegeneration and resultant alterations in this anti-inflammatory reflex. Inflammatory mediators also stimulate activation of the HPA axis, which elicits glucocorticoid release from adrenal glands. Glucocorticoids, amongst their many characteristics, are anti-inflammatory, thus neuronal

activation triggers a negative feedback loop, which can terminate the pro-inflammatory response. (Barnes, 1998). Psychological distress and neurodegeneration can cause dysregulation of this system (Magri et al., 2006, Kiecolt-Glaser et al., 2002). Such work, in addition to other bodies of research showing sympathetic nervous system alteration of the adaptive immune system (Elenkov et al., 2000), demonstrates the complex and functionally linked, bidirectional relationship between the immune system and nervous system. These studies also show how disease- or stress-dependent changes in the CNS can alter the systemic immune response to routine inflammatory events.

1.8 Sickness behaviour

Much of what is known about the CNS consequences of a systemic inflammation has been gleaned from the study of sickness behaviour (Dantzer, 2001). This concept describes a wide range of evolutionarily conserved adaptive alterations in mood, energy and motivation, initiated by the host, to aid efforts in containing and counteracting infection. The body commonly mounts a fever response in reaction to systemic inflammation; this is important for fighting invading pathogens and increasing the efficiency of the immune system. The fever response is accompanied by behavioural changes, such as reduced social activity, depression, anxiety, hyperalgesia, fatigue, anorexia, adipsia and hypersomnia (Dantzer, 2004).

While it has long been recognised that systemic inflammation alters mood, energy and motivation, these changes were previously understood to be by-products of infection and the effort exerted by the organism to fight it. In contrast to this conceptualisation, Hart (1988), influenced by identification in the 1960s of blood-borne pro-inflammatory cytokines or "factor X", which act upon the brain, proposed that these alterations represent a concerted and advantageous reorganisation of priorities by an infected individual. These stereotyped behavioural changes such as anorexia, temperature alterations and reduced energy expenditure appear to create the optimal physiological environment to mount a response to infection and injury. Specific advantages of some individual components of the sickness response have been posited, such as reduced food intake resulting in lower gut levels of iron, which can facilitate bacterial proliferation (Kluger and Rothenburg, 1979). Here, what is known about the mechanisms underlying some of the stereotyped behaviours will be briefly introduced (see Dantzer et al., 2008 for detailed review).

Thermoregulatory responses:

The alteration in body temperature in response to systemic inflammation has been studied in detail. It is commonly believed that LPS/IL-1β-induced PGE2 in the hypothalamus is fundamental to this response. Vascular COX-2 generated PGE2 binds with EP3 receptors in the median preoptic nucleus (MPO) (Lazarus et al., 2007), thus initiating GABAergic medullary signalling, which drives the fever response (Nakamura et al., 2002). Recent evidence also demonstrates a role for peripheral HPGDS-dependent PGD2 in LPS-generated fever (Gao et al., 2009): this could have important implications for understanding of the fever response.

Anxiety and the stress response

HPA activation forms an important part of the host response to invasion or injury (Besedovsky et al., 1986). Glucocorticoids (the primary one in rodents is corticosterone and in humans is cortisol) produced by the adrenal cortex have widespread effects including catabolism of energy reserves to overcome the triggering inflammatory insult, suppression of excessive inflammation and alteration of CNS function. Adrenocorticoctropic hormone (ACTH) secreted from the pituitary gland triggers the release of corticosteroids. ACTH release itself is controlled by corticotrophin-releasing factor (CRF) (Engelmann et al., 2004). LPS and IL-1β can induce PGE2 synthesis and signalling in the ventrolateral medulla and this route appears to be important in mediating CRF release and thus HPA activation (Ericsson et al., 1994). The medulla can be activated via activation of cerebral vasculature cells, which in turn activate noradrenergic and adrenergic neurons that project to the paraventricular nucleus of the hypothalamus to trigger CRF release (Ericsson et al., 1997). There is increasing evidence that both COX isozymes play important roles in the HPA response to immune challenge (Elander et al., 2009, Elander et al., 2010). The closely related anxiety response to inflammation also seems to be initiated in the brain stem. Noradrenergic projections, activated by vagal afferents and CVO macrophages, which respond to LPS and IL-1β, signal to the amgydala and ultimately drive the behavioural changes (Gaykema et al., 2007).

Behavioural depression

There is a clear reduction in motivation to engage in rewarding tasks and locomotor activity during systemic inflammation, especially following LPS and IL-1β induced inflammatory challenges. In addition, there is decreased burrowing and social exploration (Dantzer et al., 2008). Whether these activities are specifically suppressed, or there is a more general reduction in motivation, remains unclear. Increased amygdalic and hypothalamic activation, as well as deactivation of the hippocampus, have been associated with depression in behavioural activities (Dantzer et al., 2008). Specific mediators and brain regions have been implicated in these changes. Vagotomy (Konsman et al., 2000) and COX-1 inhibition (Teeling et al., 2010) can

both attenuate behavioural changes, although it is unclear if these mechanisms are linked. As with many of the sickness responses, IL-1β appears capable of behavioural suppression; this has been demonstrated with systemic administration of the cytokine and central inhibition of its actions (Konsman et al., 2008).

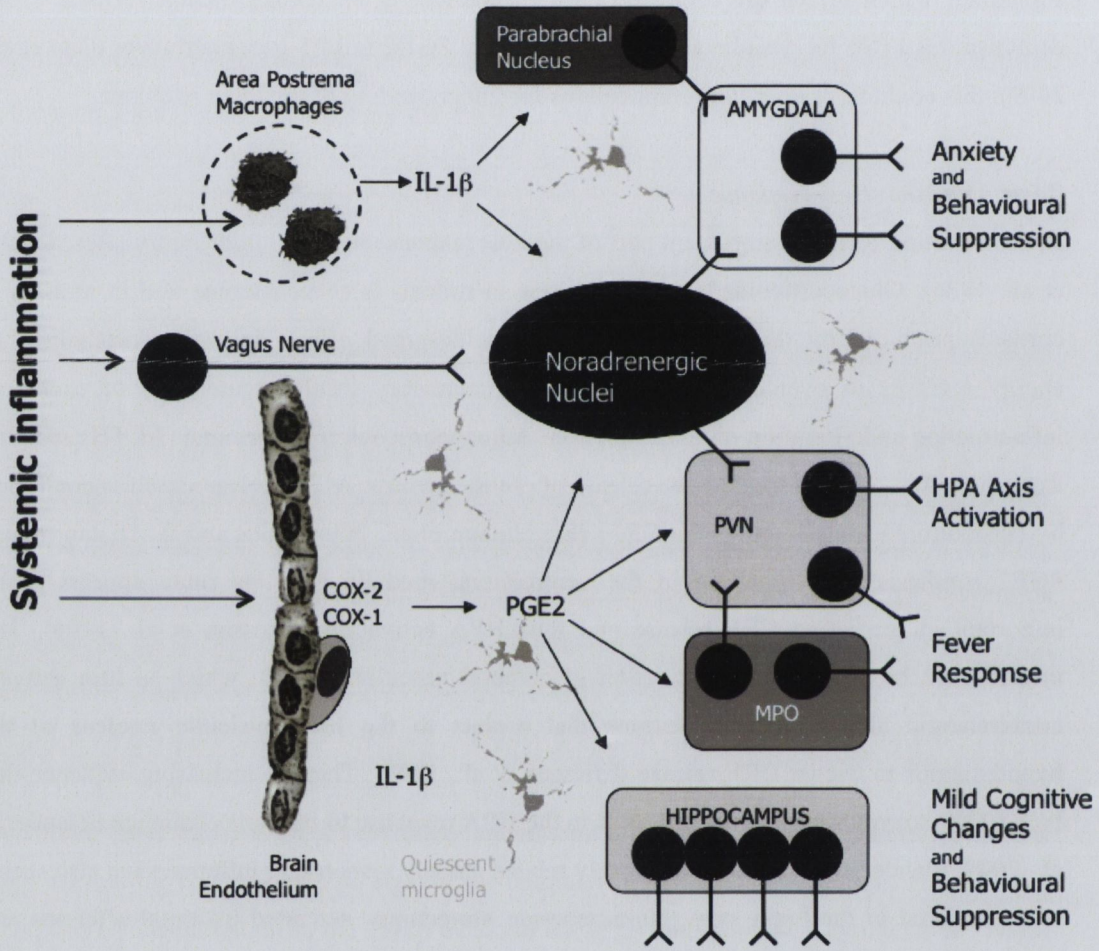

Figure 1.2 *Sickness Behaviour Responses*

This scheme represents current knowledge of the sickness behaviour response gleaned from animal research. Systemic inflammation is signalled to the brain via activation of the brain endothelium, the vagus nerve or circumventricular organs, such as the area postrema (transporters of inflammatory mediators are not shown). The endothelial cells and perivascular macrophages express COX-2 and COX-1 to synthesize PGE2 and other prostanoids. PGE2 is known to activate hypothalamic centres via EP3 and EP1 receptors to drive fever and stress responses. Anxiety responses are mediated by the amygdala, which is activated directly by both IL-1β and brainstem noradrenaline release, or indirectly by neuronal activation from the parabrachial nucleus. The hippocampus can be activated by both IL-1β and PGE2 contributing to disengagement from the environment and cognitive changes. These changes occur on a background of microglia maintained in a quiescent state (shown in grey). Taken from Cunningham (In Press).

1.9 Systemic inflammation and neuropsychiatric conditions

Over the past 15 years psychoneuroimmunology investigators have sought to assess the role of systemic inflammation and individual circulating inflammatory mediators in various conditions of altered CNS functioning, beyond sickness behaviour syndrome. Longitudinal studies have implicated elevated systemic IL-6, in particular, in many adverse outcomes. Those with higher IL-6 have been reported to have worse social relationships and sleep efficiency (Friedman et al., 2005), reduced hippocampal volume (Marsland et al., 2008) and higher rates of cognitive decline (Yaffe et al., 2004, Yaffe et al., 2003, Luterman et al., 2000). This is surprising given the paucity of evidence for IL-6 being fundamental to neuro-immune interactions. Elevations of both pro-inflammatory cytokines and prostaglandins have also been linked with neurodegenerative conditions, such AD (Tan et al., 2007, Swardfager et al., 2010, Chen et al., 2008a). Studies of depression have implicated systemic IL-1β, TNF-α and IL-6 (Miller et al., 2009, Dowlati et al., 2010). These studies demonstrate ongoing and intricate neuroimmune interactions in humans. In addition, it is obvious that the association of neuropsychiatric conditions with the elevation of pro-inflammatory cytokines mirrors aspects of classical inflammation-induced sickness behaviour responses in animals.

Given their importance in modulating the systemic response to inflammation and their implication in sickness behaviour and a host of neuropsychiatric conditions, there is a surprising lack of systematic comparisons of the effects of individual pro-inflammatory cytokines on the brain. Comparisons of the CNS effects of peripheral administration of IL-1β, TNF-α and IL-6 have concentrated on single aspects of the neuroinflammatory response, be that endothelial and parenchymal transcription of COX-2 (Lacroix and Rivest, 1998) or IκB (Laflamme and Rivest, 1999), or single aspects of sickness behaviour, such as temperature (Wang et al., 1997, Lacroix and Rivest, 1998, Laflamme and Rivest, 1999). Thus, it is difficult to compare how circulating levels of individual cytokines can differentially elicit a CNS inflammatory response, which might induce alterations in sickness behaviour. Ultimately, cytokine-induced alterations in cognitive function are of interest in this thesis and this line of investigation would also be aided by an understanding of the effects of systemic elevation of each of the cytokines. Consequently, a primary aim of this thesis was to characterise the acute systemic and CNS (hypothalamic and hippocampal) responses to peripheral administration of the classic pro-inflammatory cytokines IL-1β, TNF-α and IL-6 (Chapter 3).

1.10 Cognitive impairment during systemic inflammation

A considerable number of studies have investigated whether systemic inflammatory events can elicit acute cognitive impairments, in addition to sickness behaviour responses. It is clear that severe systemic inflammation, or sepsis, can interfere with cognition. Sepsis-associated delirium is a common complication of severe systemic inflammation in all age groups (Ebersoldt et al., 2007) However, evidence suggests that acute impairment in these situations may be related to direct brain injury, rather than arising from a controlled adaptive CNS response to inflammation. There are considerable long-term consequences of severe systemic inflammation, such as significant brain injury and long-term cognitive impairment (MacLullich et al., 2009, Iwashyna et al., 2010). Animal models support the argument that direct brain insults cause acute CNS dysfunction, during severe peripheral inflammation. Profound systemic inflammation, via cecel ligation and puncture (CLP) sepsis, can elicit acute cognitive and behavioural impairment in previously healthy animals (Shimizu et al., 1999). CLP also elicits acute vasogenic edema, increased blood flow, cytotoxicity (Bozza et al., 2009) and decreased glucose metabolism (Semmler et al., 2008), suggesting that direct brain injury may account for the acute cognitive impairment. A distinct question is whether milder, more routine systemic inflammation causes cognitive dysfunction. This is an area of contentious debate and many issues remain to be elucidated.

Immune insults which are not cytotoxic have been shown to be capable of having deleterious effects on neuronal functioning and performance in some cognitive tasks. Long-term potentiation (LTP) is the most widely used electrophysiology model for investigating plastic changes in synaptic connections and the changes that underlie maintenance of LTP have been hypothesised to parallel those that underlie memory consolidation (Lynch, 2004). It is unclear whether synaptic plasticity is required for memory but numerous studies demonstrate that inhibited plasticity occurs with memory impairment (Martin et al., 2000). Consequently, the best direct evidence that inflammation is capable of affecting neuronal substrates involved in cognitive functioning comes from LTP studies. In numerous studies, elevated IL-1β has been shown to impair the induction and maintenance of LTP in the cornu ammonis 3 (CA3) (Katsuki et al., 1990), the cornu ammonis 1 CA1 (Bellinger et al., 1995) and the dentate gyrus of the hippocampus (Cunningham et al., 1996). Ageing- or stress-related elevation, along with exogenous administration of IL-1β, has been shown to negatively affect LTP maintenance (Griffin et al., 2006, Lynch, 1998). Administration of this cytokine also negatively impacts basal synaptic function (Bellinger et al., 1993). Overexpression of IL-6 (Bellinger et al., 1995), elevated TNF-α (Cunningham et al., 1996) and increased PGE2 (Kotilinek et al., 2008) also negatively affect hippocampal LTP. Thus, endogenous and exogenous elevation of

inflammatory mediators impairs hippocampal LTP, which may be a substrate of memory. Apart from questions regarding the relevance of LTP to memory consolidation, it also remains unclear how CNS levels of inflammatory mediators generated by systemic inflammatory challenge, compare to the levels of these mediators required to interfere with neuronal functioning in the LTP model.

Peripheral and central immune challenges can impair functioning on cognitive tasks (Yirmiya and Goshen, 2011, Cunningham and Sanderson, 2008). LPS and IL-1β can induce impairments in the Morris Water Maze (MWM) (Oitzl et al., 1993, Gibertini et al., 1995). However, the metrics by which the researchers assert cognitive impairment are confounded by sickness behaviour responses, induced by the immune challenge. Some widely used cognitive tasks must be adjusted for assessment of inflammation-induced changes. Anxiety and reduced motivation are particular issues that should be taken into account (Cunningham and Sanderson, 2008).

The contextual fear conditioning task (CFC) is less vulnerable to sickness behaviour confounds and has been used to firmly demonstrate one form of inflammation-induced cognitive dysfunction. In this task, animals experience a novel environment which they explore. During exploration the animals are exposed to an auditory cue followed by a foot shock. Inflammation induced directly after this exposure session impairs their memory for the context in which the shock was encountered. Systemic LPS (Pugh et al., 1998) has been shown to be capable of interfering with consolidation of the memory of the context within which the animal received a shock. That is, animals that have had these immune challenges, display less freezing than unchallenged animals on subsequent exposure to the same context. Central IL-1β and PGE2 appear to have roles in this impairment (Hein et al., 2007, Frank et al., 2010). Thus, consolidation of contextual memory is impaired by systemic inflammation.

Maze and colleagues have implicated IL-1 and TNF-α in a model of postoperative cognitive dysfunction (POCD) using the CFC. IL-1RA-treated animals were protected from systemic LPS- and tibial fracture-induced inflammation and memory impairments (Terrando et al., 2010b, Cibelli et al., 2010). Additional work has demonstrated that systemic TNF-α is upstream of IL-1β in the surgery-induced impairment (Terrando et al., 2010a). With this work, the sensitivity of the CFC task is particularly pertinent, since the inflammatory response to both high dose of LPS and tibial fracture is considerable. Nonetheless, use of contextual fear conditioning demonstrated that relatively severe surgical trauma impairs the ability of previously healthy rodents to consolidate or lay down a new memory.

Other cognitive functions seem to be more resistant to inflammation-induced impairment. Visuospatial memory, in particular, appears to function relatively efficiently, even in the face of considerable systemic inflammation induced by live influenza virus (Jurgens et al., 2012). Models of *mycoplasma salivarium* (Swanepoel et al., 2011) and bacterial staphylococcal infections (Woodruff et al., 2011) have failed to elicit impairments in performance on the MWM. Sparkman and colleagues (2006) demonstrated that mental flexibility, but not visuospatial memory, is impaired by systemic inflammation, in an IL-6-dependent manner.

It is seems doubtful that impairment of cognitive function could be an adaptive response, unlike other aforementioned sickness behaviour responses. However, such an argument cannot be used to undermine existing reports of impairment during inflammation and highlights problems inherent in adaptationism, with regards sickness behaviour, and biology in general (Orzack and Sober, 1994). Not all traits can be adaptive; some elements of functioning are subject to the constraints of overcoming the fundamental environmental pressure (e.g. infection). Cognitive impairment, and perhaps other features of sickness behaviour, may not be adaptive; rather they might be a by-product of infection and immune efforts to eliminate the pathogen. Conversely, one could argue that improved memory would be a far more adaptive response during systemic inflammation. If some stimulus has triggered sickness in a host, it would be advantageous to remember this, to aid avoidance in the future. There is evidence for this: animals rapidly develop a taste-aversion if IL-1β is administered with palatable food (Tazi et al., 1988).

Studies in which healthy adults were given low dose immune challenges have produced equivocal and somewhat paradoxical results. Subjects in these studies report stereotypical sickness responses, such as decreased mood and increased levels of anxiety. An early study reported negative cognitive effects, in terms of verbal and non-verbal memory, in response to low-dose endotoxemia (Reichenberg et al., 2001). However, subsequent studies have failed to recapitulate these results in a consistent manner (Grigoleit et al., 2010, Grigoleit et al., 2011, Van Den Boogaard et al., 2010). In one of these studies, self-reported alertness was profoundly lower after immune challenge but objective alertness, as measured by reaction time, was actually improved (Grigoleit et al., 2011). This suggests that the conserved sickness behaviour response may act to reduce the feeling of sharpness, which would lead the host to resting. However, cognitive performance, which is independent of this judgement of perceived ability, is at least maintained, if not sharpened. Yet, within the same study it was reported that a lower dose of LPS decreased long-term memory performance, suggesting two counteracting mechanisms by which cognition can be affected by systemic inflammation. Another study has shown that LPS administration failed to induce cognitive impairment and actually elicited higher alertness as quantified by EEG analysis (Van Den Boogaard et al., 2010). Overall, it

appears that inflammation-induced alterations in facets of cognition are definitively mild in healthy animals and humans, and that pervasive cognitive impairment is not a core feature.

1.11 The consequences of combining systemic inflammation and neurodegenerative disease

Multiple clinical studies have demonstrated an association between plasma levels of inflammatory mediators and delirium (for review see Khan et al., 2011). In terms of a biomarker of delirium risk, IL-6 and IL-8 (the human homologue of mouse CXCL1) have been shown to be elevated prior to onset of delirium in hip fracture patients (van Munster et al., 2010, van Munster et al., 2008). C-reactive protein (CRP), a marker of the inflammatory response, was also shown to predict delirium incidence (Macdonald et al., 2007). Plasma inflammatory markers have also been examined in medical and surgical patients displaying cognitive dysfunction. Again, significant associations between both IL-6 and IL-8 and delirium have been shown (de Rooij et al., 2007, van Munster et al., 2010, van Munster et al., 2008, Plaschke et al., 2010). Causation cannot be implied from the associations described above and others studies have failed to reproduce positive associations (Lemstra et al., 2008). However, these studies support a large body of work which suggests that infection and other inflammatory events increase the likelihood of, and most probably can elicit, episodes of delirium.

It is difficult to tally studies demonstrating an association between elevated inflammatory markers and delirium, the profound global impairment in cognition, with studies which illustrate the mild effects of inflammatory challenge on cognition in animals and humans. However, it is important at this juncture to restate that a large body of evidence has now demonstrated how prior cognitive impairment and ageing are the primary risk factors for developing delirium. Indeed, in one biomarker study the association between elevated IL-6 and delirium was lost when adjusted for prior cognitive impairment (van Munster et al., 2010). In the aged and, particularly, the demented, mild systemic inflammatory events elicit delirium, while the same events have negligible effects in healthy individuals. That is, the relationship between mild systemic inflammation and delirium seems dependent on some prior pathology of the cognitive circuitry in the CNS. Why might systemic inflammation produce more profound effects in certain individuals? Reduced cognitive reserve, and the consequent lower threshold at which stimuli will affect cognitive function, appears to be a plausible candidate mechanism. However, a body of work from our laboratory and others has also shown that diseased and aged animals also display a different CNS inflammatory response to peripheral inflammation.

The first report of an exaggerated response to systemic inflammation in animals with neurodegenerative disease was by Combrinck and colleagues (2002). In that study, animals with a progressive neurodegenerative disease (ME7 murine-adapted form of the prion disease, scrapie, normally occurring in sheep) were shown to have an exaggerated fever and sickness behaviour response, as evidenced by exaggerated hypothermia and reduced locomotor activity, in comparison with healthy control animals after systemic LPS administration. This phenomenon was demonstrated in animals 18 weeks post-ME7 inoculation and subsequently repeated in animals 12 weeks post-inoculation (Cunningham et al., 2009), and with the viral mimetic poly I:C as the systemic inflammatory challenge (Field et al., 2010). An exaggerated sickness behaviour response has also been shown to occur in aged compared to young animals after LPS administration (Godbout et al., 2005). Notably, AD patients who experience systemic inflammatory events also demonstrate exaggerated sickness behaviour symptoms in comparison with healthy elderly individuals (Holmes et al., 2011).

The ME7 murine strain of prion disease is a terminal progressive neurodegenerative condition characterised by extracellular amyloidosis, synaptic loss, neuronal death and irreversible cognitive decline (Betmouni and Perry, 1999, Cunningham et al., 2003). Thus, ME7 mice represent a model for the progressive and irreversible cell death, synaptic loss, extracellular amyloidosis, microglial activation and cognitive decline seen in human populations with dementia.

In 2005, Cunningham et al., reported a mechanism which may explain, at least in part, the exaggerated sickness behaviour response in the aged and diseased brain to systemic inflammation. The study demonstrates how microglia are 'primed' by neurodegenerative brain to show an exaggerated response to subsequent inflammatory challenge, be it centrally or peripherally administered. In ME7 disease, microglia show morphological evidence of activation, yet display limited or muted production of inflammatory mediators. However, upon LPS challenge, the cells undergo a phenotypic switch and produce a robust inflammatory response, which is exaggerated, in comparison to that seen in normal brains, with abundant IL-1β and iNOS expression and enormous neutrophil infiltration (Cunningham, In Press). Importantly, the systemic inflammatory response to peripheral immune challenge is equivalent in diseased and normal animals (Murray et al., 2012). Hence, the CNS response is amplified, in terms of pro-inflammatory cytokines mRNA transcription and protein synthesis, in diseased animals compared to normal. The concept that the diseased brain primes microglial cells to show an exaggerated response to subsequent inflammatory challenge has been termed microglial priming (Cunningham et al., 2005). The nomenclature of microglial priming derives

from original immunology studies of macrophage priming. These studies showed that when macrophages were primed by an agent (IFN-γ) the cells produced more robust response to subsequent LPS stimulation, characterised by IL-1β and inducible nitric oxide synthase (iNOS) translation (Pace et al., 1983, Steeg et al., 1982). Subsequent studies have demonstrated that an exaggerated CNS inflammatory response is a generic phenomenon in CNS pathology and it has been observed in ageing (Godbout et al., 2005) and models of AD (Lee et al., 2002, Kyrkanides et al., 2011), PD (Godoy et al., 2008) and Wallerian degeneration (Palin et al., 2008).

There are also long-term consequences of systemic inflammation on the course of neurodegenerative disease in animal models. More severe axonal pathology and disease progression was observed in an animal model of ALS, after weekly LPS administration. Systemic inflammation also worsens AD pathology, in terms of amyloid precursor protein (APP) processing, β-amyloid deposition and tau pathology (Kyrkanides et al., 2011, Sheng et al., 2003, Kitazawa et al., 2005). Repeated administration of poly I:C cumulatively worsened ME7 disease progression (Field et al., 2010). Each individual challenge exacerbated neurological symptoms of disease but the animals recovered less as disease progressed. Single relatively modest inflammatory challenges are also capable of worsening disease pathology. One dose of LPS (500 μg/kg) induces prolonged neuroinflammation and apoptosis, and hastens progression of irreversible neurological decline in ME7 animals (Cunningham et al., 2009).

In summary, systemic inflammation superimposed on neurodegenerative disease in animal models causes exaggerated neuroinflammation, sickness responses and more aggressive disease progression. These data from animal models suggest that systemic inflammation significantly impacts on dementia progression in humans and this has heralded an entirely new area of investigation in chronic neurodegenerative disease. As previously discussed, mild to moderate systemic inflammation also elicits delirium, or acute cognitive dysfunction in this vulnerable population (Section 1.4). Recently, our laboratory has begun to investigate the acute cognitive consequences of systemic inflammation during CNS disease in an effort to understand delirium.

1.12 Systemic inflammation induces acute cognitive impairment during neurodegenerative disease: An animal model of delirium during dementia

In light of the exaggerated neuroinflammatory consequences of systemic inflammation in aged animals and animals with CNS disease, a number of research groups have begun to assess whether such animals are more susceptible to disturbances of cognition than healthy animals. Barrientos and colleagues (2006) showed that an *E. Coli* infection impaired the ability of aged

animals, but not young animals, to consolidate memory of context on the CFC task. Aged animals also demonstrated significantly higher CNS IL-1β synthesis. As stated earlier, the CFC task is particularly sensitive to inflammation-induced dysfunction (Section 1.10). Nonetheless, use of contextual fear conditioning demonstrates that aged animals are more likely to develop systemic inflammation-induced impairments of memory consolidation. A recent study by this group also demonstrates that the combination of ageing and peripheral immune challenge interact to interfere with LTP in the hippocampus (Chapman et al., 2010). The theta-burst late-phase impairment in synaptic plasticity mirrored the long-lasting impairment on memory consolidation and could be blocked with central administration of IL-1RA. However, in this model the authors were not able to demonstrate disruption of basal synaptic transmission or short-term plasticity, which are more relevant to impairment of working memory and attention, deficits fundamental to episodes of delirium (WHO, 1992, APA, 1994). Demonstrating that CNS pathology (be that from ageing or disease) confers a vulnerability to inflammation-induced impairment in these facets of cognition, requires cognitive assessment that is dynamic and etiologically valid.

Other work has shown that mental flexibility or working memory is more disrupted in immune-challenged aged animals, compared to young animals. One study, using the MWM, demonstrated reduced ability of LPS-treated aged animals to learn the new location of a hidden platform, which is moved each day of testing, compared to unchallenged aged animals or LPS-treated younger animals (Chen et al., 2008b). Again, challenged aged animals exhibited exaggerated hippocampal inflammatory markers. Our laboratory then demonstrated that animals with prior neurodegeneration also show impairment on a task, which assesses mental flexibility, during systemic inflammation (Cunningham et al., 2009). ME7 animals were impaired on learning the location of an exit on a shallow water Y-maze reference learning task after systemic LPS challenge (100 μg/kg) but weren't impaired if they had learned the exit prior to challenge. Thus, when an animal with reduced CNS function (from ageing and neurodegeneration) experiences a systemic inflammatory event, the ability to attend to or incorporate new information, rather than recall of visuospatial memory, is impaired.

The above data demonstrate acute disruption of cognition during episodes of systemic inflammation in aged and diseased animals. These conditions recapitulate a common biological context in which delirium is seen in humans. Our laboratory has recently developed a task to specifically assess acute and reversible working memory/attentional dysfunction in diseased animals given a systemic inflammatory challenge (Murray et al., 2012). Impairment in these facets of cognition form part of the core symptoms of delirium. The aim of testing diseased

animals on this task after systemic inflammatory challenge was to create the first animal model of delirium during dementia, in which to interrogate possible mechanisms.

The model again employs the superimposition of systemic LPS (100μg/kg) on a background of chronic progressive neurodegenerative disease, ME7 disease (Murray 2012). Animals are trained on a novel paddling water-based T-maze alternation task, which tests attention or working memory. On the T-maze task, animals are required to retain information for 30 seconds (the arm they had just escaped the maze through) and utilise this information to escape the maze upon re-entry by taking the exit on the opposite side of the 'T'. ME7 animals, 12 weeks post-inoculation display intact performance on this task. However, when systemically challenged with LPS, they show decreased alternation, which indicates attention or working memory impairment. Attention or working memory impairments are key features of the delirious episode as defined by DSM IV and ICD-10 definitions (APA, 1994, WHO, 1992). A recent neuropsychology study of delirium ably demonstrates that this impairment is independent of the functioning of crystallised memory. Delirious patients are able to recall information that has already been learned or consolidated but are impaired on tasks which require fluid cognitive ability; that is the ability to retain and manipulate novel information (Brown et al., 2011a).

A further strength of this task is the way in which it assesses the ability to retain information for 30 seconds. This aspect of cognition is particularly disturbed in patients with delirium but not dementia alone (Brown et al., 2011b). Another key diagnostic criterion for delirium is that the disturbance is of acute onset and reversible. (Inouye et al., 1990). On the T-maze task, animals that had intact performance show sudden inflammation-induced impairment, which has resolved 24 hours later.

Since microglial priming effects amplified CNS inflammation with equivalent systemic inflammation, it offers a plausible mechanism by which the acute impairment occurs in diseased animals when experiencing banal systemic inflammatory events. The microglial priming phenomenon now forms an important part of current understanding of inflammation-induced delirium (van Gool et al., 2010). As discussed above, there is electrophysiological evidence that inflammatory mediators affect neuronal functioning; however, the final mechanism by which neural circuitry is disrupted remains unclear. Indeed, inflammatory mediators could act on neurons themselves or alter astrocyte activity, or interfere with neurotransmitter synthesis or uptake. Evidence from our laboratory, however, indicates that heightened CNS inflammation alone is not sufficient to elicit acute cognitive dysfunction. Increasing the strength of the inflammatory challenge in normal animals does not induce

impairment. On T-maze and Y-maze tasks, it has been shown that animals challenged with LPS doses two and five times higher than that which induces impairment in diseased animals show intact performance (Murray et al., 2012, Cunningham et al., 2009). Thus, there appears to be an interaction between exaggerated inflammatory response and prior pathology which elicits the acute cognitive dysfunction. In a similar manner, relatively mild inflammatory events only induce delirium in the elderly or in those with prior cognitive impairment.

With this task, there is the potential to begin to investigate many of the poorly understood aspects of inflammation-induced delirium with molecular work and pharmacological intervention. We can investigate the factors in neurodegenerative disease which might make individuals vulnerable to systemic inflammation. Moreover, we can examine what systemic inflammatory mediators are important, how they signal to the brain and what central inflammatory mediators affect neuronal function. Studying these factors within an etiologically valid animal model of delirium during dementia makes findings more translatable to relevant clinical populations.

1.13 Objectives of the current work

Delirium is a highly prevalent condition with serious medical and economic implications. However, as a result of poorly understood pathophysiology and dearth of etiologically valid animal models, few inroads have been made on development of therapeutic intervention to improve patient treatment and prognosis. One tractable way of investigating the mechanisms of a major cause of delirium, systemic inflammation in vulnerable individuals, is to use an animal model of prior neurodegeneration. By superimposing systemic inflammation on disease, we can recapitulate a common situation in which the condition occurs. The overarching objective of this thesis is to study the CNS consequences of systemic inflammation and to use the ME7 model of prion disease to investigate the inflammatory pathways to working memory dysfunction, which has relevance to the core features of delirium.

- Detailed CNS comparisons of systemic challenges with pro-inflammatory cytokines IL-1β, TNF-α and IL-6 across compartments are lacking. This is important for identifying key mediators in LPS-induced inflammation and for understanding the inflammatory consequences of the individual pro-inflammatory cytokines. Thus, the aim of the first study in this thesis (Chapter 3) was to compare the systemic and CNS effects of LPS and the pro-inflammatory cytokines which are commonly implicated in

neuro-immune interactions. This study generated a systematic analysis of the acute effects of IL-1β, TNF-α and IL-6, along with LPS and saline, comparing the behavioural, systemic and CNS responses in C57BL/6 mice. The effects of these inflammatory molecules on locomotor activity, core body temperature, plasma inflammatory mediators, and hepatic, hypothalamic and hippocampal gene transcription were assessed 2 hours post-challenge.

- The second study in this thesis (Chapter 4) utilises the novel animal model of delirium during dementia; mimicking aspects of delirium in ME7 animals after systemic LPS. The first aim of this study was to investigate whether the progression of ME7-disease across time affects the vulnerability of animals to inflammation-induced acute episodes of cognitive impairment. To assess this, performance of ME7 animals on our novel T-maze alternation task was observed weekly, to identify the point in time where animals are on the cusp of impairment (determined by progression to permanent impairment on the task). The profile of LPS-induced impairment in animals at this point (16 weeks post-inoculation) and 12 weeks post-inoculation was then compared to determine whether there were differences in the incidence, severity or duration of induced impairment. The second aim was to identify targetable inflammatory mechanisms which have a role in these acute inflammation-induced episodes of impairment. Alterations in the prostaglandin system were assessed in ME7 animals and NSAIDs with differing COX isozyme selectivity were tested for efficacy in blocking cognitive deficits. The final aim of this study was to investigate whether blockade of systemic cytokines protects from LPS-induced sickness behaviour and neurodegeneration.

- The final study (Chapter 5) aimed to investigate the role of IL-1β in inflammation-induced acute cognitive dysfunction in animals with ongoing neurodegeneration. The contribution of IL-1β signalling to LPS-induced working memory deficits was examined using administration of IL-1RA and ME7-inoculated IL-1R1-/- animals. A further and consequent aim of this work was to assess whether systemic administration of IL-1β can elicit acute cognitive dysfunction. The peripheral and central inflammatory responses in ME7 animals to systemic administration of IL-1β were examined to support this cognitive work.

Chapter 2: Materials and Methods

2.1 Materials

Animals and *in vivo* Products

C57BL/6 mice	Harlan laboratories, UK
IL-1RI-/- / C57BL/6	Prof. Kingston Mills/Jackson Laboratory, USA
Laboratory mouse diet	Red Mills, UK
T-maze	Custom designed in TCD
Horizontal bar	Custom designed in TCD
Inverted screen	Custom designed in TCD
Thermocouple rectal probe	Thermalert , USA
Peristaltic pump	Gilson, France
Formalin solution, neutral buffered, 10%	Sigma-Aldrich, UK
Microsyringe	Hamilton, USA
Stereotaxic frame	David Kopf Instruments, USA

Treatments

Dexamethasone-21-phospate	Sigma-Aldrich, UK
Mouse recombinant IL-1β	R & D Systems, UK
Human recombinant IL-1RA	Peprotech, UK
Mouse recombinant IL-6	Peprotech, UK
Nimesulide	Sigma-Aldrich, UK
Piroxicam	Sigma-Aldrich, UK
Salmonella equine abortus Lipopolysaccharide, L5886	Sigma-Aldrich, UK
SC-560	Cayman Chemicals, USA
Mouse recombinant TNF-α	Peprotech, UK
0.9% Sodium Chloride (Sterile)	Sigma-Aldrich, UK
Sodium pentobarbital	Merial Animal Health, UK

ELISA

Corticosterone kit	Immunodiagnostic Systems, UK
Mouse CXCL1 duo set	R & D Systems, UK
Mouse IFN-β	PBL Biomedical Laboratories, USA
Mouse IL-1β duo set	R & D Systems, UK
Mouse IL-1β Quantikine	R & D Systems, UK
Mouse IL-1RA Quantikine	R & D Systems, UK
Mouse IL-6 duo set	R & D Systems, UK
Mouse MCP-1 duo set	R & D Systems, UK
Mouse PGEM kit	Cayman Chemicals, USA
Mouse PGE2 kit	Enzo Life Sciences, UK
Mouse TNF-α duo set	R & D Systems, UK
HPE Buffer	Sanquin, Netherlands
Streptavidin poly horseradish peroxidase	Sanquin, Netherlands
Substrate Solution	R & D Systems, UK

DC Protein Assay	Bio-Rad, UK
96 well maxisorb immunoplates	Nunc, UK
96 well minisorp immunoplates	Nunc, UK
Biotin-PEG_4-Hydrazide	Pierce, UK
Zeba Desalt spin Column (7K MWCO)	Pierce, UK
Mouse monoclonal anti-biotin antibody	Invitrogen, UK

Immunohistochemistry Reagents and Products

Alexafluor chicken anti-rat 594 antibody	Invitrogen, UK
Alexafluor goat anti-rabbit 488 antibody	Invitrogen, UK
Biotinylated goat anti-Flourescein antibody	Vector Laboratories, UK
Biotinylated goat-anti-rabbit antibody	Vector Laboratories, UK
Biotinylated horse-anti-goat antibody	Vector Laboratories, UK
Biotinylated horse-anti-mouse antibody	Vector Laboratories, UK
Biotinylated rabbit-anti-goat antibody	Vector Laboratories, UK
Bovine serum albumin	Sigma-Aldrich, UK
DeadEnd Fluorometric TUNEL System	Invitrogen, UK
DPX mountant	Sigma-Aldrich, UK
Goat polyclonal anti-COX-2 antibody	Santa Cruz, USA
Goat polyclonal anti-IBA-1 antibody	Abcam, UK
Harris Haematoxylin solution	VWR, UK
Histoclear II	National Diagnostics, USA
Normal chicken serum	Vector Laboratories, UK
Normal goat serum	Vector Laboratories, UK
Normal horse serum	Vector Laboratories, UK
Normal rabbit serum	Vector Laboratories, UK
Mouse monoclonal anti-Synaptophysin antibody [SY38]	Abcam, UK
PAP pen	Sigma-Aldrich, UK
Paraffin wax	Leica, Germany
ProLong Gold anti-fade reagent	Invitrogen, UK
Proteinase K from Pichia pastoris (yeast)	Roche, UK
Rabbit polyclonal anti-COX-1 antibody	Cayman Chemicals, USA
Rabbit polyclonal anti-HPGDS antibody	Cayman Chemicals, USA
Rat monoclonal anti-CD206 antibody (MR5D3)	AbD Serotec, UK
SuperFrost Plus microscope slides	Menzel-Glaser, Germany
Vectastain ABC kit	Vector Laboratories, UK

Molecular Reagents and Products

Nucleospin RNA II kits	Macherey-Nagel , Germany
RNeasy Plus mini kits	Qiagen, UK
Qiashredder columns	Qiagen, UK
RNase-free DNase I enzyme	Qiagen, UK

SYBR green PCR master mix	Roche, UK
FastStart universal probe master mix	Roche, UK
High Capacity cDNA Reverse Transcriptase Kit	Applied Biosystems, UK
Molecular grade Absolute Ethanol	Sigma-Aldrich, UK
Molecular grade water	Sigma-Aldrich, UK
Optical adhesive covers	Applied Biosystems, UK
MicroAmp 96-well Reaction Plate	Applied Biosystems, UK

General Laboratory Chemicals

Ammonium nickel sulfate	Sigma-Aldrich, UK
Boric Acid	Sigma-Aldrich, UK
Citric Acid	Sigma-Aldrich, UK
3,3' Diaminobenzidine	Sigma-Aldrich, UK
Dimethyl sulfoxide (DMSO)	Pierce, UK
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich, UK
Formic acid	Sigma-Aldrich, UK
Glycerol	Sigma-Aldrich, UK
Hydrochloric acid	VWR, USA
Heparin (bovine)	Leo Laboratories, UK
Hydrogen peroxide (H_2O_2)	Sigma-Aldrich, UK
HPLC-grade Ethanol	Sigma-Aldrich, UK
Indomethacin	FLUKA, UK
Magnesium sulfate	Sigma-Aldrich, UK
Methanol	Fisher, UK
β-mercaptoethanol	Sigma-Aldrich, UK
Pepsin from porcine gastric mucosa	Sigma-Aldrich, UK
Phosphate-buffered saline (PBS)	Thermo Fisher Scientific UK
Potassium bicarbonate	Sigma-Aldrich, UK
Sodium acetate	Sigma-Aldrich, UK
Sodium Chloride (NaCl)	Sigma-Aldrich, UK
Sodium Hydroxide	Sigma-Aldrich, UK
Sodium metaperiodate	Sigma-Aldrich, UK
Sodium phosphate dibasic (Na2HPO4)	Merck, USA
Sodium phosphate monobasic (NaH2PO4)	Merck, USA
Sucrose	Sigma-Aldrich, UK
Sulphuric acid (H2SO4)	VWR, USA
2,2,2, tribromoethanol	Sigma-Aldrich, UK
Tris-base	Sigma-Aldrich, UK
Tris-HCl	Sigma-Aldrich, UK
Triton X-100	Sigma-Aldrich, UK
Tween 20	Sigma-Aldrich, UK
Xylene	VWR, USA

General Laboratory Products

Nuclease-free PCR tube	Greiner, Germany
Needles 26G, 3/8	Braun, Ireland
Parafilm laboratory roll	Pechiney Plastic Packaging, USA
Plastic cassettes	Labonord, France
Plastic wax containers for cassettes	Labonord, France
Pipettes	Gilson, UK
Sterile Pipette tips (RNA)	Starlab, Germany
Non-sterile Pipette tips	Greiner, Germany
Standard grade No. 1 filter paper	GE Healthcare, UK
Sterile 7 ml Bijou and 30 ml tubes	Sterilin, UK
Sterile Falcon 15 ml and 50 ml tubes	Sarstedt, Ireland
Syringes (sterile 1ml)	Braun, Ireland
Tubes 200µl, 500µl, 1ml	Greiner, Germany

2.2 Methods

All solutions were made using Millipore Direct-Q 3 UV Series purified water (resistivity 18.2 MΩ cm at 25°C , < 10 ppb Total Organic Carbon and conductivity 0.056 μS/cm) and pHed using a Hanna pH 210 Bench Top pH Meter, complete with pH electrode, temperature probe and electrode holder. Chemicals were weighed on Mettler Toledo scales.

2.2.1 Animals

Female C57BL/6 mice (Harlan, Bicester, United Kingdom) were housed in groups of five at 21°C with a 12: 12 hour light dark cycle and given access to food and water *ad libitum*. Females were used in order to avoid fighting and injury in cages, which has a significant effect on behaviour. Animals were no more than three months old when challenged or inoculated with ME7 disease. All animal experimentation was performed under a license granted by the Minister for Health and Children, Ireland, with approval from the local ethical committee and in compliance with the Cruelty to Animals Act, 1876 and the European Community Directive, 86/609/EEC. Every effort was made to minimize stress on animals.

IL-1R1-/- animals on a background of C57BL/6 (strain B6.129S7-*Il1r1 tm1Imx/J*) from an established colony, originally from The Jackson Laboratory (Bar Harbor, ME), were kindly provided by Professor Kingston Mills.

2.2.2 ME7 surgery

Mice were anesthetized with intraperitoneal Avertin (2,2,2-tribromoethanol; ≈20 ml/kg body weight) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The incisor bar was set at -1 mm, to ensure an approximately level head. The scalp was incised and skull exposed. A hole was drilled in the skull on each side of the midline for bilateral infusion of 1μl homogenate of a 10% w/v ME7-infected C57BL/6 brain homogenate, prepared in sterile PBS. Injections were made into the dorsal hippocampus (co-ordinates from bregma: anteroposterior, -2.0 mm; lateral, -1.6 mm; depth, -1.7 mm), using a microsyringe (Hamilton, Reno, NV, USA) with a 26 gauge needle. The needle was left in place for 2 minutes before being withdrawn slowly. Mice were then placed in a heated recovery chamber (30°C) and finally returned to their home cage. Sucrose (5% w/v) and Carprofen (0.05% v/v; Rimadyl, Pfizer, Ireland) were added to drinking

water for two days post-surgery. Control animals were injected with a 10% w/v normal brain homogenate (NBH) in PBS, derived from a naïve C57BL/6 mouse.

2.2.3 Intraperitoneal challenges

2.2.3.1 Lipopolysaccharide

For behavioural tests, experimental groups were injected with 100 μg/kg of the bacterial endotoxin LPS (equine arbortus, Sigma, L5886, Poole, UK) prepared in non-pyrogenic 0.9% sterile saline (Sigma, Poole, UK). For molecular studies, groups were challenged at a dosage of 100, 250 or 500 μg/kg. This dose mimics a mild to moderate infection rather than severe sepsis, eliciting small changes (less than 1°C) in core body temperature and acute working memory deficits in ME7 animals (Murray et al., 2012). Control animals were injected with sterile saline (Sigma, Poole, UK) 200μl per 20 g body weight.

2.2.3.2 IL-1β

For behavioural and molecular tests, experimental groups were injected with recombinant mouse IL-1β (R&D Systems, Minneapolis, MN, USA) at a dosage of 15 μg/kg or 50 μg/kg prepared in 0.9% sterile saline. Control animals were injected with sterile saline (Sigma, Poole, UK) 200μl per 20 g body weight.

2.2.3.3. TNF-α

For behavioural and molecular tests, experimental groups were injected with recombinant mouse TNF-α (Peprotech, Rocky Hill, NJ, USA) at a dosage of 50 μg/kg or 250 μg/kg prepared in 0.9% sterile saline. Control animals were injected with sterile saline (Sigma, Poole, UK) 200μl per 20 g body weight.

2.2.3.4 IL-6

For behavioural and molecular tests, experimental groups were injected with recombinant mouse IL-6 (Peprotech, Rocky Hill, NJ, USA) at a dosage of 50 μg/kg or 125 μg/kg prepared in 0.9% sterile saline. Control animals were injected with sterile saline (Sigma, Poole, UK) 200μl per 20 g body weight. The does selected for IL-1 β, TNF- α and Il-6 injection are discussed in chapter 3. In general, doses were based upon similar and relevant experiments reported in the literature.

2.2.3.5 IL-1 receptor antagonist

Human Interleukin-1 receptor antagonist (IL-1RA; Peprotech, Rocky Hill, NJ, USA) was injected at the concentration of 10 mg/kg, prepared in sterile water, immediately after LPS challenge. Control animals were injected with endotoxin-free water (Sigma, Poole, UK) 200µl per 20 g body weight.

2.2.3.6 Dexamethasone

Dexamethasone-21-phosphate (2 mg/kg; Sigma, Poole, UK) was prepared in 0.9% sterile saline and administered 60 minutes before LPS. The solution was sonicated in 10 second on/off bursts for 5 minutes to ensure complete dissolution prior to injection. Control animals were injected with sterile saline (Sigma, Poole, UK) 200µl per 20 g body weight.

2.2.3.7 Piroxicam

Piroxicam, reportedly a selective COX-1 inhibitor (10 mg/kg; Sigma, Poole, UK), was dissolved in 0.2 M Tris-HCl (pH 8). The solution was sonicated in 10 second on/off bursts for 5 minutes to ensure complete dissolution prior to injection. It was administered 60 minutes before LPS. Control animals were injected with 0.2 M Tris-HCL 200µl per 20 g body weight.

2.2.3.8 Nimesulide

Nimesulide, a COX-2 selective inhibitor (10 mg/kg; Sigma, Poole, UK), was dissolved in 0.2 M Tris-HCl, pH 8. The solution was sonicated in 10 second on/off bursts for 5 minutes to ensure complete dissolution prior to injection. It was administered 60 minutes before LPS and control animals were injected with 0.2 M Tris-HCl 200µl per 20 g body weight.

2.2.3.9 SC-560

SC-560, a selective COX-1 inhibitor (30 mg/kg; Cayman Chemicals, Ann Arbour, MI, USA), was dissolved in 0.1 M phosphate buffer containing 24 % DMSO. It was administered 30 minutes before LPS and control animals were injected with 0.1 M phosphate buffer containing 24 % DMSO 200µl per 20 g body weight.

2.2.3.10 Biotinylated LPS

2.2.3.10.1 Biotinylation of LPS

LPS at a concentration of 1 mg/ml in 0.1 M sodium acetate was oxidised by addition of 10 mM Sodium metaperiodate at ratio of 1:1 in a light sensitive vessel for 30 minutes on ice. Oxidation was stopped by addition of glycerol (final concentration 50 mM) for 5 minutes. A 10 ml Zeba Desalt spin column (7K MWCO; Pierce, Caramlington, UK) was equilibrated with coupling buffer (PBS). To remove excess periodate and glycerol, sample was placed on column, spun at 1000 x *g* for 2 minutes and eluted. One part prepared 50 mM Biotin-PEG_4-Hydrazide (Pierce, Caramlington, UK) solution (dissolved in DMSO; Pierce, Caramlington, UK) was added to nine parts oxidised and buffer-exchanged samples and mixed for 2 hours at room temperature with gentle end to end rotation. The biotinylated molecules were then separated from non-reacted material by spin at 1000 x *g* in a desalting column pre-equilibrated with coupling buffer (0.9% NaCl). Control challenges were prepared as follows; PBS mixed for 2 hours with Biotin Hydrazide solution, as for LPS, and passed through a desalting column with 0.9% NaCl coupling buffer.

2.2.3.10.1 Injection of biotinylated LPS

Animals were injected i.p. with 100 µg/kg of biotinylated LPS in a volume of 200 µl 0.9 % sterile saline. Challenges were injected in a volume of 200 µl sterile saline. Controls were administered normal LPS or Biotin PBS.

2.2.4 Behavioural tests

For all behavioural experiments, mice were moved from the housing room and left in the test room for 15 minutes prior to beginning the task to ensure an optimal state of arousal.

2.2.4.1 Open field

The open field consisted of a plastic base (58 cm x 33cm) surrounded by walls of 19 cm. The floor of the box is divided into a grid of equal sized squares. Measurement was made of distance travelled (in terms of grid squares crossed) and total number of rears (frequency with which the mice stood on their hind legs in the maze). Naïve animals had baseline activity measured for 3 minutes when placed in the middle of the open field alone. The next day, 2 hours post-challenge, the animals' locomotor activity was measured again.

2.2.4.2 T-maze alteration task

Hippocampal-dependent working memory was assessed in mice by observing alternation behaviour in a novel water T-maze task (Murray et al., 2012). The T-maze, constructed from black perspex, was of the following dimensions (cm); long axis 67, short axis 38, depth 20 and arm width 7. Single 4 cm diameter holes were at the end of each choice arm, 2 cm from the floor and black exit holes were placed into these holes. These exit holes could be blocked to prevent space from the maze. A "guillotine" door was inserted to prevent access to one or other choice arm. Water to a depth of 2 cm at a temperature of 20 °C was poured into the maze; at this height a mouse must paddle and thus is motivated to leave the maze through an exit tube. Animals were taken with their cage mates to a holding cage. Each mouse was individually placed in the start arm of the maze with 1 arm blocked, such that they were forced to make a left or right turn. The arm sequences were chosen in a pseudo-random manner, with equal numbers of left and right turns in any one session; moreover, there were no more than 2 consecutive runs to the same arm. On making the turn the mouse could escape from the water by entering the exit hole into small tube, and then a transit tube, in which it was carried to another holding cage. The animal remained in this holding cage for a 25 second intra-trial interval, in which the guillotine door was removed and exit tube switched to the opposite arm. The mouse was then replaced in the start arm and could choose either arm. Since the exit hole which it had escaped through was blocked, the mouse must alternate from its original turn to escape. On choosing correctly the mouse escaped to the transit tube and was returned to its home cage. On choosing incorrectly the mouse was allowed to self-correct to the correct exit arm. Animals were typically trained for 14 blocks of 10 trials (inter-trial interval of 20 minutes) before other experimental manipulations are performed.

Most animals reached alternation of greater than 80%. No animals were experimentally challenged unless they performed at 70% or above for consecutive days. Furthermore, no animals that performed at lower than 80% in the last block of 5 trials or displayed evidence of a side preference on the day prior to experimental challenges were included. Some animals developed a side preference in the course of training. In such a situation a correction strategy was employed. The side preference was broken in some cases, but not all, by not allowing mice to self-correct: i.e. instead of an animal being allowed to cross the T from the incorrect arm to the correct arm, the entrance to the correct arm was closed at the moment that the incorrect arm was chosen. The animals thus returned to the start arm and once again had the option to choose the correct arm. This was repeated until the animal finally chose the correct body turn when starting from the start arm.

This correction strategy was only employed for animals displaying a complete side preference (i.e. always turning in one direction if that arm was open to them), was discontinued once their side preference was broken and was never employed within 2 days of administration of substances to examine effects on working memory.

2.2.5 Core body temperature

A thermocouple rectal probe (Thermalert TH5, Physitemp; Clifton, NJ, USA) was used to measure core body temperature. The mice were pre-adapted to measurement of rectal temperature for 2 days prior to i.p. challenges to minimise stress effects. Temperatures were taken at baseline and then at 2 hours and, in some cases, 4 hours post-challenge.

2.2.6 *Plasma ELISA*

Animals were terminally anaesthetised with sodium pentobarbital (40 mg per mouse i.p., Euthatal, Merial Animal Health, Essex, UK). The thoracic cavity was opened and blood collected in heparinised tubes directly from the right atrium of the heart. This whole blood was spun at 1.5 x *g* for 15 minutes to remove cells; the plasma was then aliquoted and stored at -20 °C. These samples were then analysed for IL-1β, TNF-α, IL-6, CXCL1, MCP-1, IL-1RA, IFN-β, Corticosterone, Prostaglandin E metabolites (PGEM) and biotinylated LPS. Samples were quantified only if the absorbance fell on the linear portion of the standard curve. In general, samples were quantified in duplicate but triplicate was used where plate space permitted

2.2.6.1 *IL-1β, TNF-α, IL-6, MCP-1 and CXCL1*

Mouse IL-1β, TNF-α, IL-6, MCP-1 and CXCL1 were quantified by sandwich-type ELISA, using R&D systems duo set kits (R&D systems, Minneapolis, MN, USA). A standard protocol was followed. The capture anti-IL-1β, TNF-α, IL-6, MCP-1 and CXCL1 antibodies were diluted 1/180 (approximately 1 μg/ml) in PBS, and used to coat a 96-well maxisorb microplate overnight (Nunc, Fisher Scientific, Leicestershire) with 100 μl per well. Plates were then washed with PBS + 0.05 % Tween and blocked with PBS + 1 % BSA (with 0.05% Tween 20 in Tris-buffered Saline for IL-1β) before addition of 100 μl samples and standards for 2 hours at room temperature. Samples were serially diluted as follows: for IL-1β and IL-6 1/4 and 1/8 and 1/12 for cytokine challenges and 1/12, 1/36 and 1/108 for LPS challenge; for TNF-α 1/2 and 1/4; for MCP-1 1/5, 1/10 and 1/20; and

for CXCL1 1/256 for cytokine challenge and 1/300, 1/600 and 1/900 for LPS challenge. Standards were in the range of 1000-4 pg/ml. Detection antibodies were used at a dilution of 1/180 and incubated on the plates for 1.5 hours. Streptavidin poly-horseradish peroxidase (HRP: Sanquin, Amsterdam, Netherlands) was diluted in 1:10,000 and incubated in the dark at room temperature for 20 minutes. Samples, standards, detection antibodies and streptavidin poly-horseradish peroxidase were diluted in high performance ELISA buffer (Sanquin; Amsterdam, Netherlands). TMB and H_20_2 were used as substrate and the reaction was stopped with 1 M H_2S0_4 before optical density was read at 450 nm with correction at 570 nm. Sample concentrations were determined by extrapolation off the standard curve.

IL-1β was also analysed using a Quantikine mouse IL-1β kit (R&D systems, Minneapolis, MN, USA). 50 µl assay diluent and 50 µl standard, control or undiluted sample was added to wells and the plate was incubated for 2 hours at room temperature. Following 5 washes with wash buffer, 100 µl IL-1β conjugate was added to all wells and the plate was incubated for 2 hours at room temperature. Standards ranged 500-7.8 pg/ml and samples were diluted 1/2, 1/4 and 1/9. The wash step was repeated and 100 µl of substrate solution was added to each well and incubated for 30 minutes at room temperature. After the addition of 100 µl stop solution, the optical density was read at 450 nm with correction at 570 nm.

2.2.6.2 IL-1 receptor antagonist

IL-1RA was analysed using a Quantikine mouse IL-1RA kit (R&D systems, Minneapolis, MN, USA). 50 µl assay diluent and 50 µl standard, control or undiluted sample was added to wells and the plate was incubated for 2 hours at room temperature. Following 5 washes with wash buffer, 100 µl IL-1RA conjugate was added to all wells and the plate was incubated for 2 hours at room temperature. Standards ranged 2000-31.2 pg/ml and samples were diluted 1/8 and 1/64. The wash step was repeated and 100 µl substrate solution was added to each well and incubated for 30 minutes at room temperature. After the addition of 100 µl stop solution, the optical density was read at 450 nm with correction at 570 nm.

2.2.6.3 IFN-β

The interferon-beta (IFN-β) ELISA was carried out using a kit supplied by PBL Biomedical Laboratories (Piscataway, NJ) and the procedure for the sandwich-type ELISA was carried out as per manufacturer's instructions. 100 µl plasma samples and standards, diluted in sample diluent,

were added to a pre-coated plate at room temperature for 60 minutes before being washed 3 times with wash solution. Samples were serially diluted 1/3, 1/9 and 1/27, while standards were in a range of 15.6-1000 pg/ml. 100 µl antibody solution (30 µl antibody/12 ml diluent) was added to each well. After 60 minutes incubation at room temperature, the plate was washed 3 times and HRP solution (36 µl HRP/12 ml concentrate diluent) was added to each well and incubated at room temperature for 60 minutes. The plate was washed 3 times and TMB substrate solution was added to each well. After a 15 minute incubation period, stop solution was added to each well and mixed gently. Optical density was read at 450 nm with correction at 570 nm. Sample concentrations were determined by extrapolation off the standard curve.

2.2.6.4 Corticosterone

The corticosterone ELISA was carried out using a kit supplied by Immunodiagnostic Systems (Tyne & Wear, UK) and the procedure for the competitive ELISA was carried out as per manufacturer's instructions. 100 µl plasma samples, calibrators and controls, diluted in sample diluent, were added to a pre-coated plate and 100 µl enzyme conjugate solution was added to all wells. The plate was then covered and incubated for 24 hours. Samples were diluted 1/10, while standards were in a range of 1.4-188 ng/ml. All wells were washed 3 times with wash solution, before addition of TMB substrate. Plate was incubated at room temperature for 30 minutes, before addition of stop solution. Optical density was read at 450 nm with correction at 570 nm. Percent binding of calibrators, controls and samples were calculated. Sample concentrations were determined by extrapolation off the standard curve.

2.2.6.5 *Prostaglandin E2 Metabolite*

Plasma levels of prostaglandin E metabolites (PGEM) were measured using a PGEM EIA kit (Cayman Chemicals Ann Arbor, MI, USA). Prostaglandin E2 is rapidly converted *in-vivo* to its 13, 14-dihydro-15-keto metabolite, which is also chemically unstable and undergoes degradation to prostaglandin A products. Prostaglandin E Metabolite (PGEM) assay is a competitive assay that converts 13,14-dihydro-15-keto PGA_2 and 13,14-dihydro-15-keto PGE_2 to a single, stable derivative that can be quantified and used as a measure of initial PGE2 concentration. The assay is based on competition between sample PGEM and a PGEM-acetylcholinesterase (AChE) conjugate (PGEM tracer), for a limited number of PGEM-specific rabbit antiserum binding sites. Since the concentration of sample PGEM varies but the PGEM tracer concentration is held constant, the amount of PGEM tracer which binds to the antiserum is inversely proportional to the amount of

sample PGEM in a well. Standards and samples were diluted in EIA buffer pre-derivitisation and PGEM buffer (EIA, carbonate buffer and phosphate buffer) post-derivitisation. Samples were serially diluted 1/80 and 1/160, while standards were in a range of 0.39-50pg/ml. Plasma samples and standards were derivatised by addition of carbonate buffer for overnight incubation at 37 °C, followed by addition of phosphate buffer and EIA buffer. 100 µl diluted plasma samples, calibrators and controls were added to the plate pre-coated with mouse anti-rabbit IgG. Control were as follows, blank, total activity, non-specific binding and maximum binding. PGEM-acetylcholinesterase conjugate was added to all wells except total activity and blank. Specific rabbit antiserum to PGEM was added to all wells except total activity, non-specific binding and blank; the plate was covered and incubated for 18 hours at room temperature. The following day, the plate was washed 5 times with wash buffer before Ellman's reagent was added to all wells; this assay was developed for 80 minutes and optical density was read at 420 nm. Non-specific binding was subtracted from maximum binding, then percent binding of standards and samples were calculated. Sample concentrations were determined by extrapolation off the standard curve.

2.2.6.6 ELISA for biotinylated LPS

Plasma levels of biotinylated LPS assay were assessed using a protocol developed in the course of this thesis. A 96-well maxisorb microplate (Nunc, Fisher Scientific, Leicestershire) was coated overnight with 100 µl per well of mouse monoclonal anti-biotin antibody (1 µg/ml; Invitrogen, Paisley, UK). The plate was washed 3 times with PBS + 0.05 % Tween and blocked with PBS + 1 % BSA before addition of 100 µl samples and standards. Samples were serially diluted 1/3, 1/9 and 1/27. Standards were in the range of 0-2000 pg/ml. The plate was washed again 3 times and 100 µl streptavidin poly-horseradish peroxidase (HRP: Sanquin, Amsterdam, Netherlands, diluted 1:10,000) was added to each well. Following another 3 washes, TMB and H_2O_2 were used as substrate and the reaction was stopped with 1 M H_2SO_4 before optical density was read at 450 nm with correction at 570 nm. Samples, standards, detection antibodies and Streptavidin poly-horseradish peroxidase were diluted in high performance ELISA buffer (Sanquin; Amsterdam, Netherlands). Sample concentrations were determined by extrapolation off the standard curve.

To arrive at this final protocol a number of optimisation steps were carried out. We biotinylated LPS (as described in Section 2.2.3.10) and hypothesised that if a biotin antibody was coated on maxisorb microplate as a capture antibody, biotinylated LPS in plasma would bind to this antibody, and would thus be quantifiable from a standard curve of serially diluted biotinylated LPS. We

constructed two standard curves from 100 ng/ml serially diluted 1:2 to a final concentration 312.5 pg/ml on plates coated with capture antibody at 1 mg/ml. At biotinylated LPS concentrations above 2500 pg/ml absorbance concentrations not correlated but these showed a linear correlation between 1000 pg/ml and 16 pg/ml (Figure 2.1). Thus, with a standard curve, unknown concentrations of biotinylated LPS could be quantified. Plasma samples of animals sacrificed 30, 45 and 60 minutes after injection of 100 μg/kg biotinylated LPS diluted 1/3, 1/9, 1/27 and 1/81 were assessed to ascertain the correct dilution of plasma for the absorbance to be in the measurable range. Sample diluted 1/81 was below detection limit and bore no relationship to sample diluted 1/27, 1/9 or 1/3. Throughout, dilutions standard to the duo kit ELISA protocol were used for detection antibody, streptavidin, TMB, H_2O_2 and H_2SO_4. A number of controls were used throughout optimisation. The plasma of animals challenged with normal non-biotinylated LPS and biotinylated PBS (PBS treated in the same way as LPS during biotinylation), were ran on all assay plates. The signal produced by these controls was always below detection limit. A TNF-α ELISA was carried out to demonstrate that biotinylated LPS was biologically active. Average detected levels were 952 pg/ml; SEM was 746 pg/ml. This high variability stemmed from a particularly high TNF-α level in one animal. The readings from animals treated with biotinylated LPS were similar to levels that we have previously reported 1 hour post-injection of normal LPS (Murray et al., 2012) if this animal was excluded (mean levels 312 pg/ml SEM 128 pg/ml). Finally, a TNF-α ELISA, without the use of a detection antibody was performed. This was to show that signal detected in the aforementioned TNF-α ELISA was from TNF-α bound to a biotinylated secondary antibody, rather than biotinylated LPS from the plasma, bound to the plate and producing a quantifiable signal. The lack of any signal in the absence of a detection antibody showed that detected signal was indicative of conserved TNF-α synthesis in animals treated with biotinylated LPS.

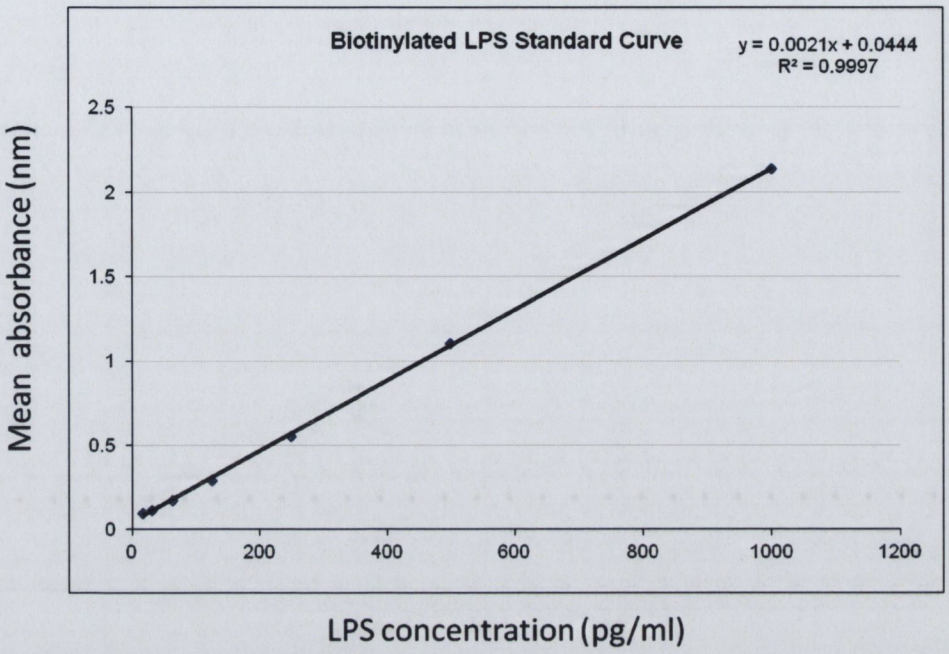

Figure 2.1 *Linear relationship between serial dilution of biotinylated LPS and optical density obtained from ELISA.*

2.2.7 ELISA for brain PGE2

2.2.7.1 Homogenisation and ethanol evaporation

PGE2 levels in hippocampal tissue were quantified using a PGE2 EIA kit (Enzo Life Sciences, Exeter, UK, previously manufactured by Assay Designs). Standards were in a range of 2500-39.1 pg/ml. Hippocampal tissue was homogenised in 100 µl homogenisation buffer (0.1 M phosphate buffer pH 7.4 containing 1 mM EDTA and 10 µm indomethacin and mixed with 1 ml 100% ethanol). Samples were then centrifuged at 3000 rpm for 10 minutes at 4°C, transferred to an empty tube and the ethanol was evaporated under a constant stream of nitrogen. Samples were then resuspended in 250 µl assay buffer, vortexed and then allowed to rest for 5 minutes. This was repeated twice more.

2.2.7.2 PGE2 assay

PGE2 levels were measured according to manufacturer's instructions. Control were as follows, blank, total activity, non-specific binding and maximum binding. Sample, standards and controls

were added to the pre-coated plate and incubated for 2 hours. Following 3 washes, 200 µl PnPP substrate solution was added to all wells and developed for 45 minutes. After addition of stop solution, optical density was read at 450 nm with correction at 570 nm. Sample concentrations were determined by extrapolation off the standard curve.

2.2.7.3 Protein assay

Sample PGE2 concentrations were normalised to protein concentration (quantified using *DC* Protein Assay [Bio-Rad, Hemel Hempstead, UK]). 5 µl hippocampus samples homogenised in homogenisation buffer were diluted 1/4 in homogenisation buffer. Bovine serum albumin, diluted in homogenisation buffer in a range 2-0.0625 mg/ml, was used as a standard curve. 25 µl working reagent A, a 5 µl dilute hippocampus sample and 200 µl reagent B were added to each well and incubated on a minisorp assay plate for 15 minutes before optical density was read at 730 nm. Sample concentrations were determined by extrapolation off the standard curve. Sample PGE2 levels were then divided by the corresponding protein concentrations to give a pg/mg of protein PGE2 concentration for each hippocampal sample.

2.2.8 RNA extraction, cDNA synthesis and quantitative PCR

2.2.8.1 Tissues for quantitative PCR

Animals were terminally anaesthetised and transcardially perfused with heparinised saline using a peristaltic pump (Anachem, Luton, UK). Brains were rapidly removed, hippocampi and hypothalami dissected out, placed in eppendorf tubes, snap frozen on liquid nitrogen and stored at -80 °C until further use. Liver samples were also taken and treated in a similar manner.

2.2.8.2 RNA extraction

Total RNA was extracted from brain and liver samples using Qiagen RNeasy Plus™ mini kits (Qiagen, Crawley, UK) or Macherey-Nagel Nucleospin® RNA II kits (Macherey-Nagel, Düren, Germany), in accordance with manufacturer's instructions. Samples were weighed to ensure each lay in the range of 20 – 40 mg. For the Qiagen RNeasy Plus kits, 600 µl Buffer RLT (with β-mercaptoethanol [β-ME], 10 µl RLT per 1 ml β-ME) was added to each sample followed by homogenisation using a motorised pestle. The sample lysates were placed in Qiagen Qiashredder

columns (Qiagen, Crawley, UK) and centrifuged at 14,800 x *g* for 6 minutes. The flow-through was collected and transferred to the genomic DNA (gDNA) Eliminator spin columns and centrifuged at 14,800 x *g* for 30 seconds. These columns were discarded and an equal volume of 70 % ethanol in the case of brain samples and 50 % ethanol for liver samples, was added to the flow-though and mixed. Samples were placed in RNeasy mini-spin columns in 2 ml collection tubes and centrifuged at 14,800 x *g* for 15 seconds. Flow-through was discarded and the columns were washed by addition of 350 µl of Qiagen Buffer RW1 followed by centrifugation at 14,800 x *g* for 15 seconds. The flow-through was again discarded and an on-column DNase digestion was performed for 15 minutes using the Qiagen RNase-free DNase I enzyme (10 µl DNase I stock solution: 70 µl Qiagen Buffer RDD per column), to ensure complete removal of contaminating gDNA. Columns were then washed with 350 µl of Buffer RW1 and centrifuged at 14,800 x *g* for 30 seconds. Flow-through was discarded and columns were inserted into new 2 ml collection tubes. 500 µl of Qiagen RPE buffer was added to each column and centrifuged at 14,800 x *g* for 15 seconds. The flow-through was discarded, 500 µl of Qiagen RPE buffer was added to the column and then centrifuged at 14,800 x *g* for 2 minutes to dry the silica membrane. The spin columns were transferred to new 2 ml tubes and centrifuged at 14,800 x *g* for 1 minute. The RNeasy mini spin columns were then placed into new 1.5 ml tubes and 30 µl RNase-free water was added directly onto the RNeasy silica-gel membranes, followed by centrifugation for 1 minute at 14,800 x *g* to elute the RNA.

After sustained technical problems with two batches of Qiagen RNA extraction kits, the Macherey-Nagel Nucleospin II kits were used for RNA extraction in later experiments. No experiments contained a mix of samples from different RNA procedures. Frozen samples were placed in RA1 buffer (350 µl) containing β-mercaptoethanol (3.5 µl) and homogenised using a motorised pestle. The homogenates were added to Nucleospin filter units and filtered by centrifugation at 13,000 rpm for 1 minute. 70 % ethanol (350 µl) was added to each sample lysate and pippetted vigorously, approximately 5 times. The resultant mixes were placed in Nucleospin RNA II columns and centrifuged at 13,000 rpm for 30 seconds, thus allowing the RNA to bind to the silica membranes. Following centrifugation, the columns were placed in new collecting tubes and membrane desalting buffer (350 µl) was added to the columns. The columns were once again centrifuged at 13,000 rpm for 1 minute before DNase (95 µl, 1:10 dilution in DNase Reaction Buffer) was pipette directly into the centre of the silica column, to digest DNA. Samples were incubated with the DNase solution for 15 minutes at room temperature. RA2 buffer (200 µl) was then added to columns and centrifuged at 13,000 rpm for 30 seconds. Columns were then placed in new collecting tubes and

RA3 buffer (600 µl, 1:2 dilution in ethanol) was added to samples before centrifugation at 13,000 rpm for 30 seconds. The resultant flow-through was discarded and the columns were washed a second time with RA3 buffer (250 µl) and then centrifuged at 13,000 rpm for 2 minutes. Lastly, columns were placed in fresh RNase-free microtubes and RNase-free water (40 µl) was added and the RNA was eluted by centrifugation at 13000 rpm for 1 minute. RNA yields were determined by spectrophotometry at 260 and 280 nm using the NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Dublin, Ireland). RNA purity was also determined using this equipment. Quantified RNA was stored at −80°C until cDNA synthesis and PCR assay.

2.2.8.3 Reverse transcription cDNA synthesis

Using a High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Warrington, UK), cDNA was synthesised from total RNA. 400 ng of total RNA were reverse transcribed per 20 µl reaction volume and 1 µl of the reverse transcription (RT) reaction was used for polymerase chain reaction (PCR). For a 20 µl volume reaction, 10 µl master mix (containing: 2 µl 10X RT Buffer; 0.8 µl 25X dNTP mix, 100mM; 2 µl 10X RT random primers; 1 µl MultiScribe™ Reverse Transcriptase; 4.2 µl RNase-free water) was added to 10 µl RNA + RNAse-free water in a nuclease-free PCR tube (Greiner Bio-One, Monroe, NC, USA). Two controls were prepared one of which replaced RNA with RNase-free water, and the other reverse transcriptase with RNase-free water. PCR tubes were placed in a DNA Engine® Peltier Thermal Cycler PTC-200 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and samples were incubated at 25°C for 10 minutes, 37°C for 120 minutes and 85°C for 5 seconds (to inactivate reverse transcriptase). Samples were held at 4°C until collection and then stored at −20°C until assay.

2.2.8.4 Quantitative PCR assay

The StepOne™ Real-Time polymerase chain reaction (PCR) system was supplied by Applied Biosystems (Warrington, UK) and used in 96-well format with a 25µl reaction volume per well for quantitative (Q)-PCR. In general, forty cycles were run with the following conditions: 2 minutes at 50°C, 10 minutes at 95°C and for each cycle 15 seconds at 95°C to denature and 1 minute at 60°C for transcription. Reagents were supplied by Applied Biosystems (SYBR Green PCR Master Mix) and Roche (FastStart Universal Probe Master [Rox] and FastStart Universal SYBR Green Master [Rox], Lewes, UK). Assays were designed using the published sequences for the genes of interest and Primer Express software (Applied Biosystems, Warrington, UK) and primer pairs were checked for specificity by standard RT-PCR using Promega PCR reagents (Promega, Southampton,

UK). Primer and probe sequences are shown in Table 1. Where possible, probes were designed to cross introns such that they were cDNA specific. In some cases the fluorescent DNA binding probe SYBR green was used in place of a specific probe. Primer and probe sequences, along with accession numbers for mRNA sequence of interest may be found in table 1. Oligonucleotide primers were resuspended in 1X TE buffer (Tris Base 10 mM, EDTA 1 mM; pH 7.5-8.0) and diluted to 10 μM working aliquots. All primer pairs were checked for specificity by standard reverse transcription (RT)-PCR followed by gel electrophoresis and each primer pair produced a discrete band of the expected amplicon size.

Assays were quantified using a relative standard curve. This was constructed from total RNA isolated from mouse brain tissue, 24 hours after intra-cerebral challenge with 2.5 μg LPS or from mouse liver tissue 2 hours after intra-peritoneal challenge with IL-1β, TNF-α or IL-6. These treatments up-regulate most target transcripts of interest in this study. In addition this standard curve was constructed using a higher concentration of RNA in the reverse transcriptase reaction than that in the samples for analysis to ensure that the cytokine transcription of all experimental animal groups would fall within the range of the standard curve constructed. Serial 1 in 4 dilutions of the cDNA synthesized from the brains or livers of immune-challenged mice were made and a curve plotted of the Ct value (cycle threshold - the cycle number at which the fluorescence of the product of transcribed gene crosses the threshold of detection) versus the log of the concentration (assigned an arbitrary value since the absolute concentration of cytokine transcripts is not known). Verification that this curve is a straight line confirms the efficiency of the PCR reaction across the entire concentration range and the equation of this line can then be used to calculate the relative concentrations of the experimental samples. Since the top standard has been assigned an arbitrary value, it follows that the calculated concentrations of all experimental samples will also have arbitrary values, but these values will be directly related to the equation of the line. All PCR data were normalised to the expression of the housekeeping gene glyceraldehyde-3-phosphate dehyrdrogenase (GAPDH).

Table 2.1: Mouse Taqman primer and probe sequences

Target	Accession no.	Oligonucleotide	Sequence	Amplicon size (bp)
Caspase-1	NM_009807.2	Forward primer	5'-GGACAAGGCACGGGACCTA-3'	132
		Reverse primer	5'-GCTGATGGAGCTGATTGAAGCT-3'	
CD11b	NM_001082960.1	Forward primer	5'-TCATTCGCTACGTAATTGGG-3'	90
		Reverse primer	5'-GATGGTGTCGAGCTCTCTGC-3'	
COX-1	NM_008969	Forward primer	5'-CCAGAACCAGGGTGTCTGTGT-3'	70
		Reverse primer	5'-GTAGCCCGTGCGAG TACA ATC-3'	
		Probe	5'-CGCTTTGGCCTCGACAACTACCAGTG-3'	
COX-2	NW_000157	Forward primer	5'-GAGTGGTAGCCAGCAAAGCC-3'	81
		Reverse primer	5'- TTTAATTGGGAACCCTTCTTTGTT-3'	
		Probe	5'-AGCAACAAAAGCGTTCTACAAAGGAACTAACCA-3	
cPGES	NM_019766	Forward primer	5'-CGCCCACCCGTTTGTCT-3'	86
		Reverse primer	5'- ATAGTCCCTTCGATCGTACCACTT-3'	
		Probe	5'- CCGTTCACCATGCAGCCTGCTTC-3'	
CRF1	NM_205769	Forward primer	5'-GGAGCCGCCCATCTCTCT-3'	64
		Reverse primer	5'-CCGGGCCATTTCCAAGAC-3'	
CXCL1	NM_008176	Forward primer	5'-CACCCAAACCGAAGTCATAGC-3'	82
		Reverse primer	5'-AATTTTCTGAACCAAGGGAGCTT-3'	
DP1	NM_008962.4	Forward primer	5'-TTTGGGAAGTTCGTGCAGTACT-3'	112
		Reverse primer	5'-GCCATGAGGCTGGAGTAGA-3'	
DP2	NM_009962.2	Forward primer	5'- TGGCCTTCTTCAACAGCGT-3'	271
		Reverse primer	5'- ACGCAGTTGGGGAATTCG-3'	
EP1	NM_013641.2	Forward primer	5'-GCAGCACTGGCCCTCTTG-3'	64
		Reverse primer	5'-TGTGCCATTATCGCCTGTTG-3'	
EP2	NM_008964.4	Forward primer	5'-TATGCTCCTTGCCTTTCAC-3'	135
		Reverse primer	5'-GACAACAGAGGACTGAGCG-3'	
EP3	NM_011196.2	Forward primer	5'-ACGGCCATCCAGCTCATG-3'	174
		Reverse primer	5'-CCGACATCTGATTGAAGATCATTT-3'	
EP4	NM_008965.1	Forward primer	5'-TGGAGGTACCATTCCTAGATCGA-3'	97
		Reverse primer	5'-GAGGAGAAGGACGCGTTGAC-3'	
FAS	NM_007987.2	Forward primer	5'-AAGAAGGACCACAACACAAATCTG-3'	99
		Reverse primer	5'-CCCTGTTAAATGGGCCACACT-3'	
		Probe	5'-TGCAGAAGGAACTGGCAGAACTCCG-3'	
GAPDH	NM_008084.2	Forward primer	5'-GACGGCCGCATCTTCTTGT-3'	65
		Reverse primer	5'-CAGTGCCAGCCTCGTCCCGTAGA-3'	
HPGDS	NM_019455.4	Forward primer	5'- AATCAAGCCAACTCTTCCATTTG-3'	208
		Reverse primer	5'- GACATTGCTCCAACGCTGTCT-3'	
		Probe	5'- TTACAATCCACCAGAGCCTCGCAATAGC-3'	
IFN-α	BX_530016	Forward primer	5'-TCTGATGCAGCAGGTGGG-3'	159
		Reverse primer	5'-AGGGCTCTCCAGACTTCTGCTCTG-3'	

Target	Accession no.	Oligonucleotide	Sequence	Amplicon size (bp)
IFN-β	X14455	Forward primer	5'-CCATCATGAACAACAGGTGGAT-3'	67
		Reverse primer	5'-GAGAGGGCTGTGGTGGAGAA-3'	
		Probe	5'-CTCCACGCTGCGTTCCTGCTGTG-3'	
IL-1β	M15131	Forward primer	5'-GCACACCCACCCTGCA-3'	69
		Reverse primer	5'-ACCGCTTTTCCATCTTCTTCTT-3'	
		Probe	5'-TGGAGAGTCTGGATCCCAAGCAATACCC-3'	
IL-1RA	NM_031167	Forward primer	5'-CTGGGAAAAGACCCTGCAAG-3'	91
		Reverse primer	5'-CCAGCAATGAGCTGGTTGTTT-3'	
		Probe	5'-TGCAAGCCTTCAGAATCTGGGATACTAACCA-3'	
IL-1R1	NM_001123382.1	Forward primer	5'-GCAATATCCGGTCACACGAGTA-3'	117
		Reverse primer	5'-ATCATTGATCCTGGGTCAGCTT-3'	
		Probe	5'-TCCTGAGCCCTCGGAATGAGACGATC-3'	
IL-1R2	NM_010555.4	Forward primer	5'- ACAAGGGAGGATCTGCATACAGA-3'	91
		Reverse primer	5'- AAGAGACTTCTTTGACTGTGGTATGGA-3'	
iNOS	U_43428	Forward primer	5'-CAGCTGGGCTGTACAAACCTT-3'	95
		Reverse primer	5'-CATTGGAAGTGAAGCGTTTCG-3'	
		Probe	5'-CGGGCAGCCTGTGAGACCTTTGA-3'	
IL-6	NM_031168	Forward primer	5'-TCCAGAAACCGCTATGAAGTTC-3'	72
		Reverse primer	5'-CACCAGCATCAGTCCCAAGA-3'	
		Probe	5'-CTCTGCAAGAGACTTCCATCCAGTTGCC-3'	
LPGDS	NM_008963.2	Forward primer	5'- CCTCACAGAGGAGGACATTGTTT-3'	82
		Reverse primer	5'- CCAGCCCTCTGACTGACTTCTC-3'	
		Probe	5'- CCCCAACCGGATAAGTGCATTCAAGAG-3'	
mPGES-1	NM_022415.3	Forward primer	5'- CCAGTATTACAGGAGTGACCCAGAT-3'	78
		Reverse primer	5'- GGAAAGGATAGATTGTCTCCATGTC -3'	
		Probe	5'- TGGAGCGCTGCCTCAGAGCCC -3'	
mPGES-2	NM_133783.2	Forward primer	5'- CAGCGGGTGTTCGGTACAC-3'	86
		Reverse primer	5'- CAGCGGGTGTTCGGTACAC-3'	
MCP-1	NM_011333	Forward Primer	5'-GTTGGCTCAGCCAGATGCA-3'	81
		Reverse primer	5'-AGCCTACTCATTGGGATCATCTTG-3'	
		Probe	5'-TTAACGCCCCACTCACCTGCTGCTACT-3'	
NLRP3	NM_145827.3	Forward primer	5'-GAGCCTACAGTTGGGTGAAATGT-3'	115
		Reverse primer	5'-CCACGCCTACCAGGAAATCTC-3'	
PAI-1	M_33960	Forward primer	5'-GGGACACCCTCAGCATGTTC-3'	79
		Reverse primer	5'-TGTTGGTGAGGGCGGAGA-3'	
		Probe	5'-TCGCTGCACCCTTTGAGAAAGATGT-3'	
PGIS	NM_008968.3	Forward primer	5'- TCAGCGGATGTTTTCCATACC-3'	62
		Reverse primer	5'- TATCCCCCGCTGACAAGGA-3'	

Target	Accession no.	Oligonucleotide	Sequence	Amplicon size (bp)
Prostamide/PGF Synthase	NM_025582.3	Forward primer	5'- CCACCCCAGTGTGATGAAGAG-3'	88
		Reverse primer	5'- TTCCAAACACATCCAAGGTTACA-3'	
		Probe	5'-CCACCGAGGACCCCACGCC-3'	
PTX3	X_83601	Forward primer	5'-ACAACGAAATAGACAATGGACTTCAT-3'	69
		Reverse primer	5'-CTGGCGGCAGTCGCA-3'	
SAA	M13522	Forward primer	5'-GCCATGGAGGGTTTTTTTCATT-3'	80
		Reverse primer	5'-CCTTTGGGCAGCATCATAGTTC-3'	
		Probe	5'-CACATGTCTCCAGCCCCTTGGAAAGC-3'	
TNF-α	M11731	Forward primer	5'-CTCCAGGCGGTGCCTATG-3'	149
		Reverse primer	5'-GGGCCATAGAACTGATGAGAGG-3'	
		Probe	5'-TCAGCCTCTTCTCATTCCTGCTTGTGG-3'	
TNF-αIP2	NM_009396	Forward primer	5'-GCTGGCAAGGCACATCCT-3'	74
		Reverse primer	5'-AGGTTGCAGTGGAGCCATTCT-3'	
		Probe	5'-CAACGCGGATGCC-3'	
uPAR	NM_011113.3	Forward primer	5'-TGCAATGCCGCTATCCTACA-3'	116
		Reverse primer	5'-TGGGCATCCGGGAAGACT-3'	
		Probe	5'-CCCTCCAGAGCACAGAAAGGAGCTTGAA-3'	
VCAM	NM_011693.3	Forward primer	5'-GATGTAAAAGGAAAAGAACATAACAAGAAC-3'	90
		Reverse primer	5'-GATGGCAGGTATTACCAAGGAAGA-3'	

2.2.9 Immunohistochemistry

2.2.9.1 Tissue preparation

Animals were terminally anaesthetised with sodium pentobarbital (40 mg/mouse i.p., Euthatal, Merial Animal Health, Essex, UK) and transcardially perfused with heparinised saline for approximately 5 minutes followed by 10 % formalin (Sigma, Poole, UK) for approximately 15 minutes. Brains were then removed post-fixed in bijou flasks over 2 days in the same fixative and then placed in PBS until embedding.

2.2.9.2 Paraffin wax embedding and sectioning of tissue

Brains for embedding were placed in plastic cassettes (Thermo Fisher Scientific, Dublin, Ireland) and transferred to a container of 70% alcohol for 20 minutes to begin the dehydration process. This was followed by 1.5 hourly changes in successively increasing alcohols: 70% alcohol II, 80%, 95%, 100% I, 100% II. Cassettes were then placed in 100% alcohol III overnight. The following day cassettes went through two five hour periods in the clearing agent Histoclear II (National Diagnostics, Manville, NJ, USA) followed by a final overnight spell in Histoclear. Brain cassettes were then transferred to and put through two changes of molten paraffin wax (McCormick Scientific, St Louis, MO, USA), heated to 60°C, each for 2 hours. Embedding moulds were filled with wax, placed on a cooling stage and brains were removed from their cassettes and placed in the mould. Cassettes were placed on top of the mould and more wax was placed on top. The wax was allowed to solidify on a cooling stage. Coronal sections (10 μm) of brains were cut on a Leica RM2235 Rotary Microtome (Leica Microsystems, Wetzlar, Germany) at the level of the hippocampus and floated onto electrostatically charged slides (Menzel-Glaser, Braunschweig, Germany) and dried at 37°C overnight.

2.2.9.3 General immunohistochemistry protocol

Sections were dewaxed in xylene and Histoclear and rehydrated through alcohols of decreasing concentration immediately before beginning a staining protocol. Immunohistochemistry was performed for the microglia marker IBA-1 (Abcam, Cambridge, UK), COX-1 (Cayman Chemicals, Ann Arbour, MI, USA), COX-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), HPGDS (Cayman Chemicals, Ann Arbour, MI, USA), synaptophysin (SY38, Abcam, Cambridge, UK), perivascular macrophage marker CD206 (MR5D3, AbD Serotec, UK) and markers of apoptosis (TUNEL, Promega, Southampton, UK). Tissue sections were dewaxed in xylene for 15 minutes

and passed to two separate vessels of Histoclear II for 10 minutes each to total clearance of wax from the tissue. Sections were then rehydrated through a series of ethanols of decreasing concentration: 100 % II, 100 % I, 95 %, 85 %, and 70 %. Following rehydration, non-specific endogenous peroxidase activity was eliminated (quenched) by incubating sections in 1 % v/v hydrogen peroxide in methanol (1 ml H_2O_2 per 100ml methanol). For some immunohistochemical reactions, antigen retrieval was performed by incubation in citrate buffer, pH 6, and microwaving twice for 5 minutes followed by 5 minutes of cooling after incubation. Slides were cooled to room temperature by the slow addition of cold tap water. Sections were washed three times in PBS for 5 minutes, encircled with a ring of wax using a liquid blocking PAP pen (Sigma, Poole, UK), and the appropriate blocking serum applied (10 % in PBS - 40 µl) and incubated at room temperature for 40 minutes. Serum was tapped off and sections were incubated in 30 µl of primary antibody. Primary antibodies were prepared in PBS unless otherwise stated. Following primary antibody incubation, sections were washed three times in PBS for 5 minutes and incubated with secondary antibody. All biotinylated secondary antibodies were supplied by Vector Laboratories (Peterborough, UK). Three 5 minute washes with PBS followed and staining was then visualized using the ABC method using peroxidase as enzyme, 0.05% v/v hydrogen peroxide as substrate, and diaminobenzidine (DAB) as chromogen. Sections were incubated in ABC solution (1 drop reagent A and 1 drop reagent B added to 5 ml PBS as per the manufacturers instruction; Vector Laboratories, Peterborough, UK) for 45 minutes followed by three washes in PBS, and finally developed in DAB solution (250 ml 0.1 M phosphate buffer, 500 µl DAB, 125 µl hydrogen peroxide). All sections (except those labelled for synaptophysin and CD206/COX-1 double staining) were counterstained with haematoxylin for 20 – 60 seconds depending on intensity desired, acidified in 1 % acid alcohol (1 % v/v hydrochloric acid in 70 % ethanol) for 5 seconds, and differentiated in Scott's tap water (500 ml distilled water, 15 g magnesium sulfate, 1 g potassium bicarbonate). Sections were dehydrated through a series of ethanols of increasing concentration for 5 minutes each (70 %, 80 %, 95 %, 100 % I, 100 % II), followed by two changes of Histoclear II for 10 minutes each, and a final incubation in xylene for 15 minutes. Slides were finally mounted in DPX (Sigma, Poole, UK) and coverslips applied.

2.2.9.4 IBA-1

Sections for the microglial marker IBA-1 were quenched for 15 minutes and incubated in citrate buffer as above. Sections were treated with pepsin for 20 minutes (prepared at 0.4 % in 0.1 M HCl and diluted 1 in 8 in 0.01 M HCl) blocked with 10 % normal rabbit serum before overnight

incubation at 4°C with the goat antibody against murine IBA-1 1/2000. Sections were then incubated in biotinylated rabbit-anti-goat secondary at 1/100 and developed using the ABC method and DAB as described above.

2.2.9.5 COX-1

Sections labelled for the prostaglandin biosynthetic enzyme COX-1 were quenched for 10 minutes, before antigen retrieval was carried out as described above. Sections were treated with pepsin for 20 minutes (prepared at 0.4 % in 0.1 M HCl and diluted 1 in 8 in 0.01 M HCl). After washing, sections were blocked with normal goat serum and incubated overnight with primary antibodies at 1/600 at room temperature. Sections were then incubated in biotinylated goat-anti-rabbit secondary at 1/100 and developed using the ABC method and DAB as described above.

2.2.9.6 COX-2

Sections labelled for the prostaglandin biosynthetic enzyme COX-2 staining were quenched for 20 minutes, incubated in citrate buffer as described above and blocked with normal horse serum in PBS for 2 hours. The goat antibody against murine COX-2 was then diluted 1/1000 and incubated on sections overnight at room temperature. Sections were then incubated in biotinylated horse-anti-rabbit secondary at 1/100 and developed using the ABC method and DAB as described above.

2.2.9.7 HPGDS

Sections labelled for the prostaglandin biosynthetic enzyme HPGDS staining were quenched for 20 minutes, incubated in citrate buffer as described above. Sections were then treated with pepsin for 20 minutes (prepared at 0.4 % in 0.1 M HCl and diluted 1 in 8), and blocked with normal goat serum in PBS for 40 minutes. The rabbit antibody against HPGDS was then diluted 1/100 and incubated on sections overnight at 4°C. Sections were then incubated in biotinylated goat-anti-rabbit secondary at 1/100 and developed using the ABC method and DAB as described above.

2.2.9.8 Synaptophysin

Sections for labelling of the synaptic vesicle protein synaptophysin were treated with 0.2 M boric acid, pH 9, 65°C for 30 minutes and cooled to room temperature. Quenching was performed as described above before addition of normal horse serum for 40 minutes. The primary antibody (SY38) was applied at 1/2000 and incubated on the sections overnight at room temperature.

Sections were then labelled using biotinylated horse-anti-mouse antibody developed using the ABC method and DAB in the presence of ammonium nickel sulphate to give a more intensely dark stain.

2.2.9.9 Double labelling and confocal microscopy

Double labelling experiments were performed using the primary antibody MR5D3 against CD206, a marker of perivascular macrophages (Galea et al., 2005) and the COX-1 primary antibody described above. The Alexafluor (Invitrogen, UK) secondary reagents were as follows: Goat anti-rabbit 488 (for COX-1), and Chicken anti-rat 594 (for CD206) used at 1:400. Non-specific peroxidase activity elimination and antigen retrieval steps were performed as described above. Sections were treated for 20 minutes with 0.4% pepsin in 0.1 M HCl and blocked in 10% normal goat serum + 10% normal chicken serum in PBS. CD206 primary antibody was incubated overnight (1:1500). The following day, sections were incubated in ant-rat 594 antibody and treated overnight with the primary antibody against COX-1 (1:600). Following addition of the anti-rabbit 488, sections were mounted in ProLong Gold (Invitrogen, Biosciences, Dublin) and coverslipped. Double-labelled sections were visualized and captured on an Olympus FV1000 confocal microscope using sequential excitation at 488 and 594 excitation (the emission maxima for these are 519 and 617 respectively) with a 60× oil (numerical aperture 1.35) objective. Images shown are single sections of 0.36 μm extracted from Z-stacks of 10-μm sections.

2.2.9.10 TUNEL

Immunohistochemistry for apoptotic cells was performed using the "Dead End" TUNEL staining kit (Promega, Southampton, UK) Non-specific peroxidase activity was eliminated as described above. Sections were then washed in 0.85% NaCl for 5 minutes before pretreatment with proteinase K (10 or 20 μg/ml) for 5 minutes. Sections were preincubated with equilibration buffer for 10 minutes and then with TUNEL buffer (25 μl per section: 22 μl equilibration buffer, 2.5 μl nucleotide mix, 0.5 μl TdT enzyme) for 2 hours at 37 °C, labelling apoptotic cells with flourescein. The reaction was stopped by addition of a 2X sodium citrate solution to sections for 15 minutes. Sections were then blocked with 10% normal goat serum for 30 minutes and incubated with biotinylated anti-flourescein antibody (5 μg/ml) and continued with the normal Avidin-Biotin-Complex for visualisation as described above. Cells that were TUNEL-positive and also showed evidence of nuclear condensation were counted in the hippocampal and thalamic regions of 10μm coronal sections of ME7 animals treated with LPS, in the presence or absence of dexamethasone-

21-phosphate. Two sections were counted and summed for each animal and groups of n = 4 or n = 5 animals were grouped to calculate average numbers of cells per treatment.

2.2.10 Quantification of synaptic loss

Synaptic density was assessed using immunolabelling of the presynaptic marker synaptophysin using the monoclonal antibody SY38 as described above. This marker showed progressively decreased density in the stratum radiatum with progressing disease. Photomicrographs at 20x magnification, centred on the corpus callosum, were taken under constant illumination. Images were converted to 16 bit grayscale in ImageJ (NIH, Bethesda, US) and transmittance was quantified using the measure function. We subtracted transmittance in the areas of interest from those for the corpus callosum and divided by transmittance in the overlying cortex to standardise for differences in density of labelling from section to section. We thus quantified the density of labelling according to the calculation:

$$\frac{\text{Transmittance (corpus callosum)} - \text{Transmittance (stratum radiatum)}}{\text{Transmittance (corpus callosum)} - \text{Transmittance (cortex)}}$$

2.2.11 Statistics

All statistical analyses were performed using GraphPad Prism 5 for Windows. In all cases data are expressed as mean ± SEM.

2.2.11.1 Chapter 3

For all measures, experimental groups were analysed using one-way ANOVA. Where a significant difference was found Bonferroni post-hoc analysis was performed. In all cases, the high dose of each proinflammatory cytokine was compared to saline/LPS. In addition, where there was not a significant difference between high dose and saline and the low dose mean was greater than the high dose, a comparison between low does and saline/LPS was made. Where appropriate, comparisons were also made between high and low doses of cytokines.

2.2.11.2 Chapter 4

For cognitive testing, two-way repeated measures ANOVA was used to compare treatments over a number of time-points. Treatment was the between-subjects factor and time was the within-subjects factor. Sickness behaviour, temperature response, ELISA and Q-PCR data were analysed by two-way ANOVA with disease state (NBH vs. ME7) and treatment (saline vs. IL-1β) or time (12 vs. 16 weeks) as the factors. Synaptic density was quantified using one-way ANOVA to compare experimental groups. Bonferroni post-hoc tests were performed following a significant ANOVA.

2.2.11.3 Chapter 5

For cognitive testing, two-way repeated measures ANOVA was used to compare treatments over a number of time-points. Treatment was the between-subjects factor and time was the within-subjects factor. Bonferroni post-hoc tests were performed following a significant ANOVA. Sickness behaviour, temperature response, ELISA and Q-PCR data were analysed by two-way ANOVA with disease state (NBH vs. ME7) and treatment (saline vs. IL-1β) as the factors. Where a significant difference was found, Bonferroni post-hoc analysis was performed. Acute peripheral IL-1β challenge elicits significant increases of inflammatory markers both peripherally and centrally, this was demonstrated in detail in chapter 1. The primary focus of the ELISA, Q-PCR, open field and temperature data experiments in chapter 3 was comparing the response of ME7 and NBH animals to acute IL-1β administration. Hence, the figures denote differences between saline administered animals (comparing NBH and ME7) and IL-β administered animals (comparing challenged NBH and ME7 animals). Where there was a main effect of treatment, Bonferroni post-hoc comparisons of saline versus IL-1β challenge within NBH and ME7 groups were also made and reported in the main text.

Chapter 3: A systematic comparative study of the peripheral and CNS effects of systemic LPS, IL-1β, TNF-α and IL-6 challenges in C57BL/6 mice

The work contained in this chapter forms the majority of a manuscript currently in preparation for *PLoS One*

3.1 Summary

Sickness behaviour mediated by systemic lipopolysaccharide inflammation (LPS) is well described and has been widely reported. However, detailed comparisons of systemic challenges with IL-1β, TNF-α and IL-6 across compartments are lacking. In the present study, we challenged female C57BL/6 mice intraperitoneally with LPS (100 μg/kg), IL-1β (15 or 50 μg/kg), TNF-α (50 or 250 μg/kg) or IL-6 (50 or 125 μg/kg). We investigated effects on core body temperature, open field activity and plasma levels of inflammatory markers at 2 hours post injection. We also examined levels of hepatic, hypothalamic and hippocampal inflammatory cytokine transcripts. Hippocampal transcription of markers of endothelial and microglial activation were also measured. As expected, endotoxin challenge elicited the widest inflammatory response. Systemic IL-1β and TNF-α challenges induced robust systemic and central inflammation with some interesting differences, namely in CXCL1, IL-6 and uPAR. IL-6 was very limited in its ability to induce systemic inflammatory responses and CNS transcripts but did induce components of the hepatic acute phase response. To study the effects of IL-1β in greater detail, we injected further animals with systemic IL-1β (15 μg/kg) and analysed the systemic and central responses across 1, 2, 4, 8 and 24 hours. This study represents a useful and novel systematic comparison of peripheral and central immune responses to the effects of LPS and classic proinflammatory cytokines. The results will form a useful source of information on responses to individual cytokines and have implications for the correlation of individual plasma cytokines with neuroinflammation and deleterious CNS conditions.

3.2 Introduction

The dogma of an 'immune-privileged' CNS has been supplanted over the past twenty years as a result of many studies that demonstrate intricate interactions between the immune and nervous systems (Steinman, 2004). It is now clear that systemic inflammation induced by conserved pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) and double stranded RNA, along with proinflammatory mediators, can trigger CNS inflammation (Konsman et al., 2002). PAMPs, prostaglandins and induced proinflammatory cytokines transduce systemic inflammation into the CNS via parallel neural and humoral routes. The afferent vagal and trigeminal nerves, activated by locally synthesised cytokines, can mediate brainstem, hypothalamic and limbic activation. Circulating PAMPs and macrophage-generated proinflammatory mediators can activate cells along the vasculature of the brain, such as endothelial cells and perivascular macrophages. Circulating inflammatory molecules can also be transported by soluble receptors or diffuse directly into the CNS at circumventricular organs (Dantzer et al., 2008).

Central inflammatory mediators resulting from systemic inflammation, acting primarily on the hypothalamus, are known to produce a coordinated set of adaptive changes, collectively known as sickness behaviour. This motivational state is thought to facilitate recovery from infection and is characterized by a febrile response, reduced social activity, hyperalgesia, fatigue, adipsia and hypersomnia (Dantzer, 2004). Contributions of IL-1β, TNF-α and IL-6 to LPS-induced sickness behaviour have been well described (Dantzer, 2001, Dantzer, 2009, Inui, 2001). In addition, other studies have investigated LPS-induced systemic inflammatory changes and the contributions made by individual cytokines (Fantuzzi and Dinarello, 1996). The effects of peripheral administration of individual cytokines have been compared in terms of temperature (Wang et al., 1997) and anorexia responses (Swiergiel and Dunn, 1999). However, comparisons of their effects on systemic and hypothalamic inflammation, in addition to sickness responses are lacking.

In recent years, researchers have sought to assess the role of individual circulating cytokines as biomarkers and indeed etiologic agents in cognitive decline and diverse clinical CNS conditions. Elevated IL-6 has been linked with reduced hippocampal volume and cognitive decline in the elderly (Marsland et al., 2008, Yaffe et al., 2003, Luterman et al., 2000, Yaffe et al., 2004) while studies of depression have implicated IL-1β, TNF-α and IL-6 (Miller et al., 2009, Dowlati et al.,

2010). The neurodegenerative conditions of Alzheimer's disease and Parkinson's disease have also been correlated with increased circulating proinflammatory cytokines (Tan et al., 2007, Swardfager et al., 2010, Chen et al., 2008a). IL-6 and IL-8 (the functional homologue of CXCL1 in mouse) have been associated with delirious episodes (Beloosesky et al., 2007, de Rooij et al., 2007, van Munster et al., 2008, MacLullich et al., 2011). However, causation cannot be implied from studies such as those above and positive findings have been poorly reproduced (Lemstra et al., 2008). Despite these studies increasingly implicating individual cytokines in CNS conditions, there is sparing information regarding the impact of each cytokine on circulating and hippocampal markers of inflammation.

Considerable basic literature demonstrates that systemic inflammatory events can specifically impact hippocampal functioning; peripheral insults can have deleterious effects on neuronal functioning and cognitive tasks (Yirmiya and Goshen, 2011, Cunningham and Sanderson, 2008). Systemic inflammation induced by LPS has been shown to be particularly damaging when it interacts with chronic neurodegeneration, producing exaggerated hippocampal inflammation (Cunningham et al., 2005), acute cognitive impairment (Murray et al., 2012) and acceleration of disease (Cunningham et al., 2009a) Ageing also confers increased vulnerability on the hippocampus to the consequences of systemic inflammatory challenges (Godbout et al., 2005, Chen et al., 2008b, Barrientos et al., 2006, Chapman et al., 2010). Low-level systemic inflammation, produced by infection, surgery or tissue trauma, has been shown to induce exaggerated sickness behaviour symptoms (Holmes et al., 2011), precipitate episodes of delirium (George et al., 1997) and contribute to progression of cognitive decline (Holmes et al., 2009) in aged and demented patients.

Though there are now many basic studies demonstrating CNS inflammatory responses to systemic inflammatory stimulation, pertinent questions remain regarding whether peripheral elevation of different cytokines initiate similar or distinct inflammatory profiles in different compartments. To delineate their potential contributions to cognitive impairment and neurodegeneration, it would be instructive to characterise and compare the hippocampal inflammation induced by individual proinflammatory cytokines. For these reasons, the objective of this study was to perform a systematic analysis of the effects of LPS (100 μg/kg), IL-1β (15 or 50 μg/kg), TNF-α (50 or 250

μg/kg) and IL-6 (50 or 125 μg/kg) in female C57BL/6 mice. The effects of these inflammatory molecules on locomotor activity, core body temperature, plasma inflammatory mediators, as well as liver, hypothalamic and hippocampal gene transcription, are compared 2 hours post-challenge, which we have previously identified as the peak of many of these responses to systemic LPS (Murray et al., 2012). Given the profound effects seen following systemic IL-1β challenge, the time course of the effects of 15 μg/kg IL-1β on peripheral and CNS inflammation was examined by sacrificing animals at 1, 2, 4, 8 and 24 hours after systemic injection.

3.3 Results

3.3.1 Impact of systemic LPS, IL-1β, TNF-α and IL-6 treatment on sickness behaviour

3.3.1.1 Core body temperature

The effect of systemic treatment (i.p.) with saline, LPS (100 μg/kg), IL-1β (15 μg/kg or 50 μg/kg), TNF-α (50 μg/kg or 250 μg/kg) and IL-6 (50 μg/kg or 125 μg/kg) on the deviation of core body temperature from baseline (t = 0) at 2 hours post-challenge in C57BL/6 female mice was examined (Figure 3.1a). A Bonferroni post-hoc test following a significant one-way ANOVA ($F = 6.552$, df 4,19, $p = 0.0017$) revealed that injection of LPS and IL-1β induced marked hypothermic responses with respect to both saline and IL-6 challenges ($p < 0.05$). TNF-α induced a one degree decreases in temperature but this was not statistically significant ($p > 0.05$) in comparison with saline treatment. In contrast, IL-6 did not produce any change in core body temperature ($p > 0.05$). Thus, peripheral administration of LPS and IL-1β both elicited a reduction in core body temperature.

3.3.1.2 Locomotor activity

Systemic LPS, IL-1β, TNF-α and IL-6 induced a clear reduction in locomotor activity 2 hours post-challenge, in comparison with saline-challenged mice (Figure 3.1b). These differences were shown by a Bonferroni post-hoc test ($p < 0.05$) following a significant one-way ANOVA ($F = 16.34$, df 4,20, $p = 0.0001$). LPS treated animals were significantly less active than the TNF-α- and IL-6-treated animals ($p < 0.05$). IL-6 low dose and high dose-treated animals also differed significantly from each other in terms of reduction in locomotor activity ($p < 0.05$). Thus, all cytokines were capable of eliciting some reduction in locomotor activity two hours post-injection.

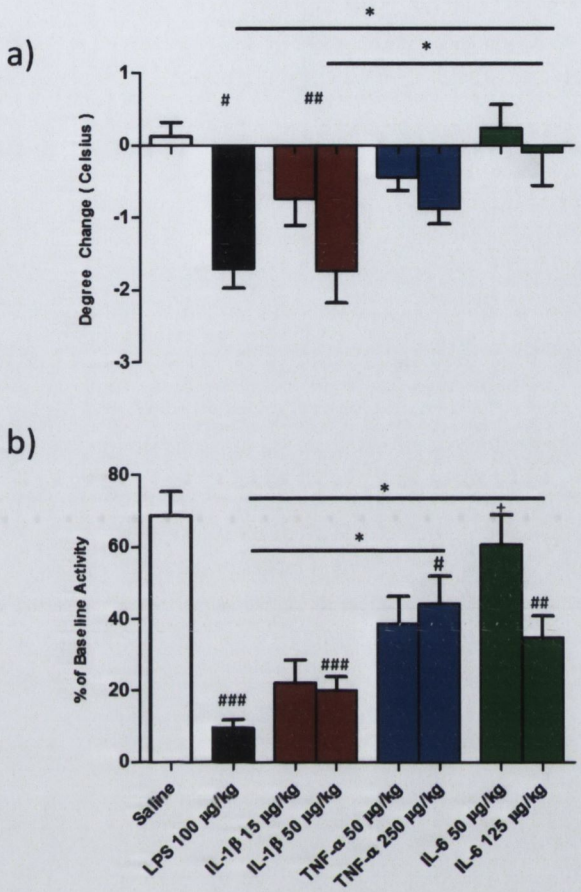

Figure 3.1 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 treatment on core body temperature and locomotor activity.* a) The deviation in core-body temperature from baseline (t=0) in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). # denotes treatment is significantly different to saline control, $p < 0.05$ ## $p < 0.01$, ### $p < 0.001$. * denotes differences between treatments $p < 0.05$ (n =5 for all groups, except LPS n =4 and 15 µg/kg IL-1β n = 3). b) Locomotor activity changes as a percentage of baseline (t=0) under the same conditions. # denotes treatment is significantly different to saline $p < 0.05$, * denotes significant difference between treatment groups indicated by lines $p < 0.05$. + denotes significant difference between low and high dose of cytokine $p < 0.05$. n = 5 for all groups, except 15 µg/kg IL-1β n = 3. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. All data have been presented as mean ± SEM.

3.3.2 Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on plasma inflammatory mediators

3.3.2.1 Proinflammatory cytokines

To assess the impact of peripheral challenge with saline, LPS (100 μg/kg), IL-1β (15 μg/kg or 50 μg/kg), TNF-α (50 μg/kg or 250 μg/kg) and IL-6 (50 μg/kg or 125 μg/kg) on proinflammatory cytokines in the plasma of mice, ELISAs were performed for IL-1β, TNF-α, IL-6 and IFN-β on blood collected 2 hours post-challenge (Figure 3.2). The appearance of the injected cytokine in the plasma was confirmed for each of the three cytokines used, but for IL-1β and TNF-α assays the injected cytokine was excluded from the analysis since injected and induced cytokine could not be distinguished. In addition, the levels of cytokines produced by the other inflammatory challenges would have appeared negligible and could not have been compared to each other. IL-1β levels after injection of IL-1β are presented below in the IL-1β time course data (Section 3.3.6.1). Inflammatory markers below detection limits in experimental groups were set to detection limit in order to facilitate statistical comparisons with detectable means in other experimental groups.

A significant one-way ANOVA ($F = 13.24$, df 3,15, $p = 0.002$) with Bonferroni pairwise tests for levels of circulating IL-1β revealed LPS and TNF-α treatments to be significantly different from saline (at detection limit) and IL-6 (at detection limit) ($p < 0.01$). Thus, IL-1β was induced by TNF-α but not by IL-6.

TNF-α was robustly induced by LPS challenge but undetectable in IL-1β- and IL-6-treated animals. Bonferroni post-hoc comparisons after a significant one-way ANOVA ($F= 11.48$, df 4,15, $p = 0.0004$) revealed these levels to be greater than those induced by saline and IL-6, which were at the detection limit of the assay ($p < 0.01$). There was no significant difference between saline, IL-6- and IL-1β-treated animals ($p > 0.05$). Therefore, IL-6 and IL-1β administration did not induce systemic TNF-α at 2 hours.

A one-way ANOVA ($F= 13.24$, df 3,15, $p= 0.002$) with Bonferroni pairwise tests for circulating IL-6 revealed that LPS and IL-1β induced significantly greater levels of IL-6 than those induced by

saline and TNF-α- ($p < 0.0001$). IL-6 levels were not elevated significantly in IL-6 animals compared to saline ($p < 0.05$), in contrast to IL-1β and TNF-α induction after concomitant challenges. In summary, IL-6 was increased by IL-1β administration but not TNF-α or IL-6 at 2 hours post-challenge.

IFN-β was detected at low levels after LPS treatment in comparison to levels induced by poly I:C challenge (Field et al., 2010) but IFN-β was not detected after saline, IL-1β, TNF-α or IL-6 treatments. A one-way ANOVA with the undetected means set at detection limit did not reject the null hypothesis ($F = 2.858$, df 4,10, $p= 0.0811$). It can be stated, however, that only LPS challenge induced any detectable plasma IFN-β.

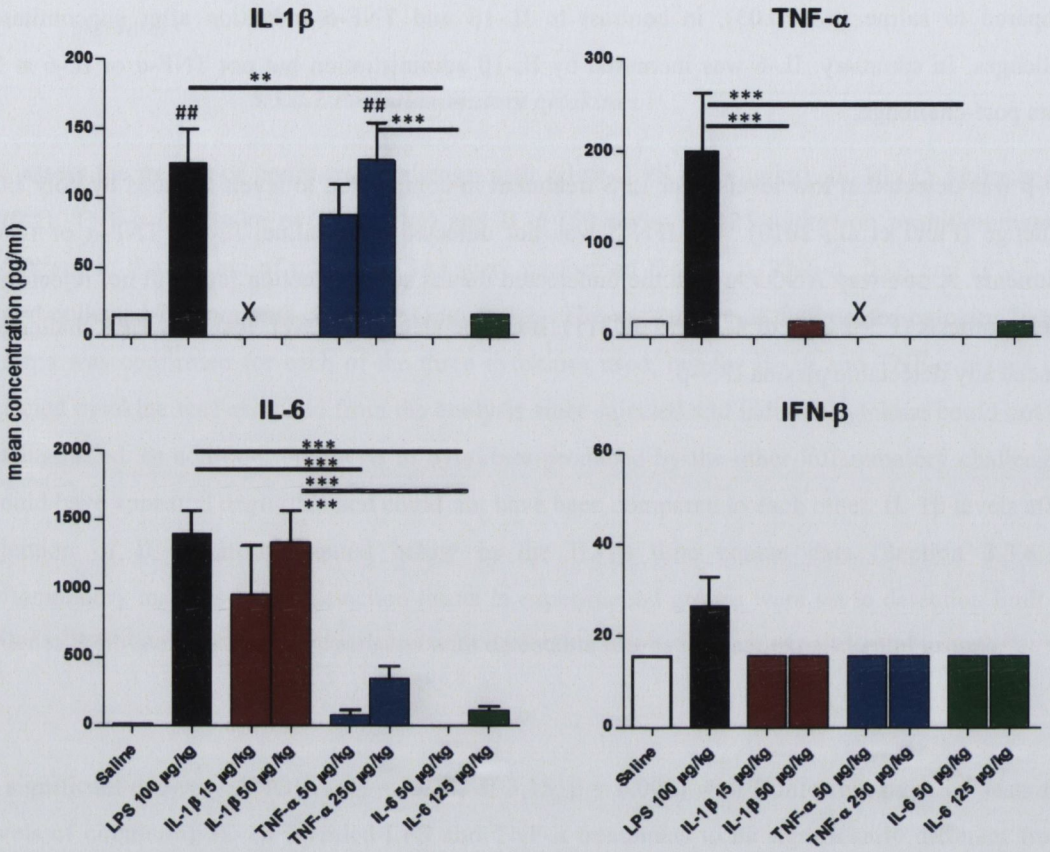

Figure 3.2 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on plasma proinflammatory cytokine levels.* IL-1β, TNF-α, IL-6 and IFN-β as measured by ELISA in plasma prepared from whole blood of C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. X denotes injected cytokine levels not shown, ## denotes treatment is significantly different to saline control $p < 0.05$. ** denotes significant difference between treatment groups indicated by lines $p < 0.01$, *** $p < 0.001$. All data have been presented as mean ± SEM, n =5 for all groups except LPS and IL-1β 50 µg/kg n = 4 and IL-1β 15 µg/kg n = 3.

3.3.2.1 Prostaglandin E2 metabolite and corticosterone

Prostaglandin E metabolite (PGEM) and corticosterone levels were also assessed in blood collected 2 hours post-challenge (Figure 3.3). One-way ANOVA ($F = 21.57$, df 4,16, $p = 0.0001$) followed by Bonferroni post-hoc comparisons showed that the LPS treatment group was significantly different to all other treatment groups for PGEM, measured as a proxy for rapidly metabolised plasma levels of PGE2 ($p < 0.001$). While the higher dose of TNF-α, IL-6 and IL-1β induced some small non significant increases in PGEM at 2 hours ($p > 0.05$), only LPS treatment resulted in robustly elevated circulating PGEM.

Following a statistically significant one-way ANOVA ($F = 3.031$, df 4,17, $p = 0.0467$), Bonferroni post-hoc pairwise comparisons revealed no significant differences in circulating corticosterone levels between the experimental groups ($p > 0.05$). Due to significant variability we cannot state that corticosterone levels are significantly elevated, but LPS, IL-1β and TNF-α all show a trend towards corticosterone induction.

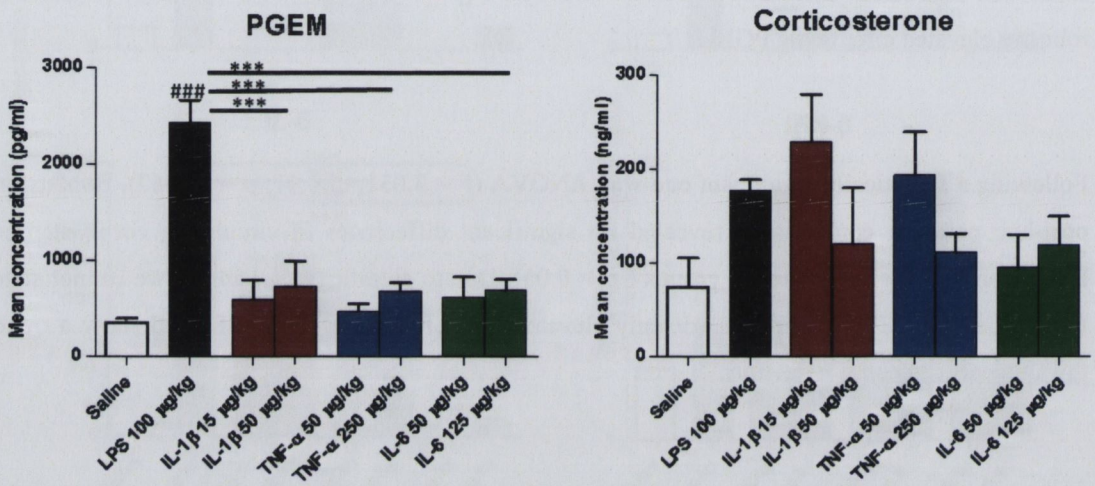

Figure 3.3 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on plasma prostaglandin E metabolite and corticosterone levels.* PGEM and corticosterone concentrations as measured by ELISA in plasma prepared from whole blood of C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. # denotes treatment is significantly different to saline $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. *** denotes significant difference between treatment groups indicated by lines $p < 0.001$. All data have been presented as mean ± SEM, n =5 for all groups except LPS and IL-1β 50 µg/kg n = 4 and IL-1β 15 µg/kg $n \geq 3$.

3.3.3 Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on liver mRNA expression

3.3.3.1 Proinflammatory cytokines IL-1β, TNF-α and downstream genes

To assess the hepatic inflammatory response in animals 2 hours post-peripheral challenge with saline, LPS (100 μg/kg), IL-1β (15 μg/kg or 50 μg/kg), TNF-α (50 μg/kg or 250 μg/kg) and IL-6 (50 μg/kg or 125 μg/kg), liver mRNA expression of proinflammatory cytokines IL-1β and TNF-α and respective downstream genes PTX3 and TNF-αIP2 was measured using quantitative PCR (Figure 3.4).

Hepatic IL-1β mRNA was induced to significantly higher levels by peripheral LPS-treatment than IL-1β, IL-6 and TNF-α-treatment ($p < 0.05$). This was demonstrated by a significant one-way ANOVA ($F = 6.270$, df 4,20, $p = 0.0019$), followed by Bonferroni comparisons ($p < 0.05$). None of the proinflammatory cytokines induced expression of IL-1β mRNA.

Similarly, PTX3 mRNA, putatively transcribed downstream of IL-1β action, was significantly induced by LPS treatment compared to saline and each proinflammatory cytokine ($p < 0.05$). This was demonstrated by Bonferroni post-hoc tests following a significant one-way ANOVA ($F = 7.911$, df 4,19, $p = 0.0006$).

TNF-α was significantly induced in LPS-treated and TNF-α-treated animals, compared to saline-treated animals. TNF-α low and high dose-treatment groups also differed significantly ($p < 0.05$). A one-way ANOVA ($F = 14.50$, df 5,24, $p = 0.0001$) and Bonferroni post-hoc tests was utilised for this analysis.

TNF-αIP2, a gene described as being expressed downstream of TNF-α (Chen et al., 2011), was robustly induced by LPS, IL-1 and TNF-α. Following a significant one-way ANOVA ($F = 95.93$, df 4,20, $p = 0.0001$), Bonferroni post-hoc comparison revealed that LPS, IL-1β and TNF-α challenges, produced significantly higher levels than saline-challenges. Levels elicited by LPS and

high dose TNF-α were significantly higher than the high dose of IL-1β and IL-6. High dose IL-1β challenge also induced significantly greater TNF-αIP2 mRNA than high dose IL-6 (all $p < 0.0001$). In summary, LPS and TNF-α-treatment elicited the most robust induction of TNF-αIP2; however, IL-1β challenge also resulted in significant induction of the gene.

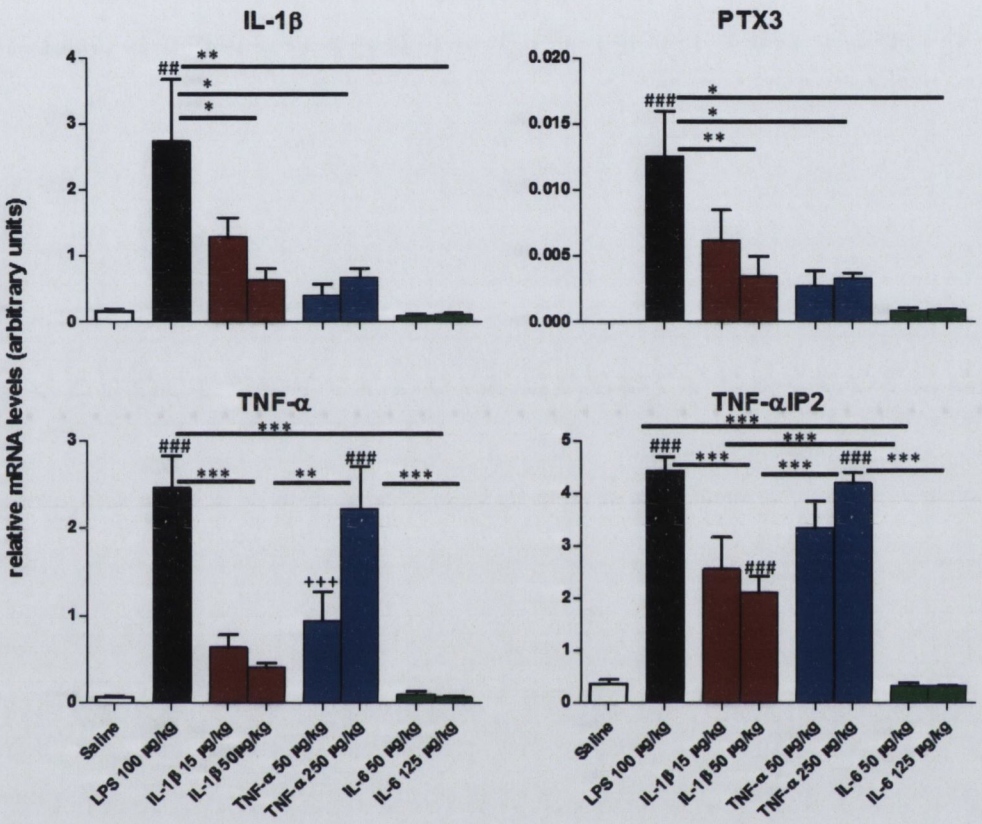

Figure 3.4 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hepatic proinflammatory cytokines IL-1β, TNF-α and downstream genes mRNA expression.* Liver expression of mRNA species for IL-1β, PTX3, TNF-α and TNF-αIP2 measured using quantitative PCR in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. ##, denotes treatment is significantly different to saline treatment $p < 0.005$, ### $p < 0.001$. * denotes significant difference between treatment groups indicated by line $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. +++ denotes significant difference between low and high dose of cytokine $p < 0.001$. All data have been presented as mean ± SEM, n = 5 for all groups.

3.3.3.2 Acute Phase Response transcription

Liver mRNA expression of acute phase proteins serum amyloid alpha 2 (SAA2), CXCL1 and MCP-1 was examined post-systemic proinflammatory challenges (Figure 3.5).

Peripheral challenge with IL-6 elicited a robust increase in SAA2. Other than eliciting a reduction in open field activity, this is the only statistically significant evidence of IL-6 induced-biological activity in the current study. Following a significant one-way ANOVA ($F = 7.858$, df 4,20, $p = 0.0006$), post-hoc tests showed that LPS, IL-1β, TNF-α and IL-6 significantly induced SAA2 mRNA expression in comparison to saline challenge. IL-6 low dose and high dose also differed significantly ($p < 0.05$).

Bonferroni post-hoc comparisons after a significant one-way ANOVA ($F = 7.453$, df 4,20, $p = 0.0008$) showed that CXCL1 was significantly induced in LPS and low dose IL-1β-treated animals compared to saline ($p < 0.05$). LPS treatment induced significantly higher CXCL1 mRNA levels than high dose IL-1β and IL-6 ($p < 0.01$). Thus, peripheral injection of the low dose of IL-1β induced higher hepatic CXCL1 mRNA levels than injection of the high dose and the levels elicited were almost equivalent to those brought about by LPS challenge. TNF-α administration induced more than a ten-fold increase in CXCL1 mRNA. Moreover, IL-6 treatment also induced a considerable fold change in transcription. However, both of these increases were not statistically significant in comparison to saline challenge ($p > 0.05$).

Following a significant one-way ANOVA ($F = 12.87$, df 4,20, $p = 0.0001$), Bonferroni comparisons revealed that LPS treatment induced significantly more hepatic MCP-1 mRNA than saline-challenge and challenge with each proinflammatory cytokine ($p < 0.05$).

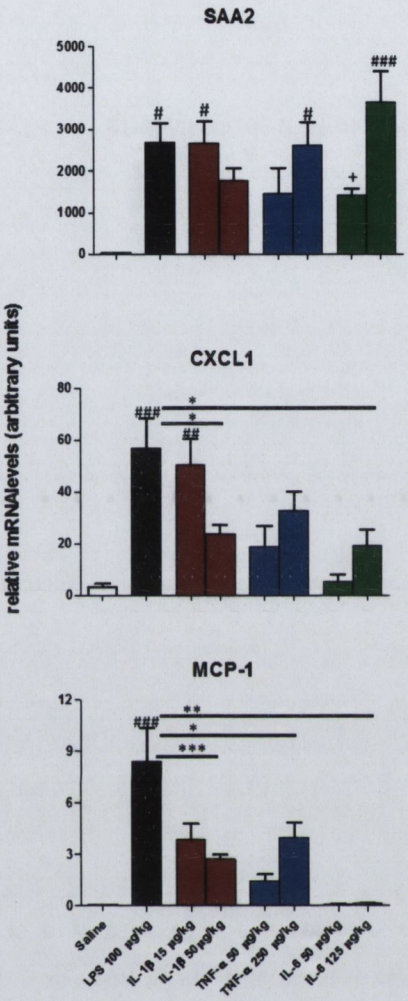

Figure 3.5 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hepatic acute phase proteins mRNA expression.* Liver expression of mRNA species for SAA2, CXCL1 and MCP-1, measured using quantitative PCR in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. # denotes treatment is significantly different to saline treatment $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. * denotes significant difference between treatment groups indicated by lines $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. + denotes significant difference between low and high dose of cytokine $p < 0.05$. All data have been presented as mean ± SEM, $n = 5$ for all groups.

3.3.4 Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hypothalamic mRNA expression

Hypothalamic mRNA expression of proinflammatory cytokines IL-1β, TNF-α, IL-6, and a marker of HPA activation, corticotrophin releasing factor (CRF) was measured (Figure 3.6) using quantitative PCR in animals 2 hours post-peripheral challenge with saline, LPS (100 μg/kg), IL-1β (15 μg/kg or 50 μg/kg), TNF-α (50 μg/kg or 250 μg/kg) and IL-6 (50 μg/kg or 125 μg/kg).

LPS and IL-1β challenges induced significant expression of IL-1β mRNA compared to saline and IL-6 challenges, demonstrated by Bonferroni post-hoc comparisons ($p < 0.01$) following a significant one way ANOVA ($F = 11.38$, df 4,20, $p = 0.0001$). There also appears to have been a trend towards a TNF-α induced increase in IL-1β mRNA but there was no significant difference between this treatment and saline treatment ($p > 0.05$).

Hypothalamic TNF-α mRNA was induced robustly by systemic LPS, IL-1β and TNF-α challenge compared to both saline and IL-6. TNF-α low dose and high dose also differed significantly. These differences were shown by Bonferroni post-hoc tests ($p < 0.001$) after a significant one way ANOVA ($F = 18.22$, df 5,22, $p = 0.0001$). Thus, peripheral administration of both TNF-α and IL-1β elicited marked hypothalamic TNF-α mRNA, as did LPS. However, IL-6 administration had no effect on mRNA levels, when compared to control treatment.

Hypothalamic IL-6 mRNA was significantly upregulated solely by LPS challenge. A significant one-way ANOVA ($F= 8.897$, df 4,19, $p=0.0003$) followed by Bonferroni post-hoc tests shows that this LPS-induced increase in IL-6 is significant compared to saline, IL-1β , TNF-α and IL-6 challenges ($p < 0.001$). When LPS treatment is excluded from the analysis, IL-1β has a significant effect on IL-6 mRNA ($p < 0.01$) in comparison with saline and IL-6 administration.

CRF mRNA was not significantly altered by acute systemic proinflammatory challenge, this was demonstrated using a one-way ANOVA ($F = 2.840$, df 4,18, $p = 0.0548$).

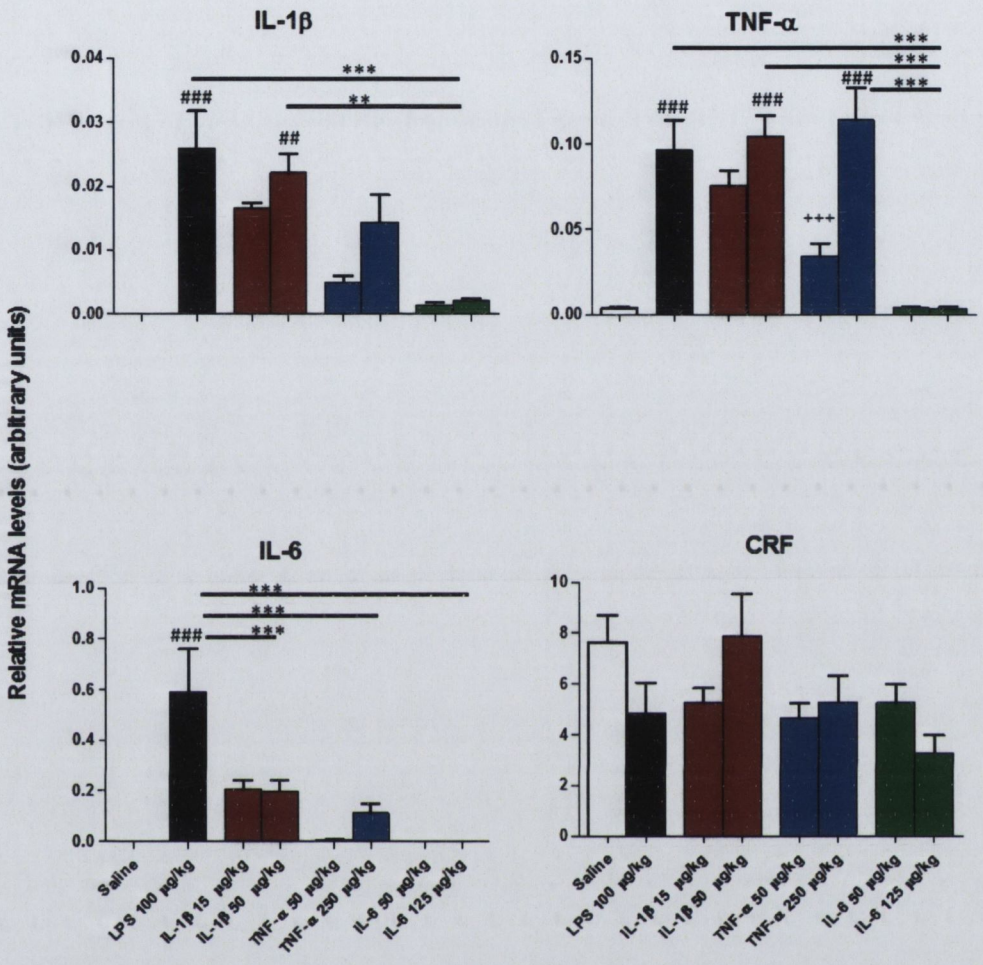

Figure 3.6 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hypothalamic mRNA expression.* Hypothalamic expression of mRNA species for IL-1β, TNF-α, IL-6 and corticotrophin releasing factor (CRF) assessed using quantitative PCR in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. ## denotes treatment is significantly different to saline treatment $p < 0.01$, ### $p < 0.001$. ** denotes significant difference between treatment groups indicated by lines $p < 0.01$, *** $p < 0.001$. All data have been presented as mean ± SEM, n = 5 for all groups, except IL-1β 15 µg/kg n = 3.

3.3.5 Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hippocampal mRNA expression

3.3.5.1 Proinflammatory cytokines

Hippocampal mRNA expression of the proinflammatory cytokines IL-1β, CXCL1, IL-6, and TNF-α was measured (Figure 3.7) using quantitative PCR in animals 2 hours post-peripheral challenge with saline, LPS (100 μg/kg), IL-1β (15 μg/kg or 50 μg/kg), TNF-α (50 μg/kg or 250 μg/kg) and IL-6 (50 μg/kg or 125 μg/kg).

Hippocampal IL-1β mRNA was significantly induced by peripheral administration of LPS, IL-1β and TNF-α in comparison to saline control challenge as demonstrated by Bonferroni post-hoc test ($p < 0.005$) after a significant one-way ANOVA ($F = 11.41$, df 43,18, $p < 0.0001$). IL-1β challenge also induced significantly higher levels than IL-6 (125 μg/kg) ($p < 0.001$).

Hippocampal CXCL1 mRNA was significantly upregulated solely by IL-1β (50 μg/kg) challenge. A significant one-way ANOVA ($F = 10.47$, df 4,18, $p = 0.0001$) followed by Bonferroni post-hoc tests shows that this IL-1β-induced increase in CXCL1 was significantly greater than saline, LPS, TNF-α and IL-6 challenges ($p < 0.01$).

IL-6 mRNA was induced robustly by systemic LPS and IL-1β in comparison with saline, TNF-α and IL-6 (125 μg/kg) challenge, shown by Bonferroni post-hoc tests ($p < 0.001$), after a significant one-way ANOVA ($F = 20.81$, df 4,16, $p = 0.0001$). TNF-α and IL-6 administration did not induce IL-6 mRNA levels that were different to saline challenge ($p > 0.05$). Thus, neither systemic TNF-α nor IL-6 induces hippocampal IL-6 transcription at 2 hours post-challenge.

TNF-α mRNA was significantly upregulated by systemic LPS, IL-1β and TNF-α challenge compared to both saline and IL-6. TNF-α low dose and high dose also differed significantly. These differences were shown by Bonferroni post-hoc tests ($p < 0.001$) after a significant one way ANOVA ($F = 22.67$, df 5,23, $p = 0.0001$).

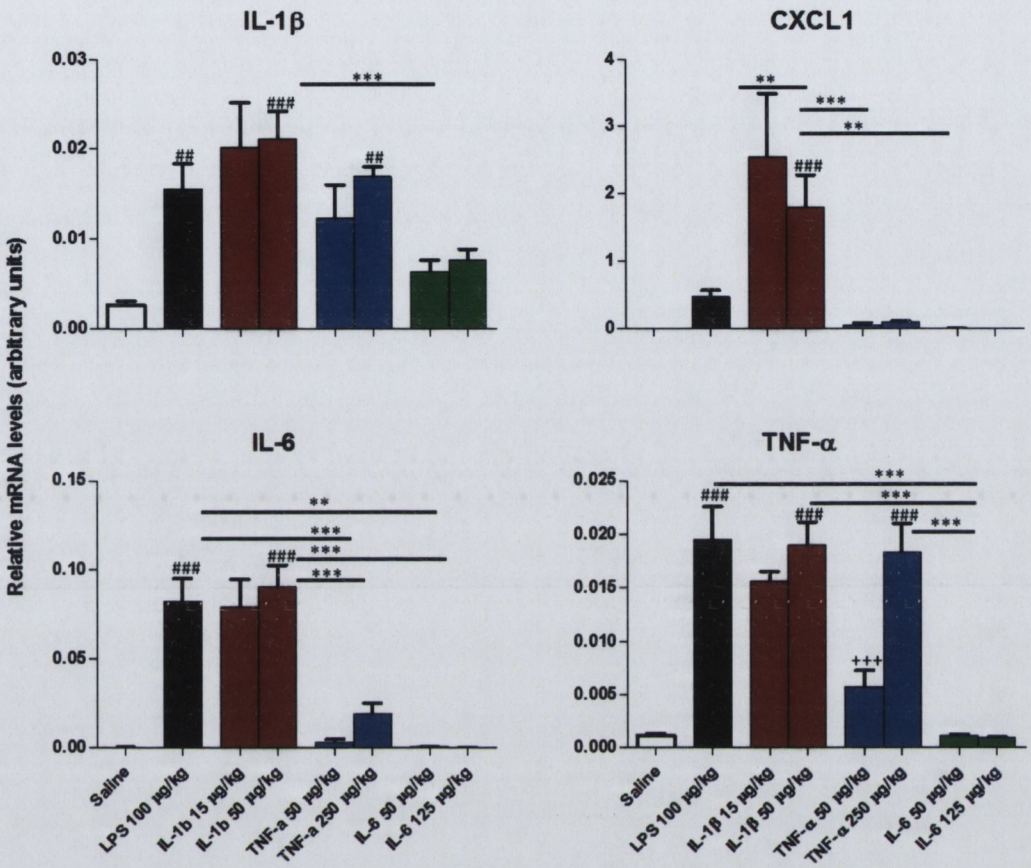

Figure 3.7 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hippocampal inflammatory cytokine expression.* Hippocampal expression of IL-1β, CXCL1, IL-6 and TNF-α mRNA measured using quantitative PCR in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. ## denotes treatment is significantly different to saline treatment $p < 0.01$, ### $p < 0.001$. ** denotes significant difference between treatment groups indicated by lines $p < 0.01$ *** $p < 0.001$. +++ denotes significant difference between low and high dose of cytokine $p < 0.001$. All data have been presented as mean ± SEM, $n = 5$ for LPS and IL-1β 50 µg/kg; $n \geq 4$ for TNF-α 250 µg/kg; $n \geq 3$ for other groups.

3.3.5.2 Type-1 interferons

Hippocampal mRNA expression of type-I interferons IFN-α and IFN-β was measured following the peripheral challenges (Figure 3.8). IFN-α mRNA was not significantly altered by systemic challenge ($F = 1.292$, df 4,18, $p = 0.3101$). Therefore, at 2 hours post-challenge, peripheral administration of LPS and the proinflammatory cytokines failed to elicit hippocampal IFN-α mRNA.

IFN-β mRNA, however, was significantly induced by LPS administration. A one-way ANOVA ($F = 4.454$, df 3,16, $p = 0.0131$) and Bonferroni post-hoc test showed that LPS treatment was significantly greater than saline, TNF-α and IL-6 (125 μg/kg) ($p < 0.05$). Thus, hippocampal IFN-β transcription is induced solely by peripheral LPS administration and not by the proinflammatory cytokines, IL-1β, TNF-α or IL-6.

3.3.5.3 Prostaglandin biosynthetic enzymes

The mRNA expression of prostaglandin biosynthetic enzymes COX-1 and COX-2 was assessed after the systemic proinflammatory challenges (Figure 3.9). COX-1 mRNA was not significantly altered by treatment as shown by a one-way ANOVA ($F = 0.1262$, df 4,18, $p = 0.9710$). COX-2 mRNA, however, was significantly altered by treatment; this was demonstrated by a one-way ANOVA ($F = 3.688$, df 4,19, $p = 0.0219$). Bonferroni post-hoc test showed that LPS treatment induced COX-2 to a significantly greater degree than saline ($p < 0.05$). The proinflammatory cytokines failed to elicit a significant increase in COX-2 mRNA. While they were not statistically significant effects, IL-1β and TNF-α treatment did increase transcription 3 fold.

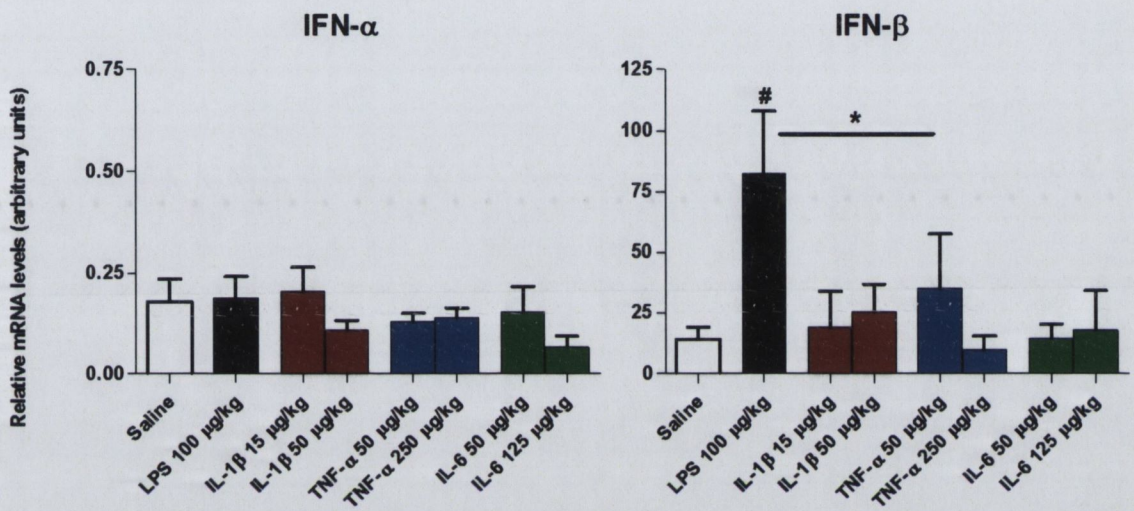

Figure 3.8 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hippocampal type-I interferon mRNA expression.* Hippocampal expression of IFN-α and IFN-β mRNA measured using quantitative PCR in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. # denotes treatment is significantly different to saline treatment $p < 0.05$. * denotes significant difference between treatment groups indicated by lines $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$. All data have been presented as mean ± SEM, $n = 5$ for TNF-α 250 µg/kg and IL-6 50 µg/kg; $n \geq 4$ for saline, LPS and TNF-α 50 µg/kg; $n = 3$ for IL-1β 15 µg/kg and IL-6 125 µg/kg .

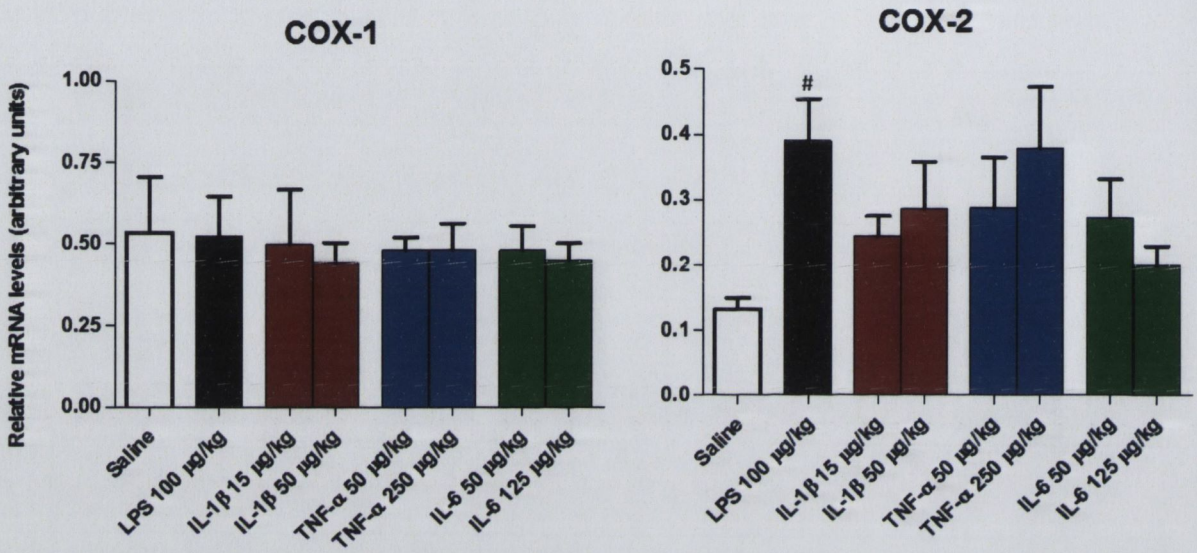

Figure 3.9 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hippocampal prostaglandin biosynthetic enzyme mRNA* expression. Hippocampal expression of the biosynthetic enzymes COX-1 and COX-2 mRNA measured using quantitative PCR in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. # denotes treatment is significantly different to saline treatment ($p < 0.05$). All data have been presented as mean ± SEM, $n = 5$ for all groups except $n \geq 4$ for TNF-α 250 µg/kg; $n \geq 3$ for IL-1β 15 µg/kg and both IL-6 groups.

3.3.5.4 Cell markers

Hippocampal mRNA expression of the endothelial marker VCAM, microglial and putative endothelial marker uPAR (Cunningham et al., 2009b) and microglial activation marker CD11b was examined (Figure 3.10). VCAM mRNA was significantly upregulated by LPS, IL-1β and TNF-α, compared to both saline and IL-6 challenges ($p < 0.001$). These differences were demonstrated by Bonferroni post-hoc tests following a significant one-way ANOVA ($F = 11.52$, df 4,19, $p < 0.0001$). Thus, LPS, TNF-α and IL-1β administration but not IL-6 administration induced upregulation of transcription of the endothelial marker VCAM.

uPAR mRNA was robustly increased by LPS treatment in comparison to saline, IL-1β, TNF-α and IL-6 treatments ($p < 0.001$). However, peripheral IL-1β administration also significantly increased uPAR mRNA in relation to saline treatment ($p < 0.05$). These differences were demonstrated by Bonferroni post-hoc tests following a significant one-way ANOVA ($F = 27.12$, df 4,19, $p < 0.0001$).

CD11b mRNA was not significantly altered by systemic challenge ($F = 0.1240$, df 4,17, $p = 0.9719$). Thus, peripheral challenge with the acute proinflammatory challengers did not result in an increased transcription of this marker of microglial activation.

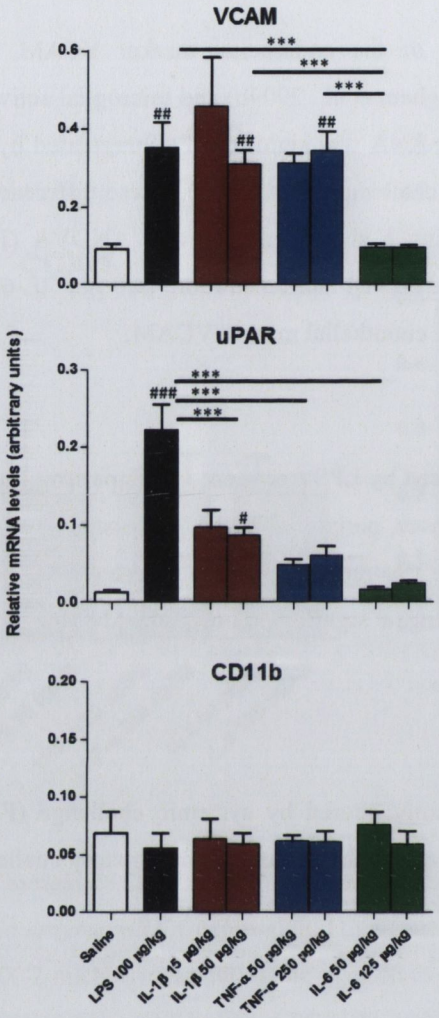

Figure 3.10 *Impact of systemic LPS, IL-1β, TNF-α and IL-6 challenge on hippocampal mRNA expression of cell activation markers.* Hippocampal mRNA expression of VCAM, uPAR and CD11b measured using quantitative PCR in C57BL/6 female mice 2 hours after systemic challenge (i.p.) with saline, LPS (100 µg/kg), IL-1β (15 µg/kg or 50 µg/kg), TNF-α (50 µg/kg or 250 µg/kg) and IL-6 (50 µg/kg or 125 µg/kg). All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between saline, LPS and each cytokine treatment at the higher dose. # denotes treatment is significantly different to saline treatment $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. *** denotes significant difference between treatment groups indicated by lines $p < 0.001$. All data have been presented as mean ± SEM, $n = 5$ for all groups except $n = 4$ for TNF-α 250 µg/kg; $n \geq 3$ for IL-1β 15 µg/kg and both IL-6 groups.

3.3.6 Impact of systemic IL-1β challenge on circulating inflammatory mediators at 1, 2, 4, 8 and 24 hours

3.3.6.1 Proinflammatory cytokines

Given the strong immune response elicited by systemic IL-1β administration in the comparative study, the effect of IL-1β across a 24 hour time course was assessed in terms of systemic, hypothalamic and hippocampal inflammatory markers. Since there were few differences between the two doses of IL-1β utilised in the comparative study, and since the lower dose is also more physiologically relevant, 15μg/kg was used in the time course study.

To assess the impact of peripheral IL-1β (15 μg/kg) challenge on proinflammatory cytokine levels in the plasma of mice, ELISAs were performed for IL-1β, CXCL1, IL-6 and TNF-α on blood collected at 1, 2, 4, 8 and 24 hours post-challenge (Figure 3.11). As in the comparative study, inflammatory markers that were below detection limits in experimental groups were set to the detection limit so as to make statistical comparisons with detectable means in equivalent experimental groups.

A significant one-way ANOVA ($F = 10.52$, df 3,16, $p = 0.0005$) with Bonferroni pairwise comparisons for circulating IL-1β revealed that the cytokine was significantly elevated in comparison to saline at both the 1-hour ($p < 0.05$) and 2-hour time points ($p < 0.01$). Note: the 2 hour time point quantifies the levels which were omitted from the analysis of the cytokine comparative study above (Section 3.3.2).This data demonstrates the acute nature of the elevation of plasma IL-1β post-IL-1β challenge.

An assay for circulating TNF-α was also performed on plasma. No detectable TNF-α was found in any experimental group. Thus, systemic administration of IL-1β (i.p.) did not elicit any detectable circulating TNF-α at any point across 24 hours post-challenge.

CXCL1 was significantly elevated at 1 and 2 hours following IL-1β injection in comparison with the saline controls ($p < 0.001$). A significant one-way ANOVA, followed by Bonferroni post-hoc tests demonstrated these differences ($F = 26.46$, df 3,16, $p < 0.0001$).

Peripheral challenge with IL-1β also led to significantly elevated circulating IL-6 protein compared to the saline groups at both 1 and 2 hours after challenge ($p < 0.0001$). A significant one-way ANOVA ($F = 43.06$, df 3,13, $p = 0.0001$) with Bonferroni pairwise tests was used to analyse the data.

A significant one-way ANOVA ($F = 976.4$, df 7,32, $p = 0.0001$) followed by Bonferroni post-hoc tests on circulating MCP-1 levels revealed that the cytokine was significantly different to saline at 1, 2 and 4-hour time points following systemic IL-1β administration ($p < 0.001$).

3.3.6.2 PGEM

PGEM, measured as a proxy for rapidly metabolised plasma levels of PGE2 was significantly elevated at 1 hour following IL-1β injection compared to saline control treatment ($p < 0.001$) (Figure 3.12). A one-way ANOVA ($F = 6.705$, df 9,33, $p < 0.0001$), followed by Bonferroni post-hoc tests revealed this statistically significant difference. By 4 hours post-IL-1β challenge PGEM levels had returned to basal levels.

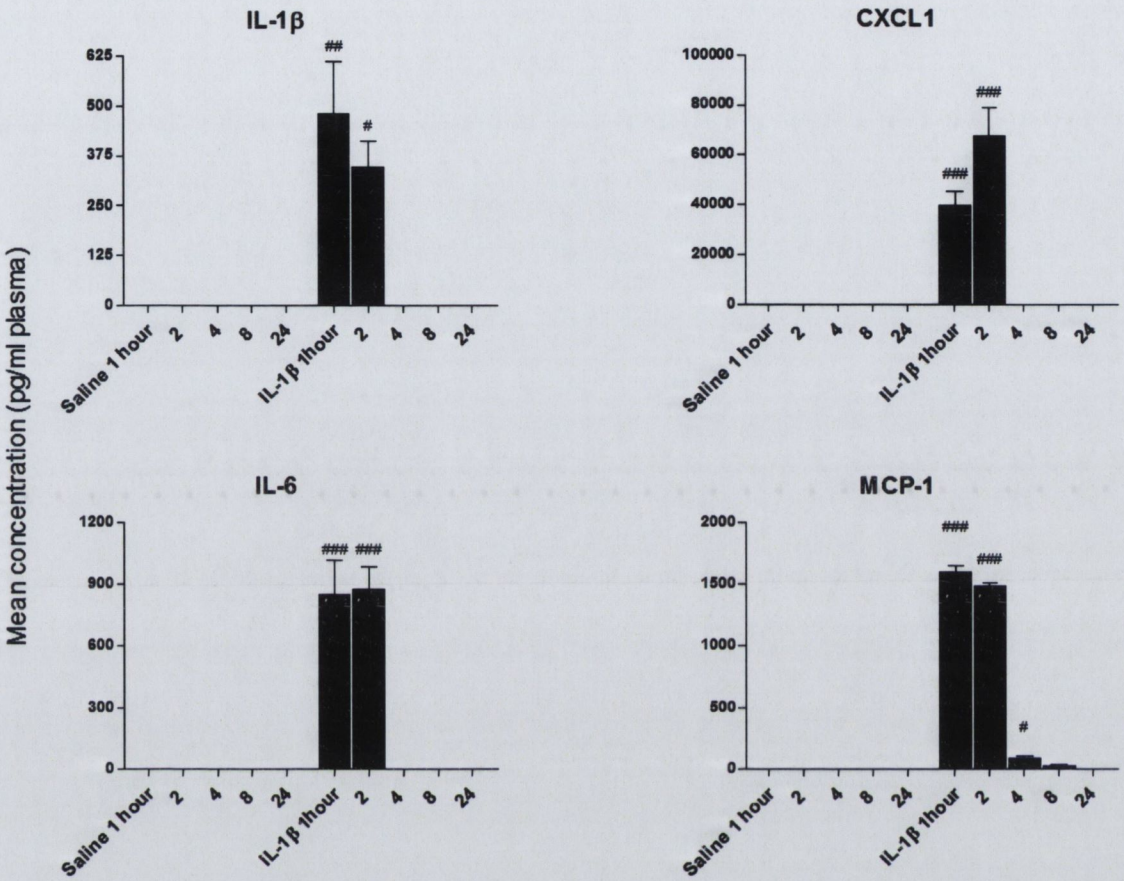

Figure 3.11 *Impact of systemic IL-1β challenge on plasma inflammatory markers at 1, 2, 4, 8 and 24 hours.* IL-1β, CXCL1, IL-6 and MCP-1 as measured by ELISA in plasma prepared from whole blood of C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. # denotes IL-1β treatment is significantly different to equivalent saline treatment $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. All data have been presented as mean ± SEM, n = 5 for all groups except n = 2 for IL-1β 1 hour in IL-6 data.

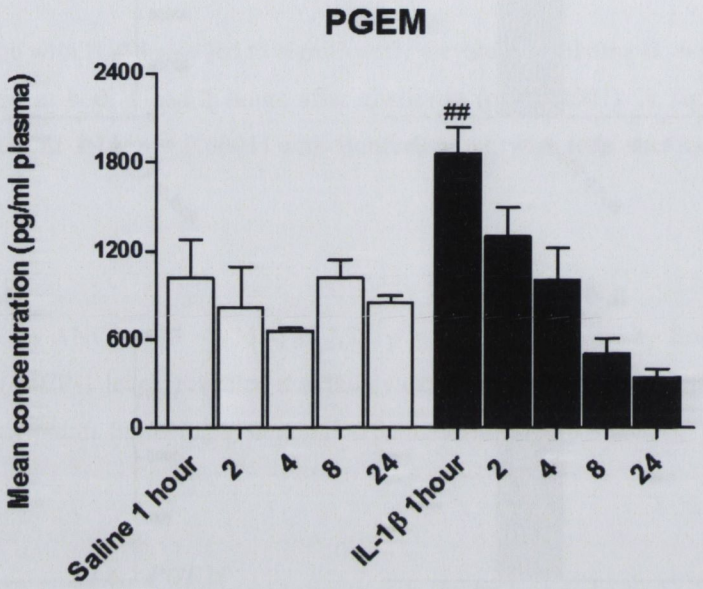

Figure 3.12 *Impact of systemic IL-1β challenge on plasma PGEM at 1, 2, 4, 8 and 24 hours.* Prostaglandin E metabolite (PGEM) as measured by ELISA in plasma prepared from whole blood of C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. ## denotes IL-1β treatment is significantly different to saline treatment $p < 0.01$. All data have been presented as mean ± SEM, n = 5 for all groups except n=3 for saline 8 and 24 hour groups.

3.3.7 Impact of systemic IL-1β challenge on hypothalamic mRNA expression at 1, 2, 4, 8 and 24 hours

3.3.7.1 Proinflammatory cytokines

Hypothalamic mRNA expression of proinflammatory cytokines IL-1β, TNF-α, IL-6, and IFN-β was measured (Figure 3.6) using quantitative PCR in tissue collected at 1, 2, 4, 8 and 24 hours post-IL-1β (15 μg/kg) challenge and compared to equivalent saline groups (Figure 3.13).

Hypothalamic IL-1β mRNA was significantly induced by IL-1β in comparison to saline control challenge at 2 hours after injection as shown by Bonferroni post-hoc test ($p < 0.01$) after a significant one-way ANOVA ($F = 3.368$, df 9,38, $p < 0.0040$).

IL-6 mRNA was induced robustly in the hypothalamus by systemic IL-1β in comparison with saline challenge at 2 hours after challenge. This was shown by Bonferroni post-hoc tests ($p < 0.001$), after a significant one-way ANOVA ($F = 9.593$, df 9,36, $p = 0.0001$).

TNF-α mRNA was also significantly upregulated by systemic IL-1β challenge compared to saline at the 1- and 2-hour time points. These differences were shown by Bonferroni post-hoc tests ($p < 0.001$) after a significant one way ANOVA ($F = 11.19$, df 9,36, $p = 0.0001$).

IFN-β mRNA expression was not significantly altered by systemic IL-1β challenge. A one-way ANOVA ($F= 1.401$, df 7,28, $p = 0.2439$) did not indicate a significant difference.

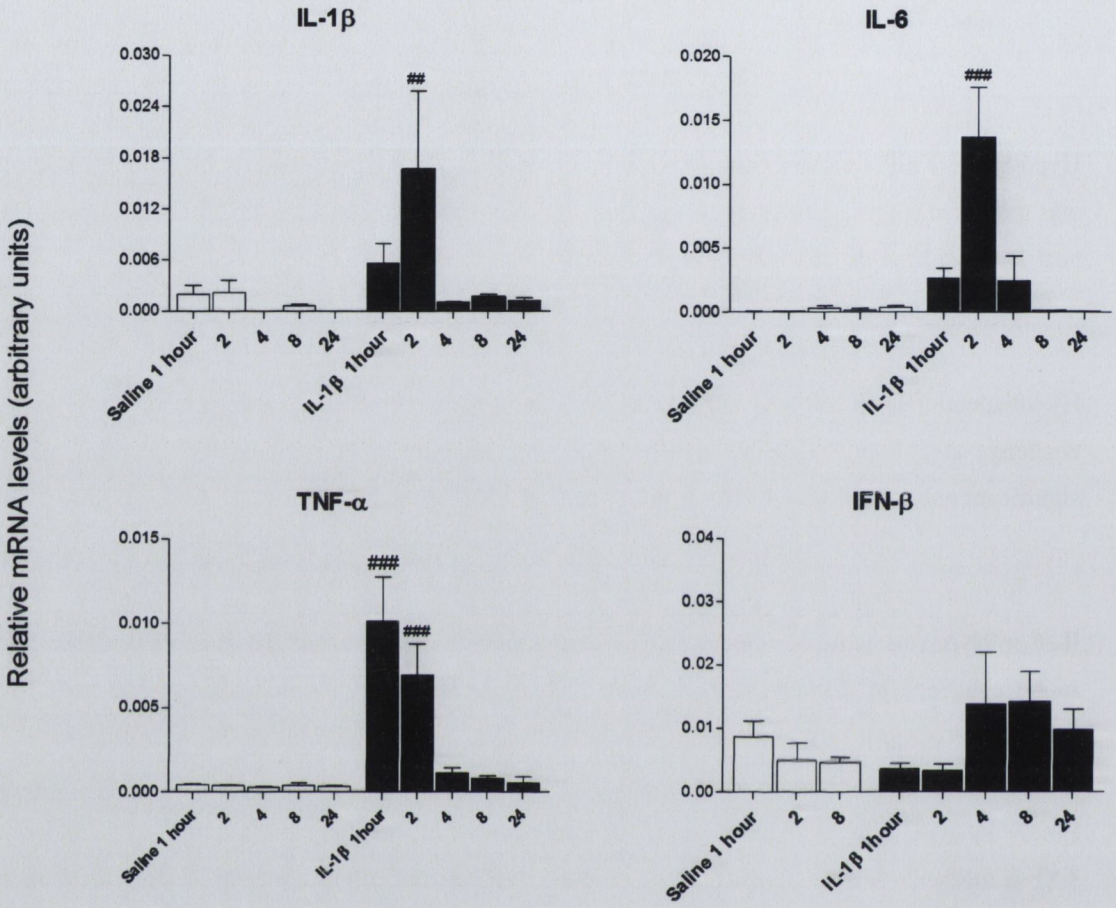

Figure 3.13 *Impact of systemic IL-1β challenge on hypothalamic proinflammatory cytokine mRNA expression at 1, 2, 4, 8 and 24 hours.* Hypothalamic expression of mRNA species for IL-1β, TNF-α, IL-6 and IFN-β was assessed using quantitative PCR in C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. ## denotes IL-1β treatment is significantly different to equivalent saline treatment $p < 0.01$, ### $p < 0.001$. All data have been presented as mean ± SEM, n = 5 for all IL-1β treated groups except 2 hour n = 4 and saline groups $n \geq 3$ saline.

3.3.7.2 Chemokines – MCP-1 and CXCL1

Hypothalamic mRNA expression of acute phase proteins MCP-1 and CXCL1 was also measured across the 24 hour time course after systemic IL-1β challenge (Figure 3.14).

Hypothalamic MCP-1 mRNA was induced by IL-1β challenge. A significant one-way ANOVA ($F = 9.920$, df 9,36, $p = 0.0001$) followed by Bonferroni post-hoc tests showed that this IL-1β-induced increase in MCP-1 was significant compared to saline control at 1 ($p < 0.05$) and 2 hours post-injection ($p < 0.001$).

CXCL1 mRNA was significantly upregulated in the hypothalamus by systemic IL-1β challenge compared to saline controls at 1 hour post-challenge. This difference was shown by Bonferroni post-hoc test ($p < 0.001$) after a significant one way ANOVA ($F = 5.689$, df 9,36, $p = 0.0001$).

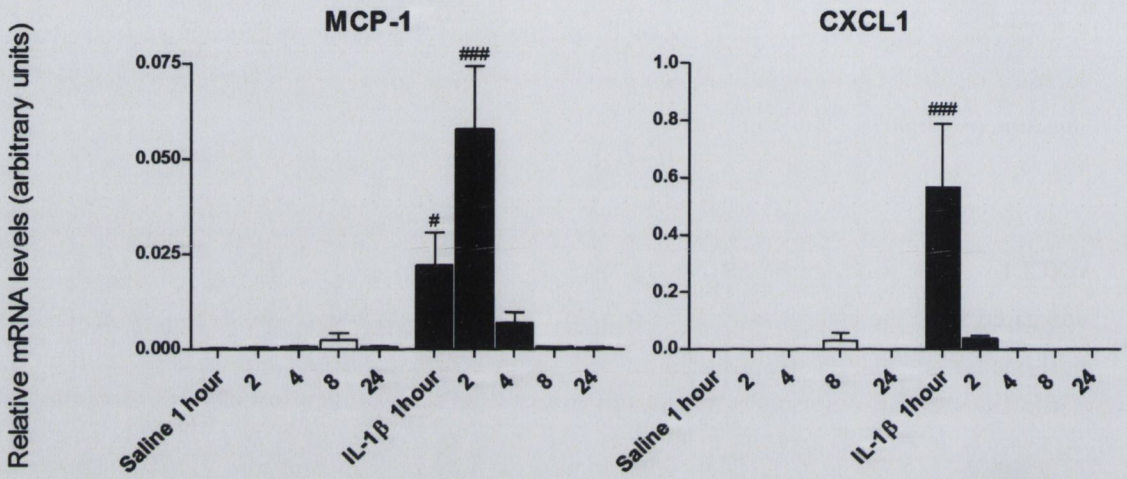

Figure 3.14 *Impact of systemic IL-1β challenge on hypothalamic acute phase protein mRNA expression at 1, 2, 4, 8 and 24 hours.* Hypothalamic expression of mRNA species for CXCL1 and MCP-1 assessed using quantitative PCR in C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. # denotes IL-1β treatment is significantly different to saline treatment $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. All data have been presented as mean ± SEM, $n = 5$ for all IL-1β treated groups except 2 hour $n = 4$ and saline groups $n \geq 3$.

3.3.8 Impact of systemic IL-1β challenge on hippocampal mRNA expression at 1, 2, 4, 8 and 24 hours

3.3.8.1 Proinflammatory cytokines IL-1β and TNF-α, and putative downstream genes PTX3 and MCP-1

Hippocampal mRNA expression of proinflammatory cytokines IL-1β, and TNF-α, and putative downstream genes PTX3 and MCP-1 was measured in animals at 1, 2, 4, 8 and 24 hours post-IL-1β (15 μg/kg) challenge and compared to equivalent saline groups (Figure 3.15).

Hippocampal IL-1β mRNA was significantly increased in animals 2 hours after i.p. IL-1β in comparison with saline challenged animals as shown by Bonferroni post-hoc test ($p < 0.001$) after a significant one-way ANOVA ($F = 4.912$, df 9,36, $p < 0.0001$). Transcription increased in IL-1β – challenged animals non-significantly at 1 hour when compared to saline. At 2 hours post-challenge, transcript levels were significantly higher than those in saline controls. Elevation was relatively short lasting and levels had dropped back to basal expression at 4 hours.

PTX3 mRNA was also upregulated by IL-1β challenge. A significant one-way ANOVA ($F = 13.29$, df 9,35, $p = 0.0001$) followed by Bonferroni post-hoc tests showed that this IL-1β-induced increase in PTX3 is significant compared to saline control levels at 2 hours post-injection ($p < 0.001$).

TNF-α mRNA was significantly upregulated by systemic IL-1β challenge compared to saline control at both 1 and 2 hours post-challenge. These differences were shown by Bonferroni post-hoc tests ($p < 0.001$) after a significant one way ANOVA ($F = 33.62$, df 9,36, $p = 0.0001$).

MCP-1 mRNA was induced robustly by systemic IL-1β in comparison with saline challenge at 1 ($p < 0.05$), and 2-hours ($p < 0.001$), shown by Bonferroni post-hoc tests after a significant one-way ANOVA ($F = 5.461$, df 9,36, $p = 0.0001$). Although it is not absolutely clear, it would appear that IL-1β and TNF-α peak at an earlier point to the putative dependent genes MCP-1 and PTX3.

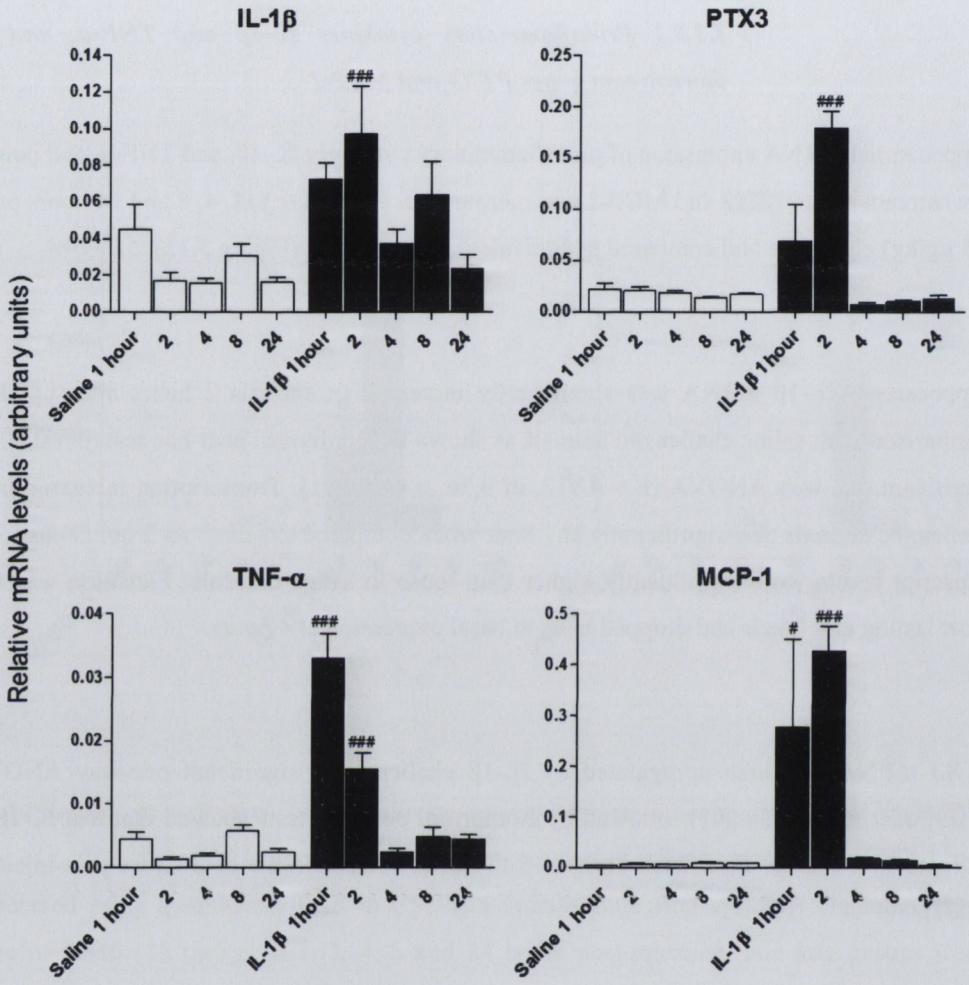

Figure 3.15 *Impact of systemic IL-1β challenge on mRNA expression of the hippocampal proinflammatory cytokines IL-1β and TNF-α and downstream genes at 1, 2, 4, 8 and 24 hours.* Hippocampal expression of IL-1β, PTX3, TNF-α and MCP-1 mRNA measured using quantitative PCR in C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. # denotes IL-1β treatment is significantly different to saline treatment $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. All data have been presented as mean ± SEM, n = 5 for all IL-1β experimental groups except 2 hours n = 4. All saline groups $n \geq 4$ except n = 3 for PTX3 at 4 hours.

3.3.8.2 Inflammatory cytokines IL-6, CXCL1and IFN-β

Hippocampal mRNA expression of IL-6, CXCL1 and IFN-β was also examined across the time course after IL-1β injection (Figure 3.16).

Hippocampal IL-6 mRNA was induced acutely by IL-1β challenge. A significant one-way ANOVA ($F = 40.87$, df 9,33, $p = 0.0001$) followed by Bonferroni post-hoc tests shows that this IL-1β-induced increase in IL-6 mRNA is significant compared to saline controls at 1 ($p < 0.05$) and 2 hours post-injection ($p < 0.001$).

Hippocampal CXCL1 mRNA was significantly upregulated by systemic IL-1β challenge compared to saline control at both 1- and 2-hours post-challenge. These differences were shown by Bonferroni post-hoc tests ($p < 0.001$) after a significant one way ANOVA ($F = 40.87$, df 9,33, $p = 0.0001$) .

IFN-β mRNA expression was not significantly altered by systemic IL-1β challenge. A one-way ANOVA ($F = 1.572$, df 9,35, $p = 0.1623$) did not indicate a significant difference amongst the experimental groups.

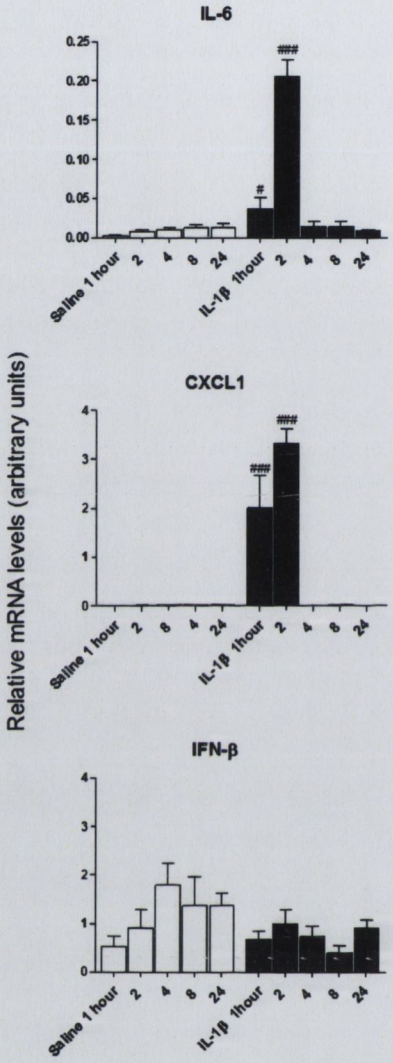

Figure 3.16 *Impact of systemic IL-1β challenge on mRNA expression of hippocampal IL-6, CXCL1 and IFN-β at 1, 2, 4, 8 and 24 hours.* Hippocampal expression of IL-6, CXCL1 and IFN-β mRNA measured using quantitative PCR in C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. # denotes IL-1β treatment is significantly different to saline treatment $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. All data have been presented as mean ± SEM, $n = 5$ for all IL-1β experimental groups except 2 hours $n = 4$. All saline groups $n \geq 4$ except $n = 2$ for IL-6 at 24 hours.

3.3.8.3 Cell activation and pathway markers

Hippocampal mRNA expression of the pleiotropic signalling molecule iNOS, microglial and putative endothelial cell marker uPAR, apoptosis–inducing FAS and plasminogen activator inhibitor (PAI)-1 was examined across the time course after IL-1β injection (Figure 3.18).

iNOS transcription was acutely elevated by systemic IL-1β challenge. At 2 hours the IL-1β treated animals express significantly higher levels of iNOS mRNA than saline-challenged controls. A significant one-way ANOVA ($F = 8.728$, df 9,35, $p < 0.0001$) followed by a Bonferroni post-hoc test ($p < 0.001$) demonstrated these differences.

FAS mRNA was induced robustly by systemic IL-1β in comparison with saline challenge at 1, 2 ($p < 0.001$) and 4 hours ($p < 0.05$) after challenge. This was shown by Bonferroni post-hoc tests after a significant one-way ANOVA ($F = 15.61$, df 9,35, $p = 0.0001$).

uPAR mRNA was significantly upregulated by systemic IL-1β challenge compared to saline at the 1 and 2 hour time points. These differences were shown by Bonferroni post-hoc tests ($p < 0.001$) after a significant one way ANOVA ($F = 45.01$, df 9,35, $p = 0.0001$).

PAI-1 mRNA was induced by IL-1β challenge. A significant one-way ANOVA ($F = 8.070$, df 7,29, $p = 0.0001$) followed by Bonferroni post-hoc tests shows that this IL-1β-induced increase in PAI-1 is significant compared to saline control at 1 ($p < 0.001$) and 2 hours post-injection ($p < 0.05$).

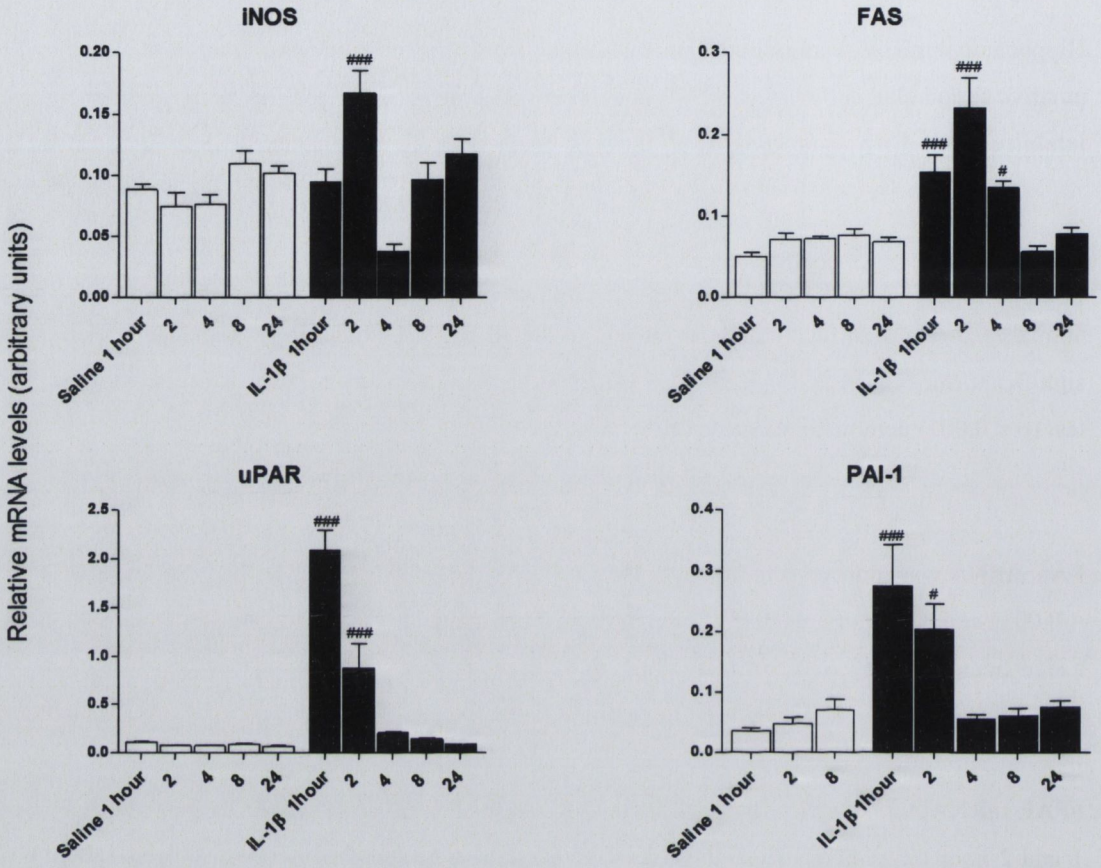

Figure 3.17 *Impact of systemic IL-1β challenge on hippocampal cell activation and pathway markers mRNA expression at 1, 2, 4, 8 and 24 hours.* Hippocampal mRNA expression of iNOS, FAS, uPAR and PAI-1 measured using quantitative PCR in C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. In the case of PAI-1 4 and 24 hour treatment groups were compared with 2 and 8 hour saline groups respectively. # denotes treatment is significantly different to saline $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. All data have been presented as mean ± SEM, n = 5 for all IL-1β experimental groups, except all 2 hour groups and the PAI 4 hour group n = 4. All saline groups $n \geq 4$ except 4 and 24 hours $n \geq 3$.

3.3.8.4 Prostaglandin biosynthetic enzymes and synthases

Hippocampal mRNA expression of prostaglandin biosynthetic enzymes COX-1 and COX-2 and prostaglandin synthases mPGES-1 and HPGDS was also examined across the time course after IL-1β injection (Figure 3.18).

COX-1 mRNA was not significantly altered by systemic IL-1β challenge. A one-way ANOVA could not reject the null hypothesis ($F = 1.229$, df 7,31, $p = 0.3171$).

COX-2 mRNA expression was significantly affected by treatment. This was shown by significant one way ANOVA ($F = 2.775$, df 7,31, $p = 0.0232$). Bonferroni post-hoc tests, however, did not identify significant differences between individual IL-1β challenge time points and the saline-injected controls ($p > 0.05$)

mPGES-1 mRNA was significantly upregulated by systemic IL-1β challenge compared to saline controls at both 1 ($p < 0.05$) and 2 hours post-challenge ($p < 0.001$). These differences were shown by Bonferroni post-hoc tests after a significant one way ANOVA ($F = 11.65$, df 7,31, $p = 0.0001$).

HPGDS mRNA expression was not significantly altered by systemic IL-1β challenge. A one-way ANOVA did not reveal any treatment-induced changes in expression of HPGDS transcription ($F = 1.324$, df 7,31, $p = 0.2723$).

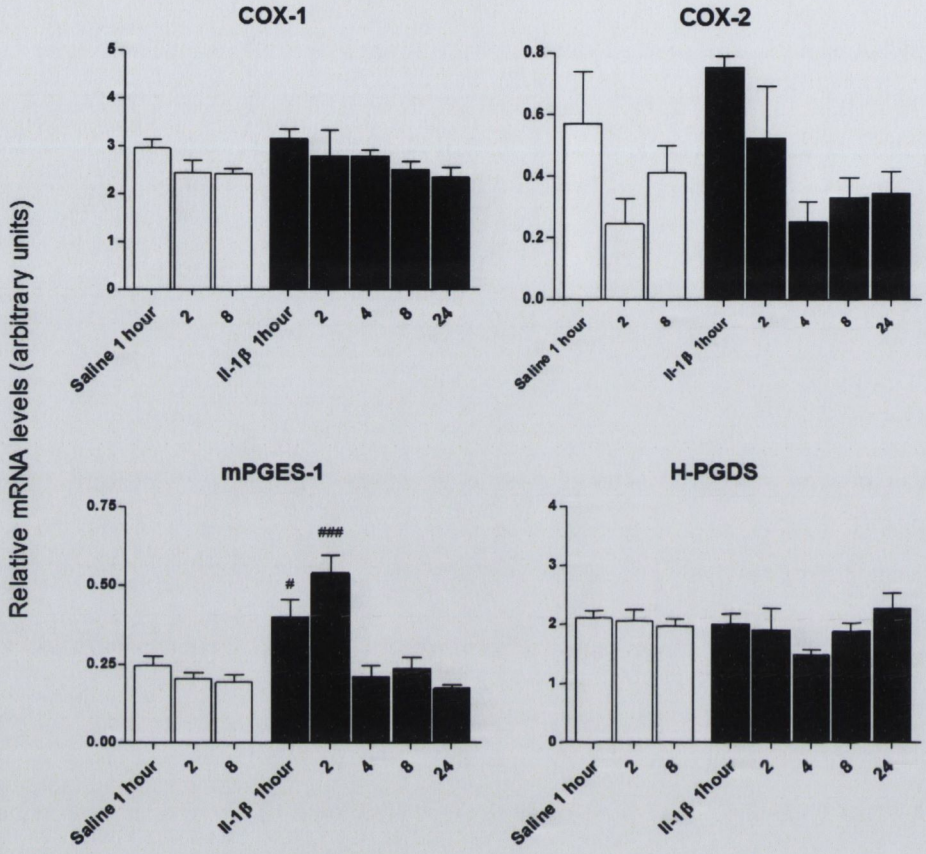

Figure 3.18 *Impact of systemic IL-1β challenge on hippocampal mRNA expression of prostaglandin biosynthetic enzymes and synthases at 1, 2, 4, 8 and 24 hours.* Hippocampal mRNA expression of COX-1 and COX-2, mPGES-1 and HPGDS measured using quantitative PCR in C57BL/6 female mice after systemic challenge (i.p.) with saline or IL-1β (15 μg/kg) at 1, 2, 4, 8 and 24 hours post-injection. All data groups were compared by one-way ANOVA, followed by Bonferroni post-hoc tests comparing differences between each IL-1β group and the equivalent saline groups. 4 and 24 hour IL-1β treatment groups were compared with 2 and 8 hour saline groups respectively. # denotes IL-1β treatment is significantly different to saline treatment $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. All data have been presented as mean ± SEM, $n = 5$ for all experimental groups except both 2 hours treatment groups $n = 4$.

3.4 Discussion

How the brain recognises and responds to peripheral inflammation is a question pertinent to diverse research areas. The vast majority of basic research has utilised the bacterial endotoxin LPS to trigger a peripheral immune response and focused on components of sickness behaviour, HPA activation, and the fever response. These studies have generally implicated circulating proinflammatory cytokines, namely IL-1β, TNF-α and IL-6, as key molecules for transduction of signal into the CNS (Dantzer et al., 2008, Konsman et al., 2002, Inui, 2001). Comparisons of the CNS effects of individual cytokines have concentrated on single aspects of the neuroinflammatory response, be that endothelial and parenchymal transcription of COX-2 (Lacroix and Rivest, 1998) or IκB (Laflamme and Rivest, 1999), or single aspects of sickness behaviour, such as temperature (Wang et al., 1997, Lacroix and Rivest, 1998, Laflamme and Rivest, 1999). In parallel, human studies have posited a deleterious and negative neuroinflammatory role for individual systemic cytokines based on associations in cross-sectional biomarker studies. For example, cytokines have been linked with cognitive decline in ageing (Yaffe et al., 2003, Yaffe et al., 2004) and the clinical conditions of delirium (van Munster et al., 2008, van Munster et al., 2010), depression (Miller et al., 2009, Dowlati et al., 2010) and dementia (Tan et al., 2007, Swardfager et al., 2010). These findings have proven difficult to interpret. This is, in part, because of a dearth of studies systematically cataloguing the systemic and central inflammatory consequences of systemic immune insults with individual cytokines.

For the reasons detailed above, the present study generated a systematic analysis of the acute effects of IL-1β, TNF-α and IL-6, along with LPS and saline, comparing the behavioural, systemic and CNS responses in C57BL/6 mice. The effects of these inflammatory molecules on locomotor activity, core body temperature, plasma inflammatory mediators, and hepatic, hypothalamic and hippocampal gene transcription were assessed 2 hours post-challenge (See Table 3.1 for summary). It was shown that IL-1β and TNF-α induce acute systemic inflammation and reduced locomotor activity. The CNS inflammatory transcription elicited by these cytokines is brief but broadly similar to that elicited by systemic LPS, although the cytokines do not induce IFN-β mRNA. IL-1β but not TNF-α induces PTX3, while hippocampal CXCL1 and IL-6 transcripts were produced to a much greater extent by IL-1β challenge. There was also evidence of activation of apoptotic pathways, acute central prostaglandin synthesis and microglial activation after IL-1β-challenge. Peripheral IL-6 was found to induce a minimal CNS response. Given the marked effects seen

following IL-1β challenge, and its frequent implication in transduction of peripheral inflammation into the CNS, the effects of systemic IL-1β on peripheral and CNS inflammation were also examined 1, 2, 4, 8 and 24 hours after administration (See Table 3.2 for summary).

The primary utility of this compendium is the systematic comparison across compartments of the effects of LPS and three proinflammatory cytokines fundamental to a systemic inflammatory response (Koj, 1996). While necessarily limited in depth and breadth, it is intended to function as a relatively concise summary of the acute systemic and central inflammatory effects of i.p. challenge with proinflammatory cytokines, which have not been systematically compared before. Additionally, facets of sickness behaviour were assessed to confirm previous observations (Dantzer et al., 2008). The presented analysis of inflammatory markers induced in the blood, liver, hypothalamus and hippocampus represents a necessary overview of similarities and differences. As expected, LPS induced the most widespread inflammatory profile and sickness behaviour symptoms. The immune response generated by LPS has been reviewed in detail elsewhere (Dantzer et al., 2008, Perry et al., 2007); the present discussion concentrates on the responses generated by the systemic proinflammatory cytokines. In addition, the intention in the context of this thesis was to provide a thorough baseline analysis of the effects of LPS and selected proinflammatory cytokines on sickness behaviour and CNS markers of inflammation in preparation for more detailed analysis of the effects of one or more of these cytokines in the context of systemic inflammation superimposed on prior pathology (Chapters 4 & 5).

Table 3.1 Comparative study summary. X denotes significant effect on measure

Measure	IL-1β	TNF-α	IL-6	LPS
Sickness Responses				
Core Body Temperature	X			X
Open Field Activity	X	X	X	X
Plasma Levels				
IL-1β	X			X
TNF-α	X	X		X
IL-6	X			X
IFN-β				
PGEM				X
Corticosterone				
Liver mRNA				
IL-1β				X
PTX3				X
TNF-α		X		X
TNF-αIP2	X	X		X
SAA2	X	X	X	X
CXCL1	X			X
MCP-1				X
Hypothalamus mRNA				
IL-1β	X			X
TNF-α	X	X		X
IL-6				X
CRF				
Hippocampus mRNA				
IL-1β	X	X		X
CXCL1	X			
TNF-α	X	X		X
IL-6	X			X
IFN-α				
IFN-β				X
COX-1				
COX-2				X
VCAM	X	X		X
uPAR	X			X
CD11b				

Table 3.2 IL-1β time course summary. X denotes significant effect on measure

Measure	IL-1β 1 hour	2 hours	4 hours	8 hours	24 hours
Plasma Levels					
IL-1β	X	X			
TNF-α	X	X			
CXCL1	X	X			
IL-6	X	X			
MCP-1	X	X	X		
PGEM	X				
Hypothalamus mRNA					
IL-1β		X			
iL-6		X			
TNF-α	X	X			
IFN-β					
MCP-1	X	X			
CXCL1	X				
Hippocampus mRNA					
IL-1β		X			
PTX3	X	X			
TNF-α	X	X			
MCP-1	X	X			
IL-6	X	X			
CXCL1	X	X			
IFN-β					
iNOS	X				
FAS	X	X	X		
uPAR	X	X			
PAI-1	X	X			
COX-1					
COX-2					
mPGES-1	X	X			
HPGDS					

3.4.1 Relevance of selected doses

Dosage for the individual immune challenges was chosen after review of the sickness behaviour literature, which has produced the majority of studies on the CNS consequences of systemic cytokines. Attempts were made to encompass the range at which the cytokines have been used across sickness behaviour studies with two doses (Table 3.3: dose studies summary pg. 118). The IL-1β doses used in the comparative study elicited broadly equivalent responses; indeed, no significant difference of effect was found in any compartment, suggesting that IL-1β does not require high systemic concentrations to be maximally active. This is consistent with studies which show that systemic blockade of IL-1β activity using IL-1RA abrogates symptoms in autoinflammatory diseases despite undetectable levels of systemic IL-1β (Church et al., 2008). Thus, the lower dose was used in assessing IL-1β effects across a 24-hour time course. The dosage range of TNF-α was larger, and reflects the higher doses of TNF-α utilised in the literature. Discernible differences in effects emerged. Interestingly, these differences were only in the induction of TNF-α mRNA- in the liver, hypothalamus and hippocampus. The TNF-α inducible protein, TNF-αIP2, was induced to almost equal levels in the liver by both dosages, this suggests that the excess TNF-α produced by the higher dose was not necessarily more biologically active. Indeed, on all other measures, the low dose was comparable to the high dose. IL-6 doses elicited broadly similar responses here. Given that IL-6 had no effect on most measures, there is some concern that the doses used for this cytokine were simply below those required for biological activity. While this may be the case, high dose IL-6 did induce a reduction in open field activity and increase in SAA2 mRNA in the liver. Low dose IL-6 did not have an effect on either of these measures. The overall lack of immune activation initiated by IL-6 will be further addressed below. Here it suffices to say that the dosage range chosen, while low, was based on the literature and the higher dose was biologically active.

LPS was injected at a dose, which mimics mild to moderate systemic inflammation, as opposed to sepsis (Cunningham et al., 2009a, Murray et al., 2012). Many sickness behaviour studies inject antibodies against individual cytokines in attempting to delineate their contribution to endotoxin-mediated systemic and central inflammation. Since, as stated above, the doses chosen for the present study were based on the sickness behaviour literature, a comparison of the relationship between the doses of the cytokines injected individually in the present study in relation to levels generated by a single LPS challenge is of interest. Analysis of plasma levels of IL-1β, TNF-α and IL-6 elicited by LPS injection, seen here and in another recent study by our group (Murray et al.,

2012), suggests that TNF-α circulates at approximately twice the concentration of IL-1β. Here we have injected TNF-α at concentrations that were 3.5-5 times higher than those of injected IL-1β. However, IL-6 was at much concentration after LPS injection - at levels 10 fold greater than TNF-α, which argues that a higher IL-6 dose might have been used in order to replicate LPS-induced levels.

A more pertinent comparison than the above, however, would be between the levels generated by the low dose of LPS utilised and the levels generated by the injected individual cytokines (Table 3.4 pg. 119). These data were excluded from the ELISA results presented since the measured levels of injected cytokine were so much higher than the levels induced by the other injected cytokines. The low dose of IL-1β and TNF-α and high dose of IL-6 generated levels equivalent to the endotoxin challenge. Therefore, it is clear that the injected doses bring about circulating levels akin to mild systemic inflammation. However, as previously stated, the present study was designed to investigate the central, particularly hippocampal and hypothalamic, inflammation induced by biologically active doses of systemic proinflammatory cytokines. It was not intended to delineate the function of each cytokine in LPS-generated systemic and central inflammation. Such a comparative study would require LPS challenge along with i.p. and i.c.v. treatment with antibodies generated against the individual cytokines. Furthermore, there is emerging evidence that LPS itself is capable of directly activating the vagal nerve (Hosoi et al., 2005) and cerebral endothelium (Chakravarty and Herkenham, 2005, Murray et al., 2012).

3.4.2 IL-1β and TNF-α elicit systemic and central inflammation

Peripheral injection of IL-1β and TNF-α induced a number of measures of systemic inflammation. IL-1β induced no significant circulating TNF-α, whereas TNF-α did induce IL-β protein synthesis. This was demonstrated both in the comparative study and the IL-1β time course study (It has also been replicated in Chapter 3). The cytokine signalling system is complex and context dependent (Ceciliani et al., 2002), but TNF-α is known to precede induction of IL-1β in lethal bacteraemia (Fong et al., 1989). In that study the authors, using a TNF antibody, show that TNF-α is necessary for the subsequent release of IL-1β. Fantuzzi and Dinarello have shown that IL-1β-deficient mice

produce equivalent levels of TNF-α to wild type animals following LPS challenge (1996). Moreover, IL-1RA administration with LPS does not alter circulating TNF-α (Granowitz et al., 1993). These results suggest that IL-1β is not essential for the synthesis of TNF-α and that IL-1β can be TNF-α-dependent but they do not demonstrate whether TNF-α can be synthesised in response to administration of IL-1β. TNF-α-like activity has been detected in the sera of IL-1β–challenged rabbits, 3.5 hours after challenge (Ikejima et al., 1990). How this result and the ones observed in the current study can be reconciled is unclear. It may be that there are inter-species differences or that the TNF-like activity assay used by Ikejima and colleagues is of a different sensitivity to the assay used in the current studies. Regardless, it is surprising that this question has not been addressed more definitively in the literature and the present results suggest further experiments are necessary. As has been demonstrated elsewhere (Koj, 1996), we found that IL-6 was induced by both TNF-α and IL-β. In the current study these immune challenges also generated plasma CXCL1 and MCP-1. Furthermore, analysis of the effects of the effects IL-1β and TNF-α (experiments performed by Edel Hennessy) across 24 hours post-administration revealed that plasma PGEM is also significantly increased.

The route by which the IL-1β- and TNF-α–mediated systemic inflammation induced central proinflammatory transcripts cannot be inferred from the analyses in the current study. There are multiple routes by which signal transduction can occur (Dantzer, 2004, Serrats et al., 2010, Gosselin and Rivest, 2008). Active transport across the blood-brain barrier, direct activation of the cerebral endothelium and cytokine stimulation of vagal nerve afferents has been shown (Banks and Kastin, 1991, Gutierrez et al., 1993, Goehler et al., 1999). The results present evidence an activation of the brain endothelium following IL-1β- and TNF-α- challenges with the hippocampal induction of VCAM and MCP-1. Endothelial cells express these markers in response to immune activation (Osborn et al., 1989, Gerszten et al., 1999, Rollins et al., 1990). Receptors for both TNF-α and IL-1β have been demonstrated on endothelial cells (Laflamme and Rivest, 1999). Endothelial and perivascular macrophages are highly receptive to circulating IL-1β and TNF-α (Gosselin and Rivest, 2007) and these cells contain the machinery to induce microglial inflammation via NFκB activation (Zhang and Rivest, 2003) and PGE2 synthesis (Turrin and Rivest, 2004). The key systemic mediator for central activation could of course be an acutely induced secondary mediator, such as PGE2, MCP-1 or CXCL1 acting alone or in synergy with the injected proinflammatory cytokines. Notably, peripheral administration of CXCL1 results in a reduction in open field activity

and burrowing (Campbell et al., 2010) and its acute elevation in plasma in the IL-1β time course is consistent with the cytokine being a plausible mediator of CNS activation.

Both TNF-α and IL-1β peripheral challenges induced proinflammatory transcripts in the hypothalamus and hippocampus. Only LPS challenge elicited IFN-β induction, there was neither central nor peripheral upregulation of type-1 IFNs after IL-1β or TNF-α challenge. The results support previous observations by our own laboratory (Murray et al., 2012) which suggest that induction of central IFN-β transcription by systemic LPS results from the direct stimulation of the endothelial cell TLR4 MyD88-independent pathway by LPS (Kawai et al., 2001). The present results also demonstrate that systemic IL-1β and TNF-α cannot elicit a peripheral or central type-1 IFN response. These findings are consistent with observations that neither cytokine elicits IRF3 phosphorylation or IRF3-dependent gene transcription (Clark et al., 2009, Clark et al., 2011), which have a central role in IFN-β gene induction (Colonna, 2007).

While both proinflammatory cytokines induced proinflammatory gene transcription in the hippocampus, IL-1β did appear to be more potent than TNF-α on a number of measures. CXCL1 and IL-6 transcripts were produced to a much greater extent by IL-1β challenge. CXCL1 induction following IL-1β was also significantly greater than that seen after LPS challenge. Neurons express CXCL1 receptors (Valles et al., 2006) and there is evidence to suggest that central CXCL1 may be neuroprotective (Watson and Fan, 2005). Why central CXCL1 would be induced so robustly by systemic IL-1β is unclear. After IL-1β but not TNF-α challenge, hippocampal PTX3 was induced. Previous evidence from our group has suggested that PTX3 is induced downstream of IL-1β protein production (Field et al., 2010) and it may have role in dementia progression (Osera et al., 2011). Notably, a recent *in vitro* study has demonstrated that elevated PTX3 impedes microglial phagocytosis of apoptotic neurons (Ko et al., 2012). The observed upregulation of uPAR transcription offers evidence of microglial activation (Cunningham et al., 2009b), although another marker of microglial activation, CD11b was not elevated. The upregulation of iNOS mRNA suggests the possibility of IL-1β-induced neurotoxicity; significant expression of iNOS protein is indicative of cellular damage (Paakkari and Lindsberg, 1995), while FAS mRNA elevation might suggest activation of apoptotic pathways (Watanabe-Fukunaga et al., 1992). Examination of the neuroinflammatory profile over 24 hours after peripheral IL-1β challenge, however, demonstrated

that activation of these pathways and the classical proinflammatory mediators was very short-lived. Almost all inflammatory transcripts had returned to baseline 4 hours after challenge. The inflammatory profile observed post-TNF-α administration (by Edel Hennessy) was similarly brief. A characterization of expression of protein for these transcripts is required to investigate this possibility further but this was not the primary aim of the current studies.

The cognitive consequences of neuroinflammation have been discussed in recent years (Yirmiya and Goshen, 2011, Cunningham and Sanderson, 2008). Currently, the mechanism by which central inflammatory mediators affect neuronal function is unclear; it may be directly, via neuronal receptors, alteration of neurotransmitters or HPA activation. Peripheral challenge with both cytokines induced increased hippocampal transcription of the major proinflammatory cytokines IL-1β and TNF-α transcription. The negative effects of central IL-1β on neuronal and memory function are clearer than that of TNF-α, although both can be deleterious (Gosselin and Rivest, 2007). Both cytokines can negatively impact neuronal and synaptic functioning in the hippocampus (Pickering and O'Connor, 2007, Cunningham et al., 1996) and both cytokines have also been shown to negatively affect memory function (Barrientos et al., 2002, Hein et al., 2010, Fiore et al., 1996). In a model of peripheral surgery-induced cognitive impairment, the memory dysfunction elicited following tibial fracture was shown to be both TNF-α and IL-1β-dependent. (Cibelli et al., 2010, Terrando et al., 2010) Moreover, the resultant central IL-1β elevation was shown to be downstream of peripheral TNF-α (Terrando et al., 2010). Central IL-1β has been shown to impact upon spatial learning in a COX-1-prostaglandin dependent manner (Matousek et al., 2010). COX-1-mediated prostaglandin elevation has recently been implicated in other inflammation-related behavioural disturbances (Teeling et al., 2010, Harden et al., 2011). The acute elevation of mPGES-1 mRNA after peripheral IL-1β administration demonstrated in the present study offers further evidence of possible acute central prostaglandin elevation (Jakobsson et al., 1999). These results, in addition to the well-documented protection from subsequent development of dementia offered by NSAIDS (McGeer et al., 1996), argue for more detailed studies of the CNS consequences of systemically elicited prostaglandins.

3.4.3 Is peripheral IL-6 important for CNS changes?

Systemic IL-6 induced no systemic inflammation; in fact only one inflammatory measure in the present study was altered by IL-6 administration, robust elevation of hepatic SAA2 mRNA, which supports previous observations that IL-6 is important in the induction of the hepatic acute phase response (Marinkovic et al., 1989, Wang and Dunn, 1998). While central IL-6 can induce a fever response (Lenczowski et al., 1999), reduce LTP in the dentate gyrus (Bellinger et al., 1995) and possibly mediate LPS-induced cognitive impairment (Sparkman et al., 2006), there is little compelling evidence that systemic IL-6 alone can induce a CNS proinflammatory cytokine response. Peripheral IL-6 challenge fails to elicit endothelial cell NFκB signalling or COX-2 transcription, which are seen after peripheral IL-1β and TNF-α challenges (Lacroix and Rivest, 1998, Laflamme and Rivest, 1999). Failure to induce the IL-6 soluble and membrane-anchored receptors and gp130 by other proinflammatory cytokines may offer a mechanistic explanation for its lack of effect (Hopkins, 2007, Rivest, 2001). It has been argued that the induction of IL-6R and gp130 by other inflammatory mediators, such as LPS, TNF-α or IL-1β, is necessary for IL-6 signalling at the brain microvasculature (Rivest, 2001). Wang and Dunn, have demonstrated an acute and short-lived HPA activation after peripheral IL-6 administration (1998). The activation peaked at 30 minutes post-challenge and had resolved by 2 hours. Thus, it may be that IL-6 administration elicited some very short-lasting CNS proinflammatory transcription, which was missed at the 2 hour time point in the current study. However, we did not find elevation of any hippocampal or hypothalamic inflammatory transcripts, some of which might have been expected to be still elevated at 2 hours. HPA activation could explain the reduced open field activity induced by IL-6 challenge; although, plasma corticosterone levels were not significantly altered. IL-6 signalling is intricate, context dependent and can be construed as pro- or anti-inflammatory (Xing et al., 1998, Jones et al., 2005). It has been reported to induce IL-1RA and soluble TNF receptor p55 expression (Hopkins, 2007). Furthermore, the IL-6/IL-6R complex interacts with gp130 to activate JAK-STAT signalling, which may contribute to inflammation resolution via SOCS3 activation (Baker et al., 2009). It is unlikely, however, that this anti-inflammatory profile was induced in the present study given the absence of TNF-α and IL-1β. While the current study does not offer evidence that IL-6 alone is anti-inflammatory, it offers further evidence of its inability to elicit a CNS inflammatory response.

When put in the context of human studies which implicate peripheral IL-6 in cognitive decline and CNS disease (Weaver et al., 2002, Wright et al., 2006, Yaffe et al., 2003, Marsland et al., 2008, Engelhart et al., 2004) the current results are not consistent with direct effects. This apparent contradiction may be explained by two factors: facilitation of other mediators and ease of measurement. Peripheral IL-6 may be important in modulating but not driving transduction of systemic inflammation into the CNS. Peripheral IL-6 does contribute to facets of sickness behaviour (Harden et al., 2011) and appears to be crucial in endotoxin-mediated fever (Harden et al., 2006). Evidence suggests that it is involved in the transport of PGE2 to its cognate receptors to initiate the fever response (Nilsberth et al., 2009). Furthermore, IL-6 knockout animals show a reduced LPS- and IL-1β-inducd sickness behaviour (Bluthe et al., 2000). Thus, while elevation of IL-6 alone may not be sufficient to elicit neuroinflammation, it does appear to work in combination with, and modulate, the biological activity of other cytokines. Secondly, IL-6 is produced at very high levels in response to inflammation. It may be that the higher levels to which it is induced simply make it the inflammatory cytokine most likely to be measureable in a number of settings. Thus, it might be that it is more easily detected and implicated than other more mechanistically important but undetected inflammatory mediators. It has been shown that plasma IL-1β is not detectable in many autoinflammatory diseases that are now known to be IL-1β mediated (Dinarello, 2009). Given the results of the present study, it seems more likely that the CNS response to acute inflammatory events elicited by infection, surgery or trauma, is being driven by TNF-α or IL-1β, rather than IL-6. It is increasingly evident that even mundane systemic inflammation has negative consequences for patients with ongoing neurodegeneration (Perry et al., 2007). Thus, peripheral IL-6, despite the limited actions, may be particularly useful as a measurable biomarker of potentially deleterious acute systemic inflammation in populations with ongoing CNS pathology. These populations are discussed further below.

3.4.4 Implications for clinical conditions

Peripheral IL-1β-and TNF-α-challenges induced relatively short-lasting and controlled increases in inflammatory transcripts. The mRNA expression of almost all the neuroinflammatory markers had resolved 4 hours after both challenges (TNF-α experiment carried out by Edel Hennessy). These findings are in agreement with Murray et al., (2012) who demonstrated that endotoxin-elicited

inflammatory transcripts in normal animals dissipates within 4 hours. That work also, however, shows that the same LPS-challenge induces prolonged and exaggerated CNS inflammation when superimposed on existing neurodegenerative disease, namely the ME7 strain of prion disease. A possible mechanism for this heightened neuroinflammation is a more robust response by disease-primed CNS microglia (Cunningham et al., 2005). Other groups have demonstrated an exaggerated response to immune challenge in other CNS disease models; transgenic models of Alzheimer's disease (Kitazawa et al., 2005, Sly et al., 2001, Brugg et al., 1995), and Parkinson's disease (Villaran et al., 2010, Godoy et al., 2008).

An underlying CNS disease burden would appear to be an important factor in studies which show associations between systemic cytokines and delirium. Episodes of delirium can be triggered by systemic inflammatory events (Maclullich et al., 2008) and confer a higher risk of developing dementia, cognitive decline and institutionalisation (Inouye, 2006). IL-6, IL-8 and CRF are associated with post-hip fracture delirium (van Munster et al., 2010, de Rooij et al., 2007, Beloosesky et al., 2007). However, prior cognitive impairment is a more important risk factor and when taken into account can negate associations of delirium with systemic cytokines (van Munster et al., 2010). Our novel animal model of delirium demonstrates acute and reversible hippocampal mediated-cognitive dysfunction when mice with neurodegenerative disease but not normal mice are challenged with LPS (Murray et al., 2012). Moreover, other preclinical studies by the group have shown that single and multiple systemic immune challenges accelerate the progression of neurodegenerative disease (Field et al., 2010, Cunningham et al., 2009a). Studies performed in collaboration with clinical colleagues have demonstrated that systemic inflammation, IL-1β and TNF-α in particular, accelerates the course of cognitive decline in dementia (Holmes et al., 2003, Holmes et al., 2009). While critical illness/sepsis can initiate delirium and associated negative outcomes in previously healthy individuals (Ebersoldt et al., 2007, Iwashyna et al., 2010), routine systemic inflammation has much more profound and deleterious effects in those with prior CNS pathology. Long-term NSAID usage has been shown to offer reduce the risk of development of dementia. NSAIDs were predicted to offer this protection by interfering with aberrant protein folding in the disease milieu (Wyss-Coray, 2006), despite the fact that their primary biological role is blockade of prostaglandin synthesis, via COX inhibition. Subsequent prospective NSAID intervention trials have shown that NSAIDs fail to influence the decline in cognitive function in Alzheimer's disease (Aisen et al., 2003, Lyketsos et al., 2007, Jaturapatporn et al., 2012). However,

aforementioned literature suggests that circulating inflammatory mediators, induced by banal infections, surgery or trauma, can induce further cognitive dysfunction and decline in populations that already have underlying CNS burdens. Thus, it is possible that the original epidemiological observations of NSAID-mediated protection from dementia reflected the beneficial effects of long-term inhibition of systemic inflammation. Appropriately designed long-term randomised control trials may yet demonstrate that NSAIDs actually offer protection from cognitive decline to AD patients, via inhibition of systemic prostaglandins. Indeed, populations with co-morbidities may have been important in the epidemiological studies that first demonstrated a role for NSAIDs in protection against subsequent dementia (McGeer et al., 1996, in 't Veld et al., 2001, Vlad et al., 2008).

In such a context, it can be seen how systemic inflammation might also negatively affect the course of cognitive ageing in general. Chronic low-grade systemic inflammatory factors increase in ageing, as do age-related inflammatory diseases, such as obesity, diabetes, hypertension, atherosclerosis and Crohn's disease (Franceschi et al., 2000, Licastro et al., 2005). Moreover, the ageing brain is increasingly burdened and, thus, more vulnerable to inflammatory insults. Ageing results in loss of synaptic input in the neocortex, while age-related neuroinflammation has been shown to impair neuronal function (Griffin et al., 2006, Lynch, 2010). In addition, microglial priming and an exaggerated response to systemic neuroinflammation has been demonstrated in aged animals (Godbout et al., 2005). Prospective observational studies have demonstrated that elevated systemic inflammation (defined as elevated CRP and IL-6) contributes to cognitive decline (Yaffe et al., 2003, Yaffe et al., 2004). However, other prospective studies have failed to find evidence for an association between circulating cytokines and cognitive decline in the elderly (Wilson et al., 2003, Dik et al., 2005, Schram et al., 2007). One study of note showed that an association between cognition and elevation of peripheral cytokines disappeared when cardiovascular disease was taken into account (van Exel et al., 2003). This again suggests the importance of underlying disease burden in the link between systemic inflammation and negative CNS outcomes. As such, the relationship between peripheral cytokines and CNS dysfunction appears to be strengthened by both peripheral and central inflammatory conditions. Whether peripheral inflammation is a cause or consequence of mood disorders is still a matter of debate. However, it seems clear that systemic cytokines are elevated in depression (Miller et al., 2009,

Dowlati et al., 2010) The role of innate immunity in depression presents a fertile and novel area for potential therapeutic intervention in depression research (Dantzer et al., 2011).

3.4.5 Summary

Undoubtedly, there are synergistic and complex interactions between the individual cytokines and other mediators in response to infection and peripheral tissue damage. However, a systematic comparison of the effects of the key messengers, IL-1β, TNF-α and IL-6 is necessary given the lack of information concerning the effects of these proinflammatory cytokines on CNS inflammation. The present study offers a systematic comparison of the effects of peripheral challenge with the most widely implicated proinflammatory cytokines on systemic and central inflammation in normal animals. While systemic inflammation has been associated with CNS dysfunction, evidence suggests that it may be most relevant in the context of CNS vulnerability arising from disease or ageing. The present results call for further study of the effects of systemic IL-1β and TNF-α in exacerbating ongoing CNS disease.

Table 3.3 Summary of studies based on which immune challenge doses were decided

Challenge	Reference	Dose
LPS	(Cunningham et al., 2009a, Murray et al., 2012)	100 μg/kg
IL-1β	(Gosselin and Rivest, 2008)	10 μg/kg
	(Swiergiel and Dunn, 2007)	5/15/50 μg/kg
	(Elander et al., 2007)	30 μg/kg
	(Mormède et al., 2003)	100 μg/kg
TNF-α	(Wang et al., 1997)	50 μg/kg
	(Bluthé et al., 1994)	125 μg/kg
IL-6	(Wang et al., 1997, Wang and Dunn, 1998)	50 μg/kg

Table 3.4. Comparison of plasma cytokine levels generated by i.p. injection of the concomitant cytokine and LPS

	IL-β (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)
LPS (100μg/kg)	125	200.49	1393.77
IL-1β (15μg/kg)	284.70		
IL-1β (50μg/kg)	1309.55		
TNF-α (50μg/kg)		269.45	
TNF-α (250μg/kg)		32719.26	
IL-6 (50μg/kg)			958.35
IL-6 (125μg/kg)			1335.22

Chapter 4: the roles of synaptic loss and prostaglandins in acute cognitive dysfunction: animal model of delirium during dementia

The prostaglandin work contained in this chapter forms the majority of a manuscript currently in revision for the *Journal of Neuroscience*. The neurodegeneration data forms part of a paper published in the *Journal of Neuroinflammation*- Murray CL*, Skelly DT* and Cunningham C (2011) Exacerbation of CNS inflammation and neurodegeneration by systemic LPS treatment is independent of circulating IL-1b and IL-6. Journal of Neuroinflammation. 8:50. * denotes both authors contributed equally to the work

4.1 Summary

Delirium, the acute and severe disruption of facets of cognition and behaviour, is a highly prevalent neuropsychiatric syndrome, particularly in dementia patients. Those with prior cognitive impairment are most at risk to develop delirium and systemic inflammation is a common trigger in this population. Our laboratory has previously modelled delirium during dementia by demonstrating that animals with chronic neurodegenerative disease (the ME7 model of prion disease) show acute working memory impairment during a systemic inflammatory event. In the present study, we have used this model to demonstrate that the progression of disease, in this model of dementia, increases the incidence, severity and duration of LPS (100 μg/kg i.p.)-induced acute episodes of cognitive impairment. This increased susceptibility is associated with a decrease in the density of hippocampal pre-synaptic terminals. There is also evidence of increased expression of cyclooxygenase, and prostaglandin synthases and receptors, which is indicative of a disease-related amplification of the CNS prostaglandin system. Accordingly, hippocampal PGE2 is elevated by disease. Pre-treatment with the COX-1 inhibitors piroxicam and SC-560, but not the COX-2 inhibitor nimesulide, provided significant protection against LPS-induced working memory deficits in diseased animals. Piroxicam blocked systemic and central PGE2 synthesis but did not affect proinflammatory cytokine levels in the blood or brain. These data indicate a role for COX-1-mediated prostaglandins in acute cognitive dysfunction and have relevance for understanding how dementia and inflammation interact to produce delirium. In this chapter, we also assessed the hypothesis that systemic cytokines play a role in the exacerbation of CNS inflammation in ME7 disease. We demonstrated, using the synthetic glucocorticoid dexamethasone-21-phosphate to inhibit systemic cytokine synthesis, that in LPS (500 μg/kg) –challenged animals systemic cytokines are required to induce hypothermia but not sickness behaviour or acute neurodegeneration. Furthermore, it was shown that intraperitoneally administered LPS is detectable in plasma. These results indicate that LPS itself, binding at the cerebral vasculature, can transduce inflammatory signals into the CNS, which interact with, and exacerbate, ongoing pathology.

4.2 Introduction

Delirium is a highly prevalent and complex neuropsychiatric syndrome (Inouye, 2006) comprising profound acute disturbance of consciousness and cognitive function (APA, 1994, WHO, 1992). While it can occur in patients of any age, the highest incidence is in the elderly and demented (Elie et al., 1998, Young and Inouye, 2007), with a prevalence of 22-89% in these populations (Fick et al., 2002). Episodes of delirium lead to very significant lengthening of hospital stays and are known to contribute to subsequent cognitive decline (MacLullich et al., 2009), acceleration of dementia (Fong et al., 2009, Kat et al., 2008) and to shorten time to permanent institutionalisation and death (Witlox et al., 2010). Given its deleterious consequences and large economic and societal burdens (Leslie et al., 2008), delirium is increasingly recognised as an important area of research. However, its pathophysiology is poorly understood (Maclullich and Hall, 2011) and a lack of animal models has certainly contributed to this impasse.

Systemic inflammation resulting from infection, injury or surgery is amongst the most common triggers of delirium (Maclullich et al., 2008). Clinical studies have demonstrated an association between plasma levels of inflammatory mediators and delirium (Khan et al., 2011). Systemic IL-6 and IL-8 (the human homologue of mouse CXCL1) have been shown to be elevated prior to the onset of delirium in hip fracture patients (van Munster et al., 2010, van Munster et al., 2008). In another study, c-reactive protein (CRP), a marker of the inflammatory response, was also shown to predict delirium incidence (Macdonald et al., 2007). There is also some evidence that altered activity of the hypothalamic-pituitary-adrenal (HPA) axis is a factor in delirium (McIntosh et al., 1985, Robertsson et al., 2001b, Pearson et al., 2010). However, there are no studies demonstrating a causative role for any one of these inflammatory mediators in any studies of delirium. There is also a complete absence of research on the role of prostaglandins in delirium in vulnerable aged populations. This is surprising when one considers the diverse biological roles for the many prostaglandin subtypes, the clear role for prostaglandins in a plethora of inflammatory responses (Miller, 2006) and implication of prostaglandin receptors in neurological conditions (Andreasson, 2010, Cunningham and Skelly, 2011).

Aging and dementia are the major risk factors for delirium. It is on this background of prior cognitive impairment that systemic inflammation commonly induces delirium (Cunningham and Maclullich, In Press). Our laboratory has exploited these etiological factors to create the first animal model of delirium during dementia (Murray et al., 2012). Dementia was modelled using the

ME7 model of prion disease, which shows progressive extracellular amyloidosis, synaptic loss, neuronal loss and a well-characterised sequence of affective, cognitive and neurological changes (Cunningham et al., 2003, Guenther et al., 2001). By challenging normal and ME7 animals, 12 weeks post-inoculation, with a relatively low dose of bacterial endotoxin lipopolysaccharide (LPS, 100 μg/kg) to mimic Gram negative bacterial infection, this study demonstrated that systemic inflammation induces acute and transient working memory deficits in animals with prior neurodegenerative disease but does not affect normal animals. This is consistent with data in clinical populations. Inattention and short term memory deficits of acute onset and transient course are key features of the DSM-IV and ICD-10 definitions of core features of delirium (APA, 1994, WHO, 1992) and thus this model has good validity for performing mechanistic studies in delirium.

In the current study we hypothesised that the extent of the prior degenerative state of the brain alters susceptibility to acute exacerbation of cognitive function. To this end, we examined the susceptibility of animals at different stages of the neurodegenerative process to acute LPS-induced deficits. Furthermore, we hypothesised that underlying neurodegenerative disease leads to progressive changes in the prostaglandin signalling system and that cyclooxygenase inhibition using selective NSAIDs would protect against the acute working memory deficits induced by systemic LPS.

In the previous chapter, it was shown that systemic administration of LPS into normal animals elicits robust systemic inflammation, sickness behaviour and upregulation of inflammatory markers in the hippocampus and hypothalamus. Our group has previously shown that LPS-challenged ME7 animals display an exaggerated hypothermia and sickness behaviour response (Combrinck et al., 2002). Moreover, a single LPS challenge can induce acute neurodegeneration and hasten neurological decline in diseased animals (Cunningham et al., 2005, Cunningham et al., 2009). An exaggerated neuroinflammatory response to systemic immune challenge has been demonstrated in other CNS disease models; Wallerian degeneration (Palin et al., 2008) and transgenic models of Alzheimer's disease (Lee et al., 2002, Sly et al., 2001). Systemic inflammation has now been shown to have more severe outcomes for patients with prior CNS pathology. Our group in collaboration with others have demonstrated that cognitive decline is accelerated in AD patients who experience systemic inflammatory events (Holmes et al., 2009).

A second aim of this chapter was to investigate the mechanism by which systemic inflammatory events initiate deleterious outcomes in the diseased brain. More specifically, we addressed the

hypothesis that systemic cytokines play a role in transduction of signals into the CNS to elicit acute changes, namely hypothermia, sickness behaviour and acute neurodegeneration. ME7 and NBH animals were treated with systemic LPS (500 μg/kg) in the presence or absence of the synthetic glucocorticoid dexamethasone-21-phosphate, which inhibits systemic cytokine synthesis.

4.3 Results

4.3.1 Disease-induced progression of cognitive decline and synaptic loss

4.3.1.1 Disease progression induces gradual cognitive decline

Since ME7 disease is a progressive terminal neurodegenerative disease, inoculated animals will inevitably reach a point in disease where they can now long perform a cognitive task. We monitored performance on our novel paddling T-maze alternation task over a number of weeks to identify the stage at which ME7-diseased animals can no longer maintain intact performance on the task as a result of irreversible cognitive decline (intact performance is considered consecutive days above 80% performance; Murray 2012). We hypothesised, that immediately prior to this point in disease, animals with intact T-maze performance are at risk for greater subsequent inflammation-induced impairment than that induced in animals 12 weeks post-inoculation.

A large number of ME7- and NBH- inoculated mice were assessed on T-maze performance between 3 and 5 times per week from 12 until 17 weeks post-inoculation. Performance by NBH animals remained at approximately 90% between 13 and 17 weeks, while ME7 animals, as a group, showed slightly lower alternation rates (80%) that remained stable until week 15, declining significantly thereafter (Figure 4.1). Repeated measures ANOVA analysis showed that performance by ME7 and NBH differs. There was an interaction between disease and time ($F = 10.82$, df 4,156, $p < 0.0001$) and main effects of disease ($F = 25.74$, df 1,39, $p < 0.001$) and of time ($F = 9.24$, df 4,156, $p < 0.0001$). The first time at which there was a significant post-hoc difference (Bonferroni, $p < 0.05$) was 15 weeks post-inoculation. At 16 weeks there was also a significant difference ($p < 0.001$) but this was driven by the inability of some animals to maintain alternation, while most retained intact performance. However, by 17 weeks ($p < 0.001$) the majority of ME7-diseased animals showed considerable working memory impairment, evidenced by the drop in mean from 70% to 60 % between these weeks.

In summary, between 15 and 16 weeks into ME7 disease, most animals maintain intact T-maze alternation performance but are on the precipice of disease-induced task failure.

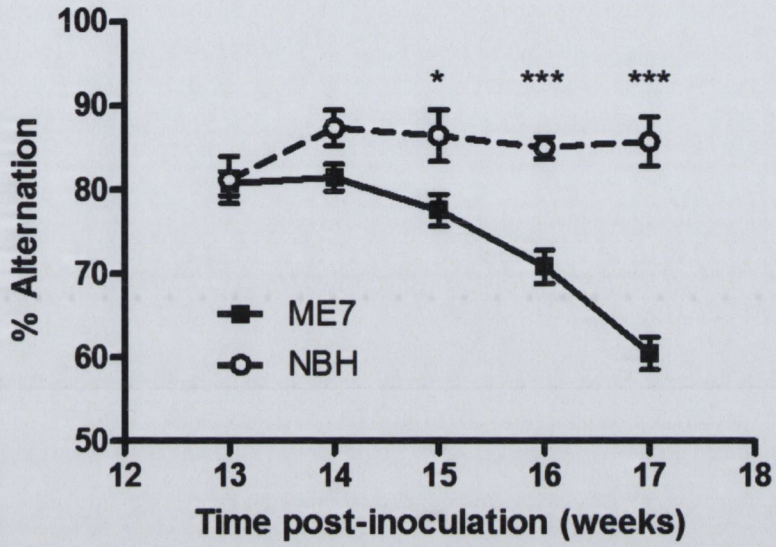

Figure 4.1 *Disease-induced progression of cognitive decline.* The working memory performance of ME7 and NBH-inoculated animals was assessed using the paddling T-maze alternation task. Animals were trained to acquire the task (consistently above 80%) and were then assessed between 3 and 5 times per week from 13 to 17 weeks and average weekly performances of the groups were calculated and analysed. A two-way ANOVA revealed an interaction between time and disease and main effects of time and disease. Subsequent Bonferroni post-hoc tests revealed significant differences between ME7 and NBH groups, which are denoted by $*p < 0.05$ and $***p < 0.001$. ME7 n = 30, NBH n = 11. All data have been presented as mean ± SEM.

4.3.1.2 Disease progression induces progressive loss of pre-synaptic terminals

ME7-diseased animals showed largely intact performance on the T-maze alternation task from 12 weeks post-inoculation until 16 weeks. Given that the T-maze task is hippocampal dependent (Murray et al., 2012) and the association between decreased synaptic density and cognition (Terry et al., 1991, Sze et al., 1997), we investigated whether there was quantifiable synaptic loss at the level of the hippocampus between 12 and 16 weeks post-ME7 inoculation. Comparisons were made between diseased animals at these time points and 16 week NBH control animals.

Synaptic density was assessed using immunolabelling with the pre-synaptic marker synaptophysin (antibody: SY38). Images of sections were taken and, after correction for staining variability (as detailed in Chapter 2 Section 2.2.10), the synaptic density of labelling in the stratum radiatum was quantified. Synaptophysin showed progressively decreased density in the stratum radiatum with progressing disease (Figure 4.2 a-c and d-f). When comparing NBH with ME7 at 12 weeks and 16 weeks post-inoculation one-way ANOVA revealed an effect of disease ($F = 86.54$, df 2, 16, $p < 0.0001$). Bonferroni post-hoc tests showed a significant difference between NBH and 12 week diseased animals ($p < 0.001$) and a significant difference between 12 weeks and 16 weeks diseased animals ($p < 0.05$). Thus, it can be concluded that as disease progresses the synaptic density in the dorsal hippocampus decreases progressively. Challenge of ME7 animals with LPS does not produce a significant change in this synaptic density at 72 hours post-challenge (data not shown).

Therefore it was shown that 12 weeks post-inoculation ME7 animals display robust synaptic loss compared to NBH animals. At 16 weeks, where animals are on the cusp of irreversible cognitive failure on the T-maze task, there is even greater loss of synaptic density.

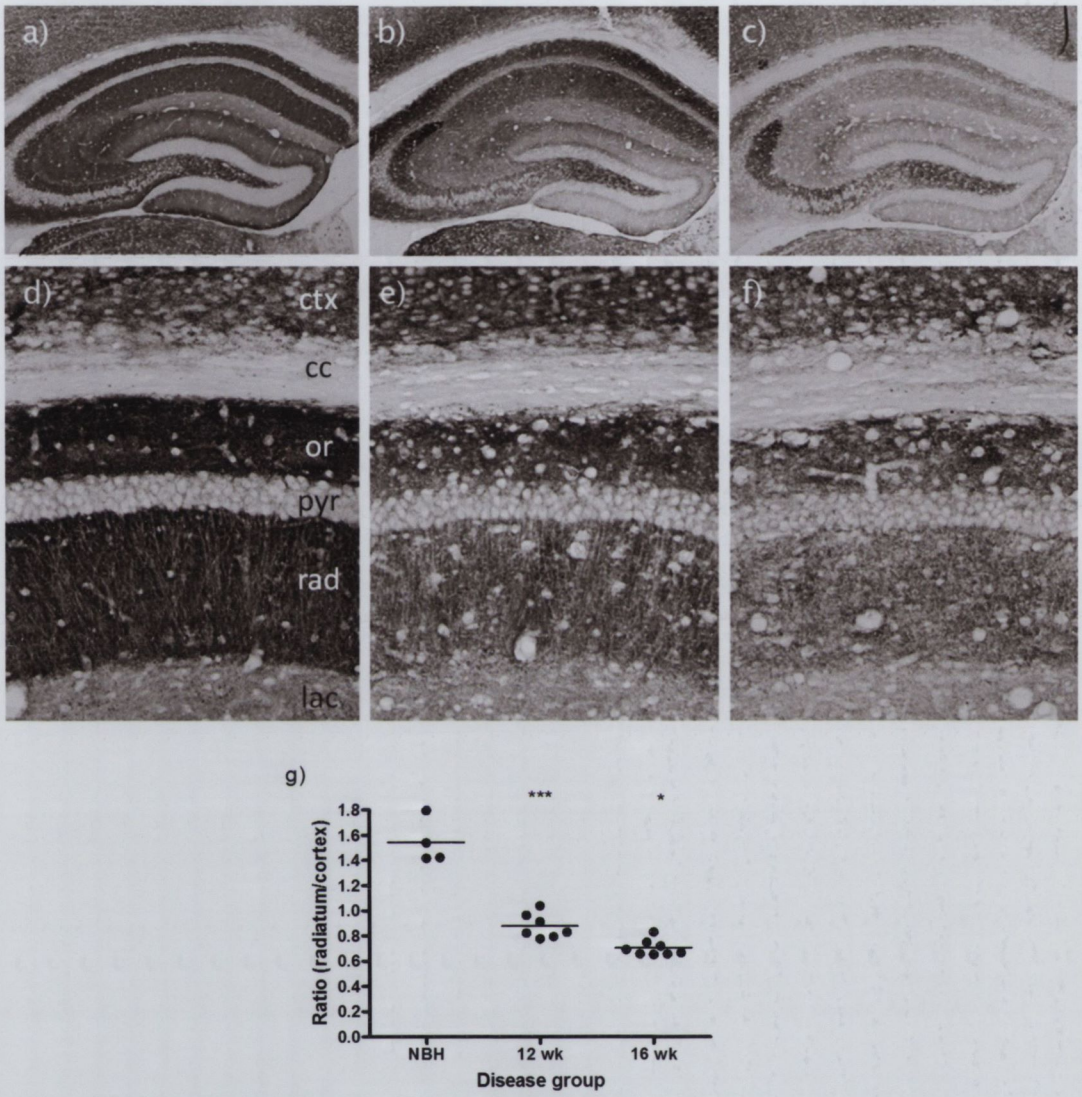

Figure 4.2 *Visualisation and quantification of disease-induced progression of synaptic loss.* Density of pre-synaptic terminals, as represented by immunolabelling with antibodies (SY38) against the synaptic vesicle protein synaptophysin, decreases as neurodegenerative disease (ME7) progresses. Hippocampus x4 (a - c) and CA1 stratum radiatum x20 (d - f) of NBH-inoculated (a, d) and ME7-inoculated animals at 12 (b, e) and 16 (c, f) weeks post-inoculation, ctx. cortex; cc corpus callosum; or, oriens; pyr, pyramidal; rad, radiatum; lac, lacunosum molecular. Scale bar = 100μm. Synaptic density was calculated using imageJ and plotted (g) for NBH (n = 4) and ME7 at 12 (n = 7) and 16 weeks (n = 8). Groups were compared by one-way ANOVA and Bonferroni post-hoc tests, NBH vs. 12 week denoted by *** $p < 0.001$ and ME7 16 weeks vs. ME7 12 weeks * $p < 0.05$).

4.3.2 Progression of disease increases the incidence, severity and duration of acute exacerbations of cognitive function.

(An experiment by Carol Murray contributed a number of animals to the current data).

In the present study we aimed to challenge animals later in disease to assess whether this would elicit greater systemic inflammation-induced cognitive dysfunction. Since previous cognitive work focussed on 12 week animals and 16-week ME7 animals displaying intact performance are on the cusp of impairment on the task in the absence of immune challenge (figure 4.1), we administered LPS (100 μg/kg i.p.) to ME7 animals at these points in disease and control animals (NBH). Regardless of the time point post-inoculation, only ME7 animals that were still performing the task consistently above 80% alternation were challenged for the study.

As can be observed in figure 4.3, ME7 animals at 12 and 16 weeks showed equivalent performance prior to LPS challenge. Saline-challenged ME7 animals maintained intact alternation performance across the entire experiment. In addition, NBH animals challenged with LPS (100 μg/kg i.p.) did not show any decrease in working memory performance (alternation) between 3 and 9 hours.

ME7 animals at 12 weeks post-inoculation showed decreased alternation at 3 hours post-LPS, consistent with previously described experiments (Murray et al., 2012). However, this impairment was not present in all 12 week LPS-challenged animals and the impairments had resolved by 24 hours. In contrast, a greater number of LPS-challenged ME7 animals at 16 weeks post-inoculation showed decreased alternation performance; in comparison to that displayed by 12 week ME7 animals, this was a more profound and prolonged impairment. It peaked at 7 hours and remained incompletely resolved at 24 hours. Repeated measures ANOVA showed an interaction between time and treatment ($F = 2.12$, df 12, 240, $p < 0.0163$) and main effects of treatment ($F = 18.34$, df 2, 40, $p < 0.0001$) and time ($F = 7.55$, df 6, 240, $p < 0.0001$). Bonferroni post-hoc tests show that the working memory of ME7 + LPS animals at 16 weeks remained impaired with respect to saline controls between 3 and 9 hours ($p < 0.001$). 16 week ME7 animals challenged with LPS were also significantly impaired with respect to ME7 + LPS (12 weeks) in the block of 5 trials from 7-9 hours ($p < 0.05$). Thus, LPS has more robust effects on working memory performance at 16 weeks compared to 12 weeks post-disease inoculation.

In summary, these data demonstrate that progression of disease predicts increased incidence, severity and duration of systemic inflammation-induced acute cognitive impairment.

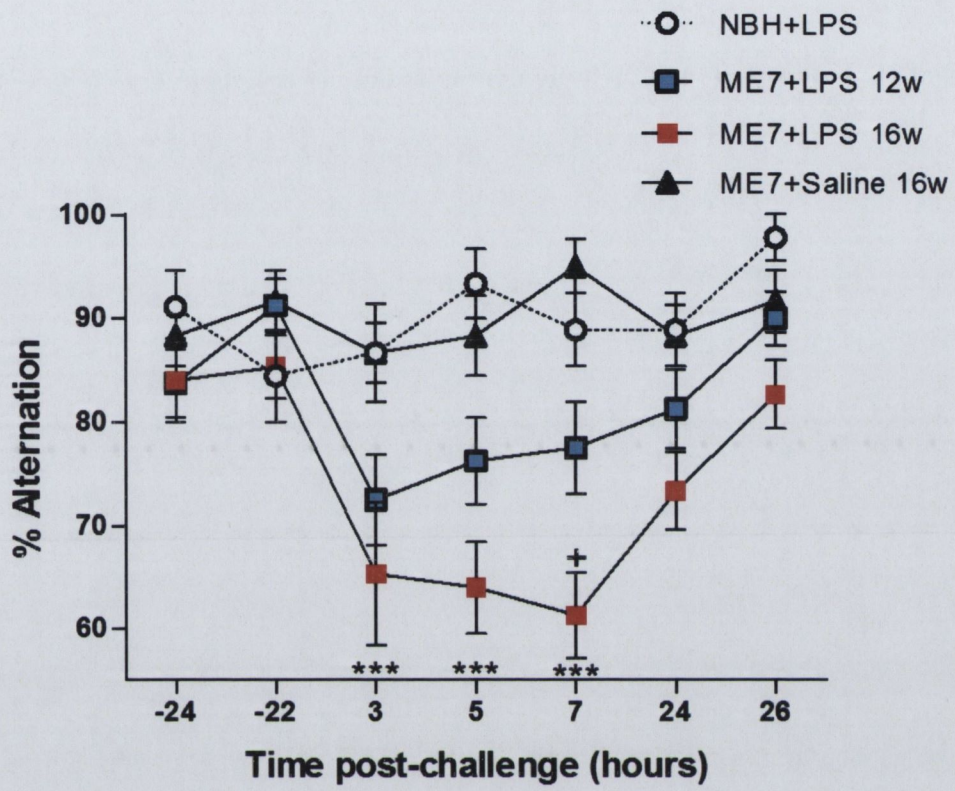

Figure 4.3 *Progression of disease increases the incidence, severity and duration of acute exacerbations of cognitive function.* T-maze alternation of NBH animals challenged with LPS, 100 μg/kg, versus ME7 animals at 12 or 16 weeks post-inoculation challenged with LPS (100 μg/kg) and ME7 animals at 16 weeks challenged with sterile saline. All animals were assessed 24 hours before acute challenge, tested for 5-6 hours post-challenge and reassessed 24 hours after challenge. A two-way ANOVA revealed main effects of time and disease and an interaction between time and disease. Significant Bonferroni post-hoc differences between ME7 + LPS (16 weeks) and ME7 and saline are denoted *** $p < 0.001$ and between ME7 + LPS (16 weeks) and ME7 (12 weeks) by + $p < 0.05$. NBH + LPS n=9, ME7 12w + LPS n=16, ME7 16w + LPS n=15, ME7 16w + Saline n=12. All data have been presented as mean ± SEM.

4.3.3 Prostaglandin biosynthetic isozymes COX-1 and COX-2 in disease

In the preceding section we have presented evidence that progression of neurodegenerative disease confers a propensity to greater acute-onset cognitive dysfunction in response to systemic inflammation. Moreover, we have demonstrated that there is progressive synaptic loss associated with this disease progression. We aimed to identify possible targets for pharmacological intervention in this model of delirium during dementia. Given evidence that prostaglandins are readily induced in response to inflammation (Tilley et al., 2001) and play a role in the CNS response to acute inflammation (Dantzer et al., 2008, Teeling et al., 2010), we examined hippocampal transcription of the prostaglandin biosynthetic enzymes COX-1 and 2, the prostaglandin E and D receptors and the prostaglandin D, E, F and I synthases as a function of disease progression.

Transcription of the two common isozymes of COX: COX-1 and COX-2- was assessed in ME7 animals and NBH animals, 12- and 16-weeks post inoculation (Figure 4.4). Hippocampal COX-1 mRNA expression was increased in ME7 animals with progressing disease. Analysis by two-way ANOVA revealed an interaction between disease and time ($F = 13.41$, df 1,16, $p = 0.0021$). In addition, there were main effects of disease ($F = 110$, df 1, 16, $p < 0.0001$) and time ($F = 22.76$, df 1, 16, $p = 0.0002$). Bonferroni post-hoc comparison showed that COX-1 transcripts were higher in 16 week ME7 animals in comparison with 12 week ME7 animals ($p < 0.001$).

COX-2 mRNA also showed a disease-associated increase, although this was less marked and was not yet obvious at 12 weeks. There was an interaction between disease and time ($F = 6.21$, df 1, 16, $p = 0.0241$) and main effects of disease ($F = 11.75$, df 1, 16, $p = 0.0034$) and time ($F = 6.2$, df 1, 16, $p = 0.024$). Bonferroni post-hoc comparison showed that COX-2 transcripts were higher in 16 week ME7 animals, in comparison with 12 week ME7 animals ($p < 0.001$).

Thus, it can be stated that COX-1 and COX-2 mRNA are increased in ME7 disease, particularly by 16 weeks post-inoculation.

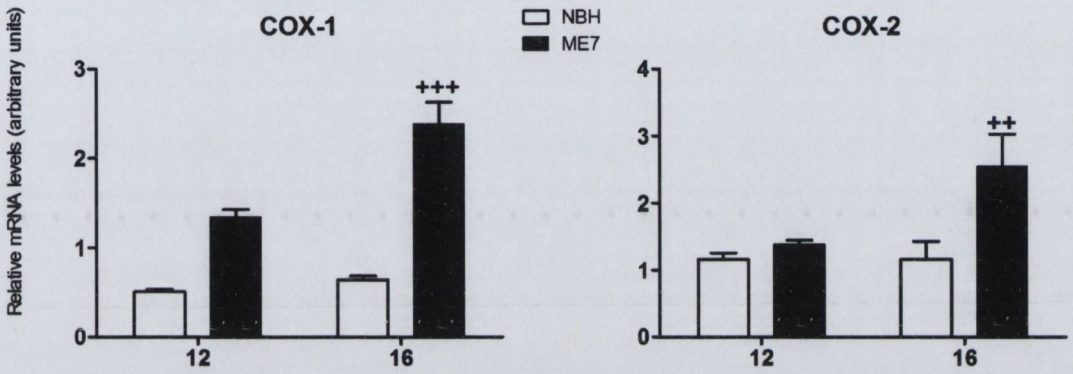

Figure 4.4 *Hippocampal mRNA expression of the prostaglandin biosynthetic isozymes, COX-1 and COX-2, in disease.* The expression of mRNA species for the prostaglandin biosynthetic isozymes COX-1 and COX-2 was assessed using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and results are denoted by ++ $p < 0.01$, +++ $p < 0.001$. $n = 4$ for all groups except ME7 at 12 weeks ($n = 8$). All data have been presented as mean ± SEM.

4.3.4 Hippocampal transcription levels of prostaglandin E synthase isozymes and receptor subtypes in disease

4.3.4.1 Prostaglandin E synthase isozymes

We then examined hippocampal mRNA levels of prostaglandin-E synthase isozymes, mPGES1, mPGES2 and cPGES in ME7 animals at 12 weeks and 16 weeks and NBH animals at the same time points post-inoculation (Figure 4.5). There was a significant disease-induced elevation in transcript levels of mPGES1, while expression was also significantly increased as diseased progressed. A two-way ANOVA revealed no interaction between disease and time ($F = 2.18$, df 1, 15, $p = 0.1609$). There were main effects of disease ($F = 53.85$, df 1, 15, $p = 0.0001$) and time ($F = 8.59$, df 1, 15, $p = 0.0261$). Bonferroni post hoc tests showed there was a significant difference between mRNA levels at 12 weeks and 16 weeks of disease ($p < 0.05$).

There were no significant disease-related alterations in the mRNA levels of mPGES2 or cPGES. Analysis of mPGES2 data by two-way ANOVA showed that there was no interaction between disease and time ($F = 5.20$, df 1, 15, $p = 0.3370$). Furthermore, disease ($F = 3.91$, df 1, 15, $p = 0.4032$) or time alone ($F = 14.02$, df 1, 15, $p = 0.1242$) did not significantly affect mPGES2 transcription.

Similarly, cPGES mRNA levels were neither affected by an interaction between disease and time ($F = 1.34$, df 1, 15, $p = 0.5598$) nor by time ($F = 16.61$, df 1, 15, $p = 0.0528$) or disease alone ($F = 17.07$, df 1, 15, $p = 0.0501$). However, as is evident from the p values, there was a trend towards both disease and time individually affecting cPGES transcription.

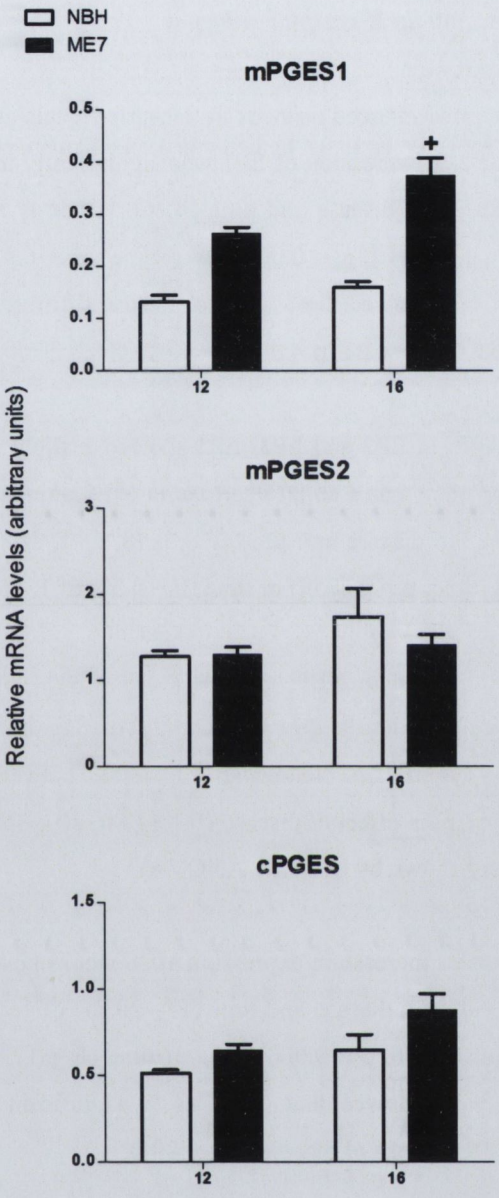

Figure 4.5 *Hippocampal mRNA expression of prostaglandin E synthase isozymes in disease.* The expression of mRNA species for the prostaglandin E synthase isozymes, mPGES-1, mPGES-2 and cPGES, was assessed using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and results are denoted by + $p < 0.05$, ME7 12 weeks vs. ME7 16 weeks. n = 4 for all groups except ME7 at 16 weeks (n = 7). All data have been presented as mean ± SEM.

4.3.4.2 Prostaglandin E receptor subtypes

There were also significant disease-induced changes in transcript levels of prostaglandin E receptor subtypes (Figure 4.6). The mRNA expression of EP1 was significantly increased with progressing disease. There was an interaction of disease and time ($F = 6.19$, df 1, 16, $p = 0.0242$) and main effects of disease ($F = 19.96$, df 1, 16, $p = 0.0004$) and time ($F = 6.97$, df 1, 16, $p = 0.0178$). Bonferroni post-hoc comparisons showed there was a significant difference between EP1 mRNA levels at 12 weeks and 16 weeks of disease ($p < 0.01$).

There were less marked changes in EP2 and EP3. EP2 showed a slight increase in NBH animals between 12 and 16 weeks but there was a slight decrease in ME7 animals across the same period. There was an interaction between disease and time ($F = 6.58$, df 1, 16, $p = 0.0207$) but no main effect of disease ($F = 0.17$, df 1, 16, $p = 0.6859$) or time ($F = 0.32$, df 1, 16, $p = 0.5776$).

Similarly EP3 was slightly increased in NBH animals at 16 weeks with respect to NBH at 12 weeks, while in ME7 EP3 mRNA levels dropped slightly in the same period, although these changes were not statistically significant. Neither an interaction between disease and time ($F = 15.03$, df 1, 16, $p = 0.0954$) nor main effect of disease ($F = 11.00$, df 1, 16, $p = 0.1490$) or time ($F = 0.77$, df 1, 16, $p = 0.6934$) were shown by two-way ANOVA.

In contrast, EP4 showed a marked increase in expression at 16 weeks post-ME7-inoculation. There was a significant interaction between disease and time ($F = 20.71$, df 1, 16, $p = 0.0003$) and main effects of disease ($F = 26.04$, df 1, 16, $p = 0.0001$) and of time ($F = 17.87$, df 1, 16, $p = 0.0006$). Bonferroni post-hoc comparisons showed that there was a significant difference between EP4 mRNA levels at 12 weeks and 16 weeks of disease ($p < 0.001$).

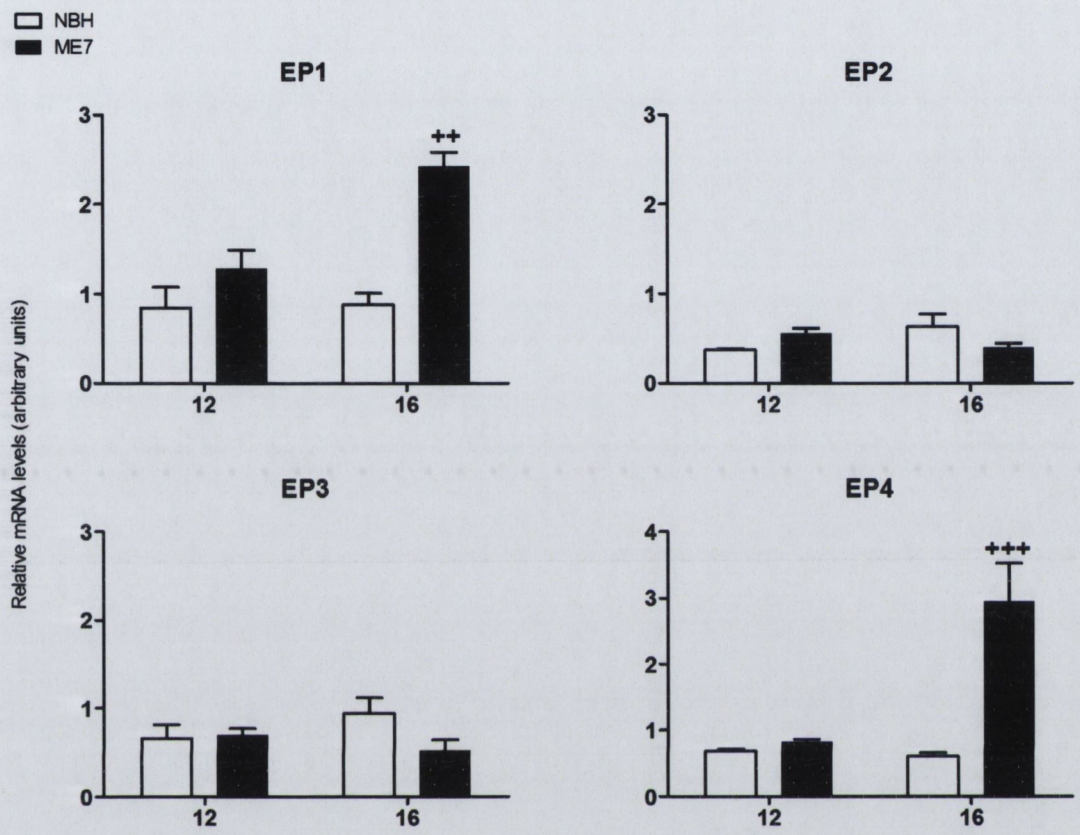

Figure 4.6 *Hippocampal mRNA expression of prostaglandin E receptor subtypes in disease.* The expression of mRNA species for the prostaglandin E receptor subtypes, EP1-4 was assessed using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and results are denoted by ++ $p < 0.01$, +++ $p < 0.001$, ME7 12 weeks vs. ME7 16 weeks. n = 4 for all groups except ME7 at 12 weeks (n = 8). All data have been presented as mean ± SEM.

4.3.5 Hippocampal transcription levels of prostaglandin D synthase isozymes and receptor subtypes in disease

Hippocampal mRNA levels of prostaglandin D synthase isozymes, HPGDS and LPGDS, and receptor subtypes, DP1 and DP2, were examined in ME7 animals, at 12 weeks and 16 weeks, and NBH animals, at the same time points post-inoculation (Figure 4.7). There was a significant disease-induced increase in HPGDS, while expression was also significantly increased as disease progressed. A two-way ANOVA revealed an interaction between disease and time ($F = 10.60$, df 1, 15, $p = 0.0053$). There were main effects of disease ($F = 23.02$, df 1, 15, $p = 0.0001$) and time ($F = 5.209$, df 1, 15, $p = 0.0261$). Bonferroni post hoc tests showed that HPGDS was elevated at 16 weeks ($p < 0.001$) in diseased animals compared to NBH controls. There was also a significant difference between mRNA levels at 12 weeks and 16 weeks of disease ($p < 0.01$).

LPGDS mRNA levels in the hippocampus were not altered by ME7 disease. There was neither an interaction between disease and time ($F = 1.299$, df 1, 15, $p = 0.2723$), nor main effects of disease ($F = 1.120$, df 1, 15, $p = 0.3066$) or time ($F = 0.1368$, df 1, 15, $p = 0.7167$) individually. However, there appeared to be a trend towards differences between ME7 animals and NBH 16-weeks post-inoculation.

There were no significant changes in DP1 and DP2 mRNA. Hippocampal DP1 transcription was not altered by an interaction of disease and time ($F = 2.53$, df 1, 14, $p = 0.5234$) and there was no main effect of disease ($F = 0.08331$, df 1, 14, $p = 0.7771$) or time ($F = 2.296$, df 1, 14, $p = 0.1520$) on transcription levels, as tested by two-way ANOVA.

Similarly, with hippocampal DP2 mRNA there was neither an interaction between disease and time ($F = 0.01598$, df 1, 14, $p = 0.9012$) nor main effects of disease ($F = 0.8309$, df 1, 14, $p = 0.3774$) or time ($F = 0.2041$, df 1, 14, $p = 0.6583$) shown by two-way ANOVA.

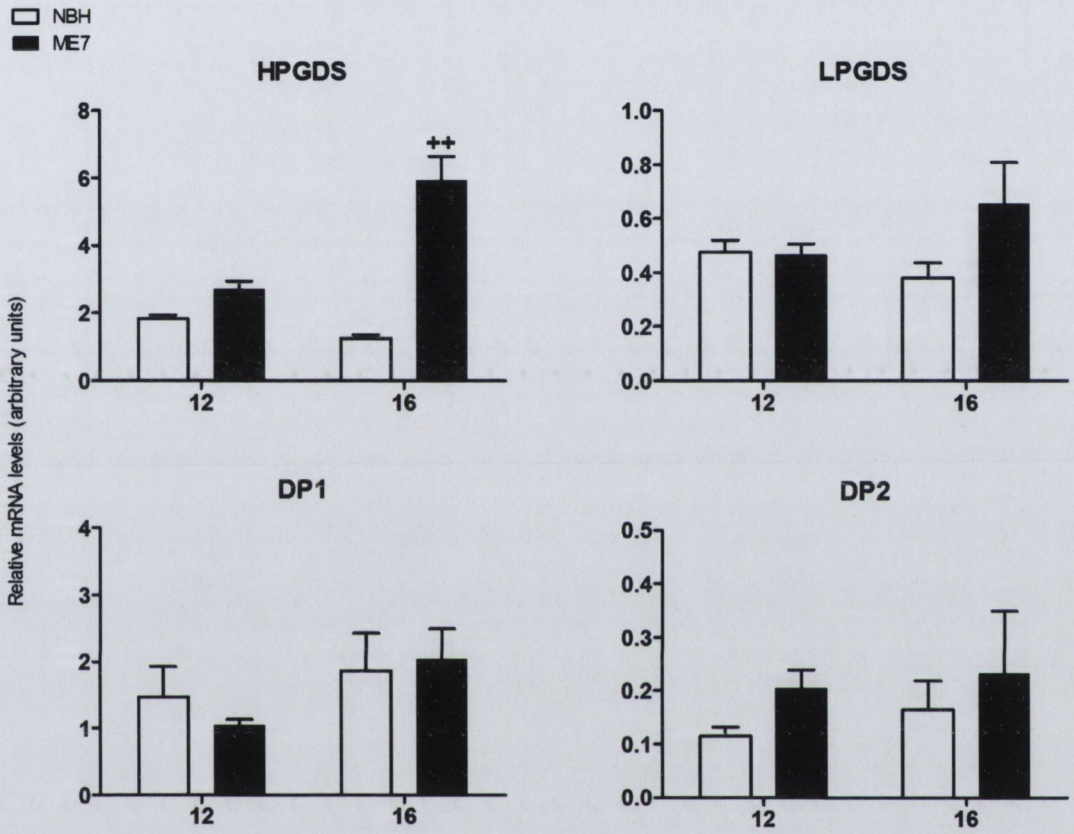

Figure 4.7 *Hippocampal mRNA expression of prostaglandin D synthase isozymes and receptor subtypes in disease.* The expression of mRNA species for the prostaglandin D synthase isozymes, HPGDS and LPGDS, and receptor subtypes, DP1 and DP2, was assessed using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and results are denoted by ++ $p < 0.01$, ME7 12 weeks vs. ME7 16 weeks. For HPGDS and LPGDS, n = 4 for all groups except ME7 at 16 weeks n = 7. For DP1 and DP2, n = 4 for all groups except NH at 16 weeks n = 6. All data have been presented as mean ± SEM.

4.3.6 Hippocampal transcription levels of prostaglandin F/prostamide synthase and prostaglandin I/prostacyclin synthase in disease

Hippocampal mRNA expression of prostaglandin F/ prostamide synthase and prostaglandin I/ prostacyclin synthase were examined in ME7 animals, at 12 weeks and 16 weeks, and NBH animals, at the same time points post-inoculation (Figure 4.8).

Hippocampal prostaglandin F/prostamide synthase mRNA expression in ME7 was elevated by progression of ME7 disease. There was neither an interaction between disease and time ($F = 1.129$, df 1, 14, $p = 0.0745$) nor main effect of disease ($F = 0.9199$, df 1, 14, $p = 0.3538$) revealed by two-way ANOVA. There was, however, a main effect of time ($F = 8.233$, df 1, 14, $p = 0.0124$). A Bonferroni post-hoc test revealed that this main effect was driven by an increase in ME7 animals between 12 and 16 weeks ($p < 0.05$).

Prostaglandin I/prostacyclin synthase mRNA levels in the hippocampus were not altered by ME7 disease. There was neither an interaction between disease and time ($F = 1.495$, df 1, 13, $p = 0.2432$), nor main effects of disease ($F = 3.761$, df 1, 13, $p = 0.0745$) or time ($F = 3.335$, df 1, 13, $p = 0.0909$) individually demonstrated by two-way ANOVA.

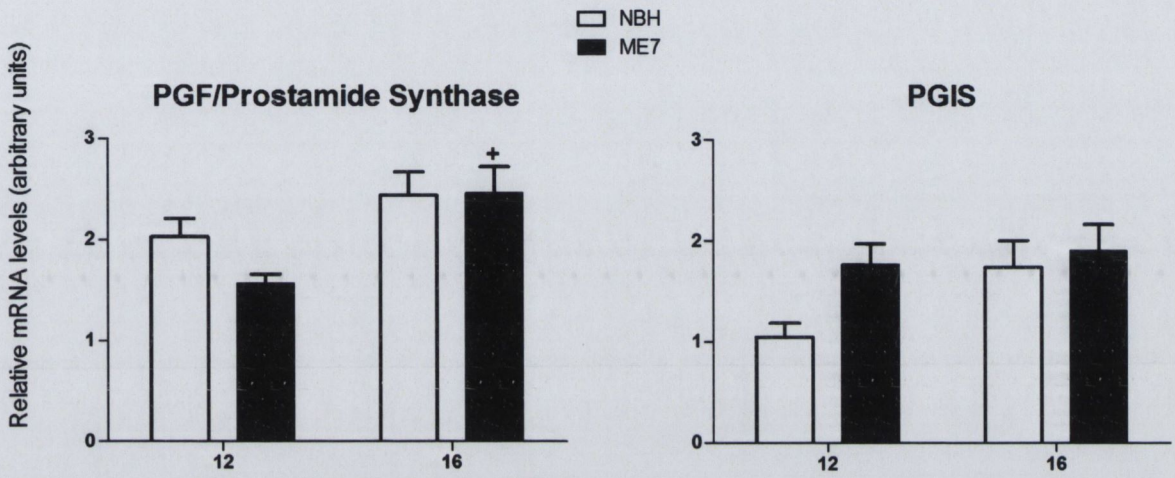

Figure 4.8 *Hippocampal mRNA expression of prostaglandin F2/ prostamide synthase and prostaglandin I2/ prostacyclin synthase in disease.* The expression of mRNA species for prostaglandin F/ prostamide synthase and prostaglandin I/ prostacyclin synthase was assessed using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and results are denoted by + $p < 0.05$, ME7 12 weeks vs. ME7 16 weeks. n = 4 for all groups except ME7 at 16 weeks (n = 7 for prostaglandin F/ prostamide synthase and n = 6 for prostaglandin I/ prostacyclin synthase). All data have been presented as mean ± SEM.

4.3.7 Hippocampal transcription levels of prostaglandin biosynthetic isozymes COX-1 and COX-2 in disease after LPS treatment

Given the observed alterations in components of the prostaglandin system in ME7 disease and our interest in the interaction between ME7 disease and systemic inflammation, we assessed the effect of LPS (i.p. 100 μg/kg) on hippocampal transcription of components of the prostaglandin system, measured previously in disease, in 16 week ME7 animals and NBH controls 2 hours post-challnge.

At 2 hours post-challenge with LPS, there was no significant change in COX-1 mRNA transcript levels in either the NBH or ME7 groups (Figure 4.9). Two-way ANOVA demonstrated no interaction between treatment and disease ($F = 0.6888$, df 1, 21, $p = 0.4159$), nor main effect of LPS treatment ($F = 0.2501$, df 1, 21, $p = 0.622$).

Hippocampal COX-2 mRNA transcripts were increased 2 hours after LPS challenges. There was no interaction between treatment and disease ($F = 0.9761$, df 1, 21, $p = 0.3344$) but there was a main effect of treatment alone ($F = 11.33$, df 1, 21, $p = 0.0029$). While elevated COX-2 mRNA expression was induced in both ME7 and NBH animals, this was only significant in NBH animals as demonstrated by Bonferroni post-hoc comparisons ($p < 0.05$).

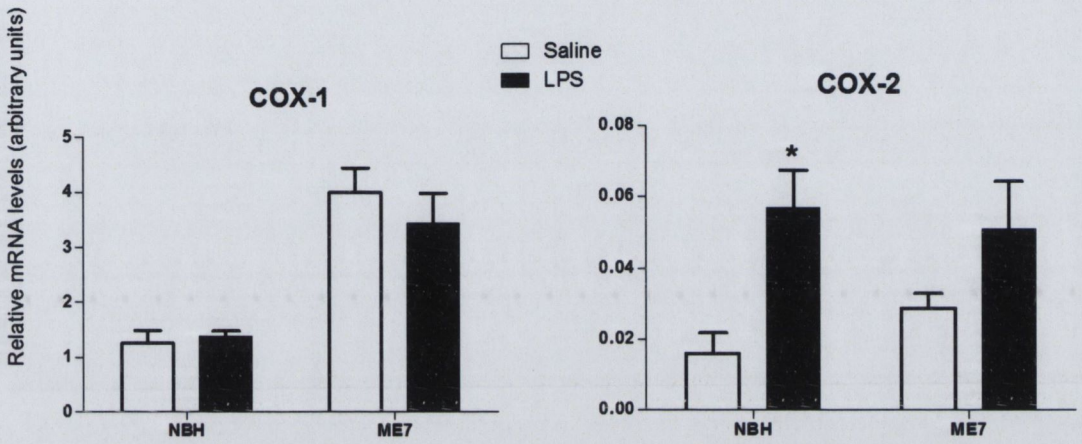

Figure 4.9 *Hippocampal mRNA expression of the prostaglandin biosynthetic isozymes COX-1 and COX-2 in ME7 disease after LPS treatment.* Hippocampal expression of mRNA species for the biosynthetic enzymes COX-1 and COX-2 in NBH and ME7 animals 16 weeks post-inoculation, 2 hours post-LPS (i.p. 100 μg/kg) or saline challenge using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and results are denoted by * $p < 0.05$, NBH + LPS vs. NBH + saline. n = 6 for NBH + saline, n = 5 for NBH + LPS and n = 7 for other groups. All data have been presented as mean ± SEM.

4.3.8 Hippocampal transcription levels of prostaglandin E synthase isozymes and receptor subtypes in disease after LPS treatment

4.3.8.1 Prostaglandin E synthase isozymes

At 2 hours post-LPS challenge, hippocampal mPGES-1 mRNA transcripts were increased (Figure 4.10). There was no interaction between treatment and disease ($F = 0.09141$, df 1, 16, $p = 0.7663$) but there was a main effect of treatment alone ($F = 37.27$, df 1, 16, $p = 0.0023$). mPGES-1 mRNA expression was clearly elevated after LPS challenge in both ME7 and NBH animals, this was only significant in NBH animals, as demonstrated by Bonferroni post-hoc comparisons ($p < 0.05$).

There was no significant change in mPGES-2 mRNA transcript levels in NBH or ME7 animals post-LPS challenge. Two-way ANOVA demonstrated no interaction between treatment and disease ($F = 1.280$, df 1, 16, $p = 0.2746$), nor main effect of LPS treatment ($F = 3.527$, df 1, 16, $p = 0.0787$).

Similarly, cPGES mRNA expression was not increased by LPS challenge in NBH or ME7 animals 2 hours after systemic LPS administration. Neither an interaction between treatment and disease ($F = 3.012$, df 1, 16, $p = 0.1019$), nor a main effect of LPS treatment ($F = 0.5221$, df 1, 16, $p = 0.4804$) alone were shown by two-way ANOVA.

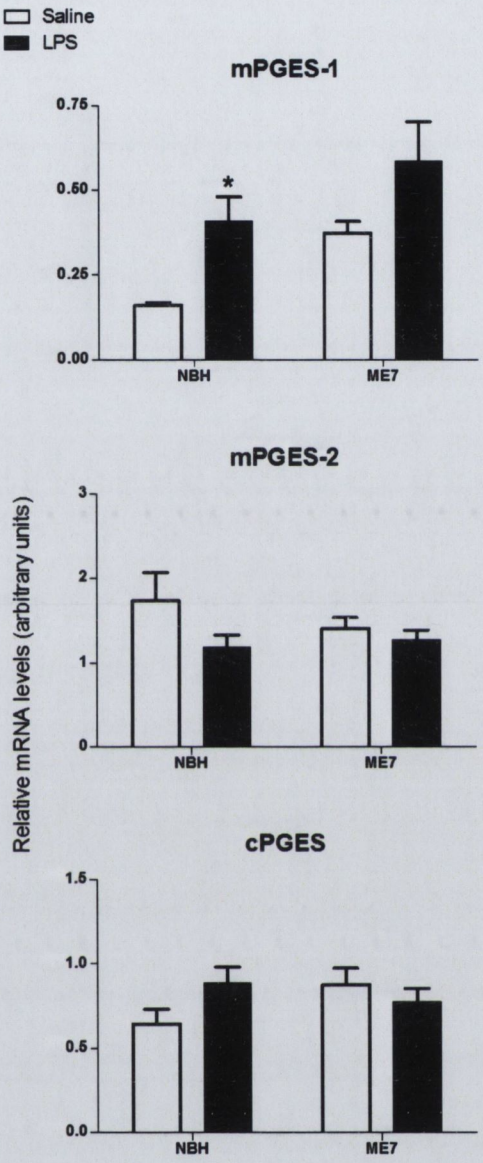

Figure 4.10 *Hippocampal mRNA expression of the prostaglandin E synthase isozymes in ME7 disease after LPS treatment.* Hippocampal expression of mRNA species for the prostaglandin E synthase isozymes mPGES-1, mPGES-2 and cPGES in NBH and ME7 animals 16 weeks post-inoculation, 2 hours post-LPS (i.p. 100 μg/kg) or saline challenge using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and results are denoted by * $p < 0.05$, NBH + LPS vs. NBH + saline. n = 4 for NBH + saline and ME7 + LPS, n = 5 for NBH + LPS and n = 7 for ME7 + saline. All data have been presented as mean ± SEM.

4.3.8.2 Prostaglandin E receptor subtypes

LPS challenge did not induce a significant change in the mRNA expression of the prostaglandin receptors EP1-4 in NBH or ME7 animals (Figure 4.11). There were slight decreases in some transcript expression patterns but these changes were not marked.

Hippocampal EP1 mRNA was not altered 2 hours after systemic administration in NBH or ME7 animals, as demonstrated by two-way ANOVA ($F = 2.000$, df 1, 21, $p = 0.1727$). Furthermore, there was no interaction between LPS treatment and disease ($F = 0.4304$, df 1, 21, $p = 0.0511$).

Similarly, two-way ANOVA showed that hippocampal EP2 mRNA was not altered significantly by an interaction between disease and LPS treatment ($F = 0.1.493$, df 1, 21, $p = 0.2353$) or LPS treatment alone ($F = 0.0312$, df 1, 21, $p = 0.8613$).

There was no significant change in EP3 mRNA transcript levels in NBH or ME7 animals post-LPS challenge. Two-way ANOVA demonstrated no interaction between treatment and disease ($F = 2.256$, df 1, 21, $p = 0.1480$), nor main effect of LPS treatment ($F = 2.952$, df 1, 21, $p = 0.1005$).

EP4 mRNA expression was not increased by LPS challenge in NBH or ME7 animals 2 hours after systemic LPS administration. Neither an interaction between treatment and disease ($F = 0.3853$, df 1, 21, $p = 0.5414$), nor a main effect of LPS treatment ($F = 0.1042$, df 1, 21, $p = 0.7500$) alone were shown by two-way ANOVA.

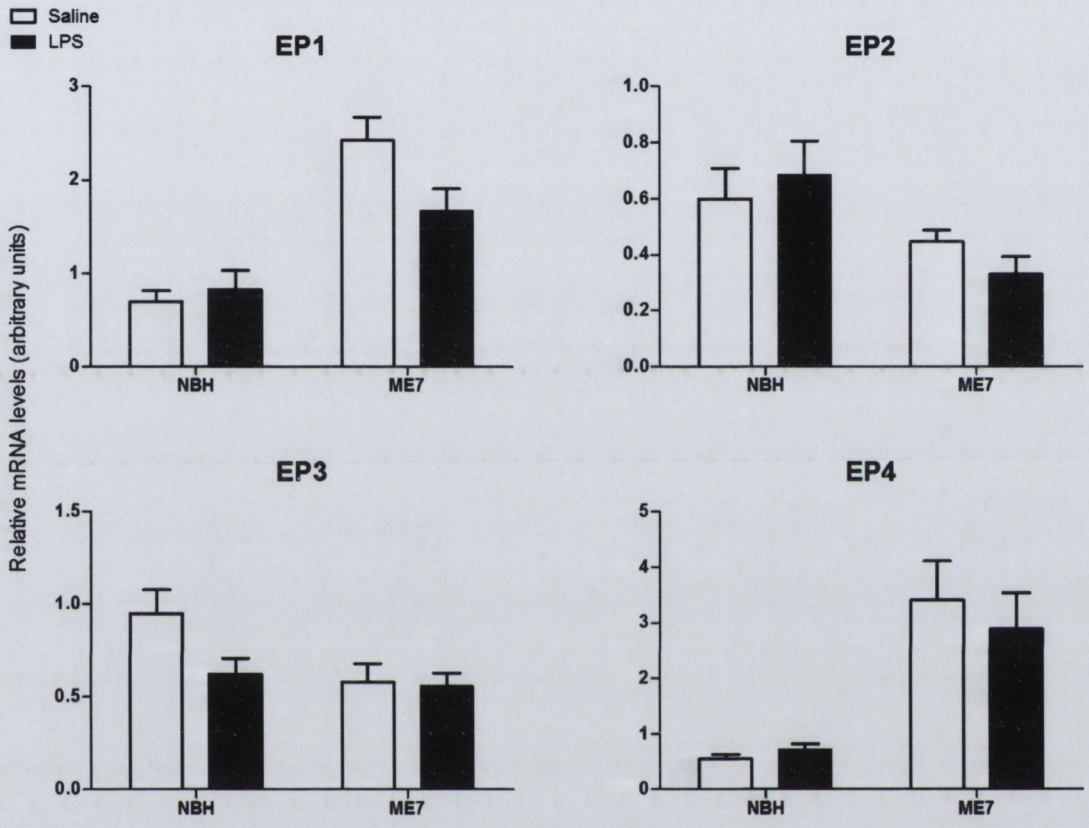

Figure 4.11 *Hippocampal mRNA expression of the prostaglandin E receptor subtypes in NBH and ME7 animals after LPS treatment.* Hippocampal expression of mRNA species for the prostaglandin E receptor subtypes, EP1- EP4, in NBH and ME7 animals 16 weeks post-inoculation, 2 hours post-LPS (i.p. 100 μg/kg) or saline challenge using quantitative PCR. Data were analysed by two-way ANOVA. n = 6 for NBH + saline, n = 5 for NBH + LPS and n = 7 for other groups. All data have been presented as mean ± SEM.

4.3.9 Hippocampal transcription levels of prostaglandin D synthase isozymes and receptor subtypes in disease after LPS treatment

Hippocampal mRNA levels of prostaglandin D synthase isozymes, HPGDS and LPGDS, and receptor subtypes, DP1 and DP2, were examined in 16 week ME7 animals and NBH animals, 2 hours after peripheral administration of LPS (Figure 4.12).

At 2 hours post-LPS challenge, there was no significant change in HPGDS mRNA transcript levels in NBH or ME7 animals. Two-way ANOVA demonstrated no interaction between treatment and disease ($F = 1.785$, df 1, 16, $p = 0.2002$), nor main effect of LPS treatment ($F = 0.1733$, df 1, 16, $p = 0.6828$).

Similarly, LPGDS mRNA expression was not increased by LPS challenge in NBH or ME7 animals, 2 hours after systemic LPS administration. Neither an interaction between treatment and disease ($F = 0.8863$, df 1, 16, $p = 0.3624$), nor a main effect of LPS treatment ($F = 0.0050$, df 1, 16, $p = 0.9447$) alone were shown by two-way ANOVA.

Hippocampal DP1 mRNA transcripts were decreased by LPS treatment. There was no interaction between treatment and disease ($F = 0.03395$, df 1, 15, $p = 0.8563$) but there was a main effect of treatment alone ($F = 12.05$, df 1, 15, $p = 0.0034$). Although DP1 mRNA expression was clearly decreased after LPS challenge in both ME7 and NBH animals, this was only significant in ME7 animals, as demonstrated by Bonferroni post-hoc comparisons ($p < 0.05$).

There was no significant change in DP2 mRNA transcript levels in NBH or ME7 animals post-LPS challenge. Two-way ANOVA demonstrated no interaction between treatment and disease ($F = 0.1617$, df 1, 15, $p = 0.6933$), nor main effect of LPS treatment ($F = 1.877$, df 1, 15, $p = 0.1655$).

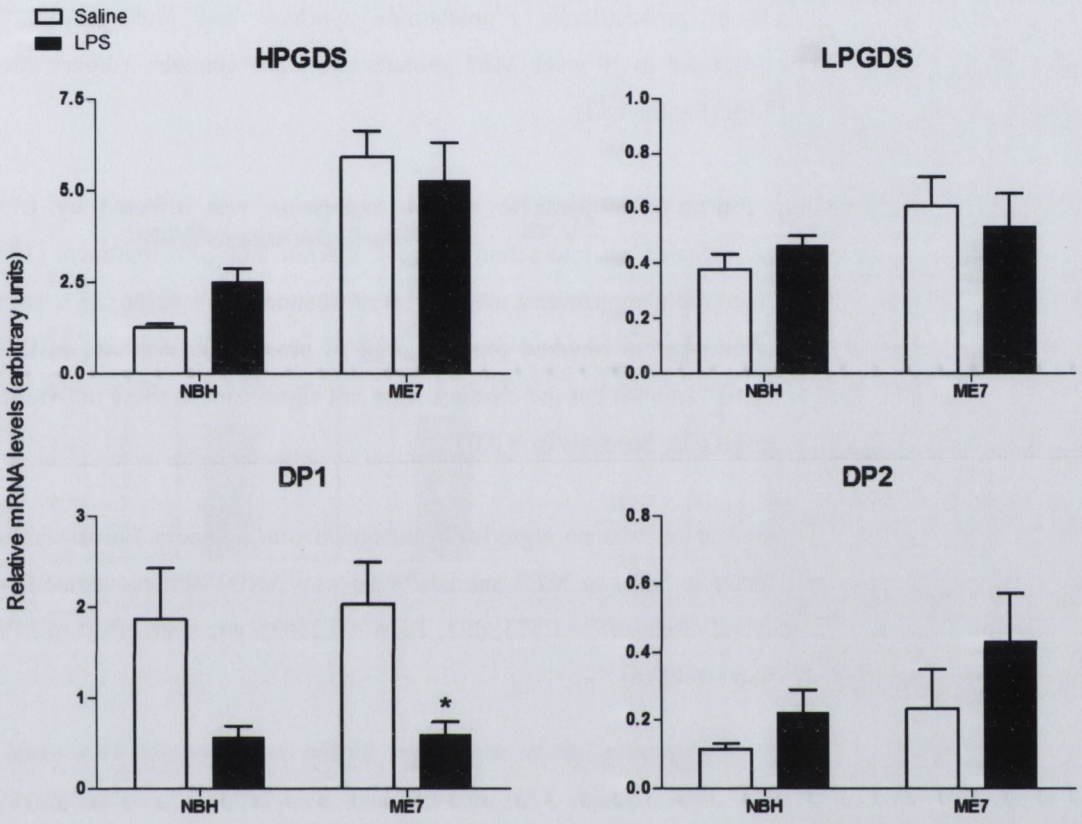

Figure 4.12 *Hippocampal mRNA expression of the prostaglandin D synthase isozymes and receptor subtypes in ME7 disease after LPS treatment.* Hippocampal expression of mRNA species for the prostaglandin D synthase isozymes, HPGDS and LPGDS, and receptor subtypes, DP1 and DP2, was assessed in NBH and ME7 animals 16 weeks post-inoculation, 2 hours post-LPS (i.p. 100 μg/kg) or saline challenge using quantitative PCR. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and are denoted by * $p < 0.05$ ME7 + LPS vs. ME7 + saline. For HPGDS and LPGDS n = 7 ME7 + saline, n = 5 NBH + LPS and n = 4 for other groups. For DP1 and DP2, n = 6 ME7 + saline, n = 5 ME7 + LPS and n = 4 for other groups. All data have been presented as mean ± SEM.

4.3.10 Hippocampal transcription levels of prostaglandin F/prostamide synthase and prostaglandin I/prostacyclin synthase in disease after LPS treatment

Hippocampal mRNA levels of prostaglandin F/prostamide synthase and prostaglandin I/ prostacyclin synthase were examined in 16 week ME7 animals and NBH animals, 2 hours after peripheral administration of LPS (Figure 4.13).

Hippocampal prostaglandin F/prostamide synthase mRNA expression was affected by LPS treatment. Two-way ANOVA revealed no interaction between disease and LPS treatment ($F = 0.5661$, df 1, 16, $p = 0.4627$) but did demonstrate a main effect of treatment ($F = 6.386$, df 1, 16, $p = 0.0224$). Systemic LPS administration lowered prostaglandin F/ prostamide synthase mRNA expression in both NBH and ME7 animals but the changes were not significant in these individual groups as analysed by Bonferroni post-hoc tests ($p > 0.05$).

At 2 hours post-LPS challenge, there was no significant change in prostaglandin I/prostacyclin synthase mRNA transcript levels in NBH or ME7 animals. Two-way ANOVA demonstrated no interaction between treatment and disease ($F = 1.573$, df 1, 16, $p = 0.2303$), nor main effect of LPS treatment ($F = 4.533$, df 1, 16, $p = 0.0515$).

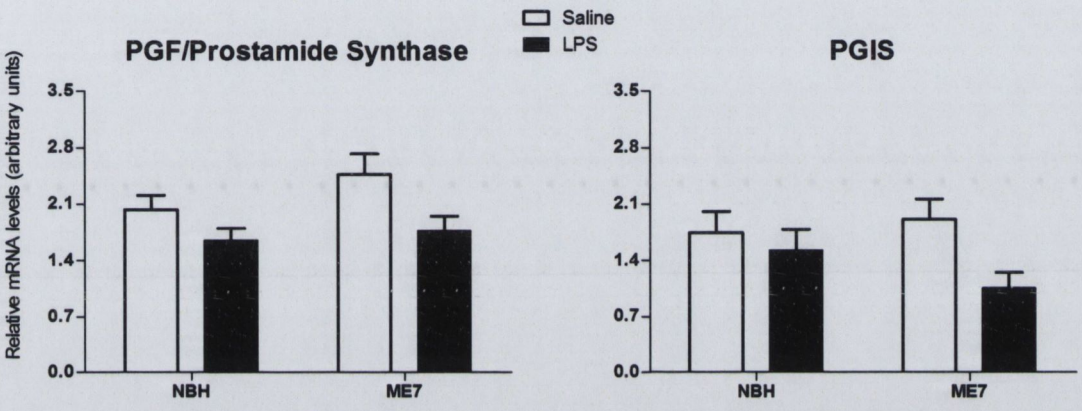

Figure 4.13 *Hippocampal mRNA expression of the prostaglandin F/prostamide synthase and prostaglandin I/ prostacyclin synthase in ME7 disease after LPS treatment.* Hippocampal expression of mRNA species for prostaglandin F/ prostamide synthase and prostaglandin I/ prostacyclin synthase in NBH and ME7 animals 16 weeks post-inoculation, 2 hours post-LPS (i.p. 100 μg/kg) or saline challenge using quantitative PCR. Data were analysed by two-way ANOVA. n = 4 NBH + saline, n = 6 ME7 + saline, n = 5 for other groups. All data have been presented as mean ± SEM.

4.3.11 Immunohistochemical detection of cyclooxygenases and prostaglandin D synthase in the hippocampus

4.3.11.1 Immunohistochemical detection of COX-1 and COX-2 on microglia

We used IBA-1 to show the distribution of microglial cells in the hippocampus and these cells can be seen to increase significantly in number from NBH to 12 weeks ME7 and again to ME7 16 weeks (Figure 4.14 a-c). This density was not appreciably altered after LPS challenge (Figure 4.14 d).

COX-1 showed similarly increased numbers as disease progressed (Figure 4.14 e-h) and these cells show a perinuclear labelling pattern but are clearly identifiable as microglia (Figure 4.14 i). However, there are also a number of vascular-associated cells that can be clearly distinguished from the unlabelled endothelial layer. These cells appear to be perivascular macrophages and seem to increase with disease but are not altered by systemic LPS (Figure 4.14 j-m).

COX-2 expression appears to be microglial and is increased by disease (Figure 4.14 n-j). COX-2 expression is also increased in the cerebral vascular endothelium upon systemic LPS challenge (insets).

Hence, microglia are increased in the hippocampus of diseased animals. Moreover, these microglia express COX-1 and COX-2. Endothelial cells express COX-2 after peripheral LPS stimulation. Vascular-associated cells that are not endothelial cells also express COX-1.

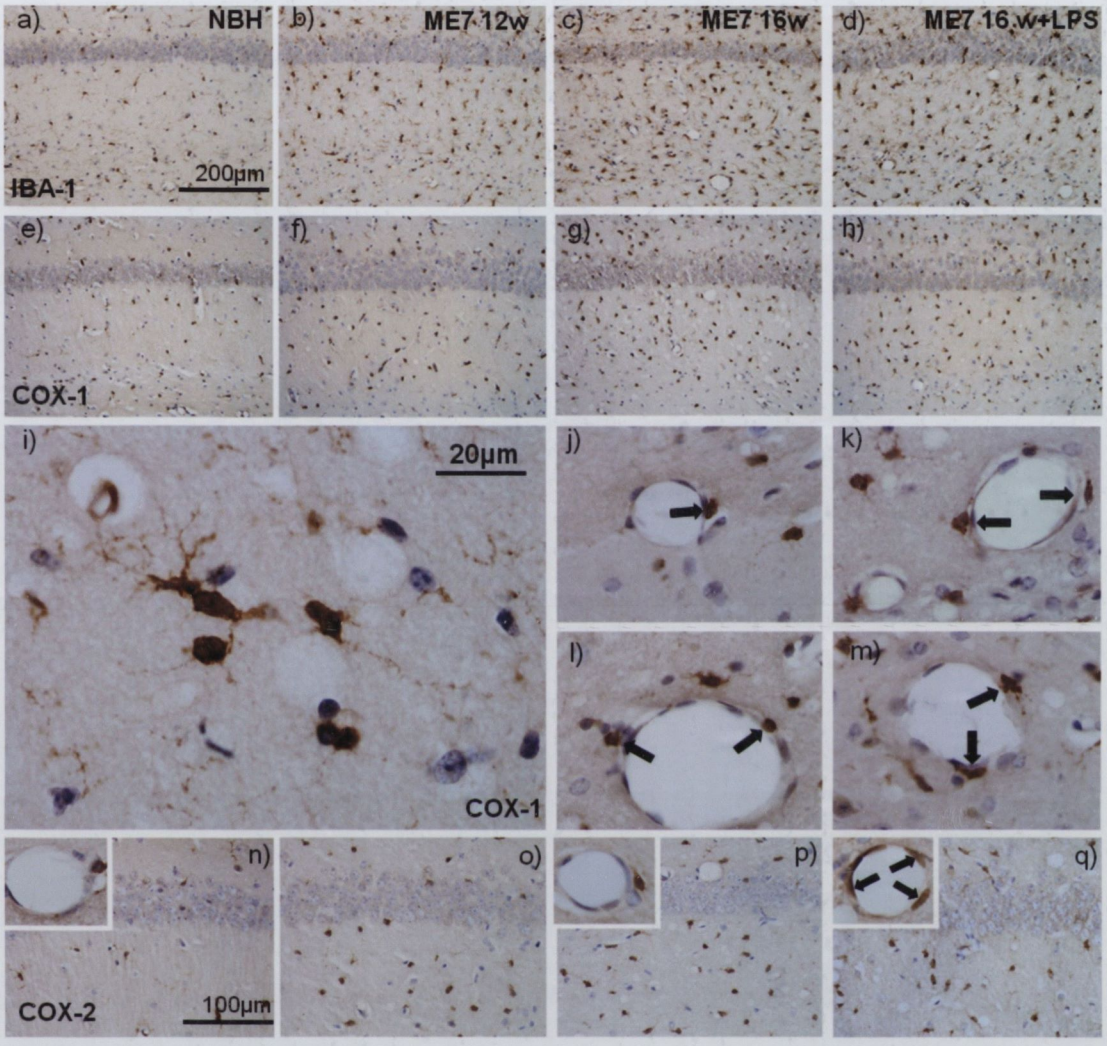

Figure 4.14 *Immunohistochemical detection of microglia and cyclooxygenases.* a-d) The distribution of microglia in the CA1 hippocampal region of normal and diseased brains was examined using the microglial marker IBA1 (x20, scale bar = 200µm). e-h). Expression of COX-1 in the same region of normal and diseased brains show increased density of positive cells 16w > 12w > NBH (x20, scale bar = 200µm). i) The COX-1-positive cells show clear microglial morphology at 100x (scale bar=20µm). j-m) Hippocampal blood vessels (100x) show a population of COX-1-positive cells that are clearly distinguishable from the endothelial layer (arrows). n-q) Expression of COX-2 shows similar distribution in microglial cells (x40, scale bar = 100µm).. Insets show that the endothelial layer is COX-2 negative in NBH and in disease but becomes COX-2 positive upon challenge with LPS (arrows).

4.3.11.2 Immunohistochemical detection of COX-1 on perivascular macrophages

To confirm that the COX-1-expressing vascular-associated cells observed previously were perivascular macrophages, double immunofluoresence labelling for COX-1 (Alexafluor-488, green) and CD206 (Alexafluor-594, red) was performed on hippocampal sections of diseased and normal brains.

In both ME7 (Figure 4.15 a-c) and NBH animals (Figure 4.15 d-f), most, if not all, cerebral cells that were positive for CD206, a marker of perivascular macrophages (Galea et al., 2005), also stained positive for COX-1. To ensure that CD206 staining in the red channel was not the result of spectral overlap from the green flourophore, an ME7 control section was imaged on the confocal microscope. The fluorescent antibody to capture CD206 but not COX-1 was incubated on the control section (Figure 4.15 g-i). The control section still demonstrated CD206 staining, demonstrating that the vascular cells were indeed perivascular macrophages and not strong COX-1 staining crossing over from the green channel.

Thus, perivascular macrophages in diseased and normal animals express COX-1.

4.3.11.3 Immunohistochemical detection of HPGDS expression on microglia

Prostaglandin D synthase isozyme HPGDS expression in the hippocampus of NBH and ME7 animals 16 weeks post-inoculation was investigated.

In sections from both ME7 and NBH animals, HPGDS expression was detected on cells in a perinuclear labelling pattern but these cells are clearly identifiable as microglia. These cells increase in number in ME7 disease and are further increased with the progression of disease (Figure 4.16 a-c), in a similar manner to IBA-1 expression. Peripheral administration of LPS does not visibly alter density of expression (Figure 4.16 d).

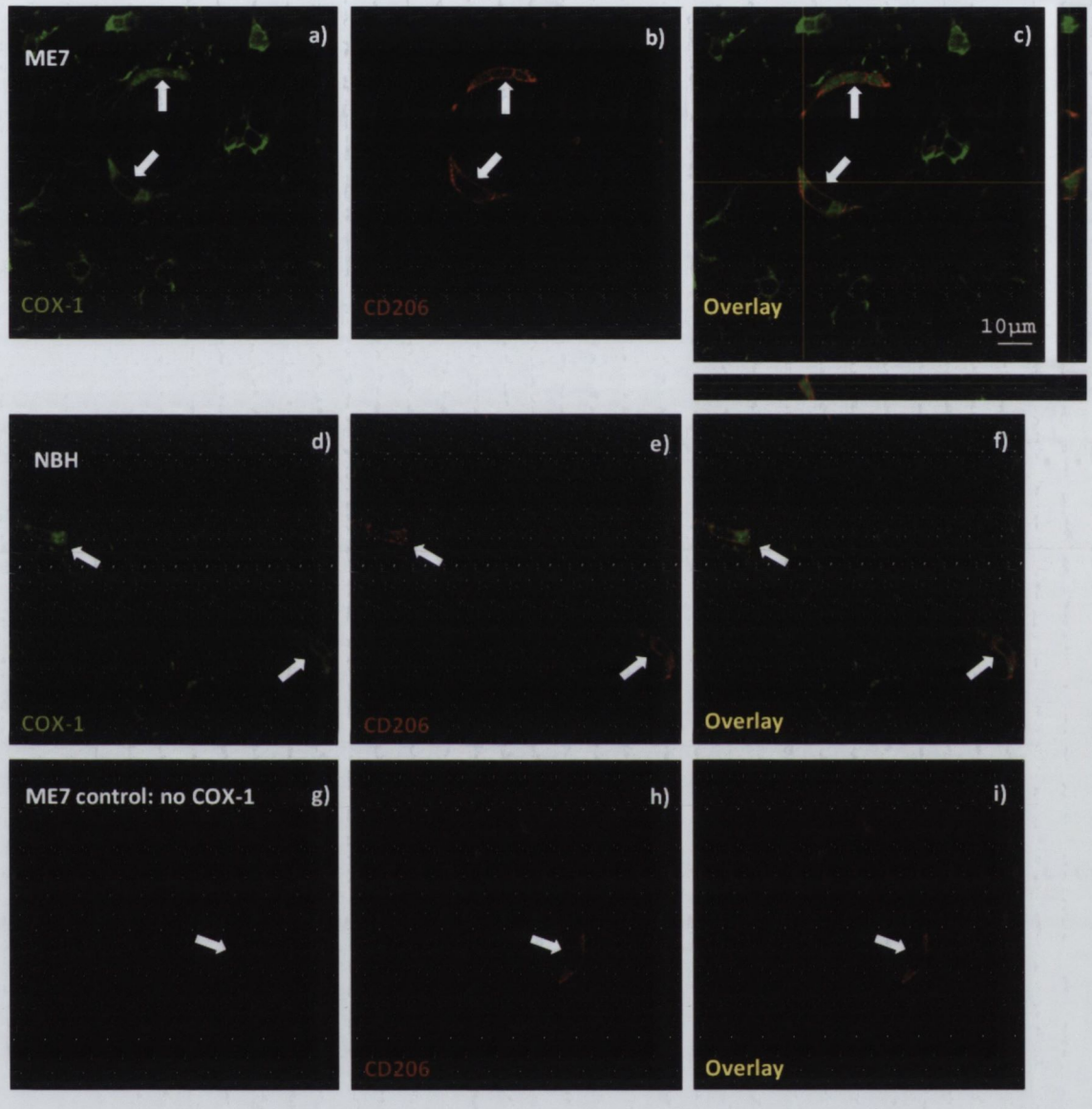

Figure 4.15 *Immunoflourescent labelling of perivascular macrophages with COX-1.* Double immunofluoresence labelling for COX-1 (488 nm) and CD206 (594 nm) was performed on hippocampal sections from ME7 and NBH animals to assess whether perivascular macrophages express COX-1. The Double immunofluoresence demonstrates double-labelled perivascular macrophages (arrows) in diseased (a-c) and normal brains (d-f). An ME7 control, labelled with a fluorescent antibody to detect CD206 but not labelled with an antibody to detect COX-1 still demonstrates CD206 staining in the absence of COX-1 (g-i). (60x, scale bar = 10 µm).

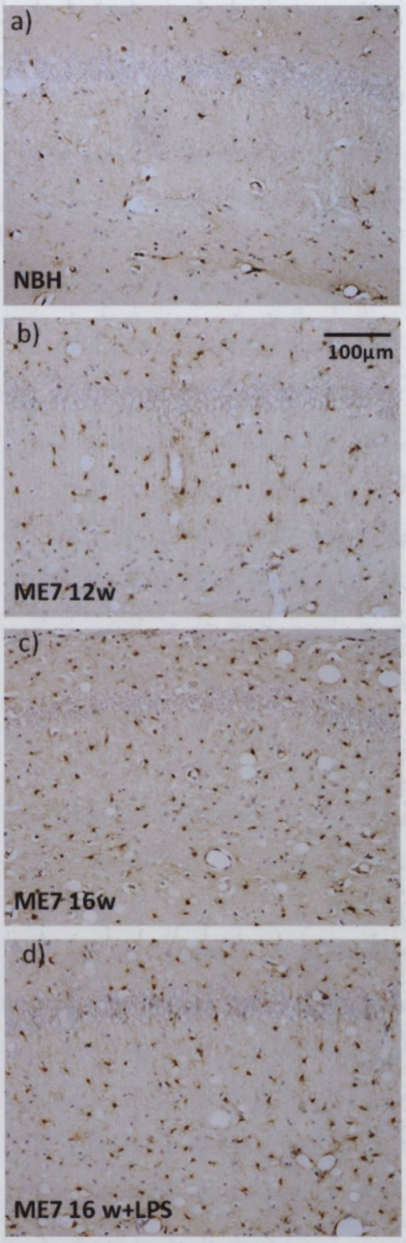

Figure 4.16 *Immunohistochemical demonstrations of HPGDS expression on microglia.*

The expression of prostaglandin D synthase isozyme HPGDS in the CA1 hippocampal region of normal and diseased brain (16 weeks post-inoculation) was examined (a-d, x20, scale bar = 100µm). The staining demonstrates increased density of positive cells in ME7 disease, 16w > 12w > NBH. The HPGDS-positive cells show clear microglial morphology.

4.3.12 Investigation of the contribution of COX-2-derived prostaglandins to acute cognitive dysfunction

4.3.12.1 Peripheral administration of COX-2-specific inhibitor nimesulide does not protect against inflammation-induced cognitive dysfunction.

We have shown induction of COX-2 by PCR and immunohistochemistry related to both disease and LPS. We also demonstrated similar induction of mPGES-1 mRNA, which is described to couple with COX-2. Consequently, we assessed the ability of a COX-2 inhibitor to prevent the acute-working memory deficits induced by LPS in ME7 animals. Pre-treatment of animals with the specific COX-2 inhibitor nimesulide (10 mg/kg) did not protect against LPS (100 μg/kg)-induced working memory deficits on the T-maze alternation task (Figure 4.17 a). There was no significant interaction between time and treatment ($F = 0.5217$, df 6, 102, $p = 0.7907$) or main effect of treatment ($F = 1.200$, df 1, 17, $p = 0.272$) but there was a main effect of time ($F = 9.07$, df 6, 102, $p < 0.0001$), as assessed by two-way ANOVA.

4.3.12.2 Peripheral administration of COX-2-selective inhibitor nimesulide does not inhibit PGE2 synthesis in plasma or brain.

We assessed whether peripheral administration of nimesulide impacted circulating prostaglandin levels after systemic LPS challenge. Prostaglandin E metabolite (PGEM) was assessed as a proxy for rapidly degraded plasma levels of PGE2. In ME7 animals, at 2 hours post-LPS (100 μg/kg) there was a significant increase in PGEM levels but nimesulide (10 mg/kg) failed to inhibit this elevation (Figure 4.17 b). Bonferroni post-hoc tests after a significant one-way ANOVA ($F = 7.210$, df 2, 11, $p = 0.0100$) showed LPS-induced PGEM ($p < 0.05$ vs. ME7 + nimesulide). However, nimesulide administration does not affect this increase ($p > 0.05$ vs. ME7 + LPS).

We also assessed whether peripheral administration of nimesulide affects hippocampal PGE2 levels (Figure 4.17 c). LPS-treated ME7 animals did not show any acute PGE2 elevation 2 hours post-LPS challenge in comparison to ME7 animals treated with nimesulide alone, assessed by one-way ANOVA. Animals treated with both LPS and nimesulide showed no significant difference compared to animals treated with nimesulide alone or LPS alone. Thus, hippocampal PGE2 levels were not altered by administration of nimesulide.

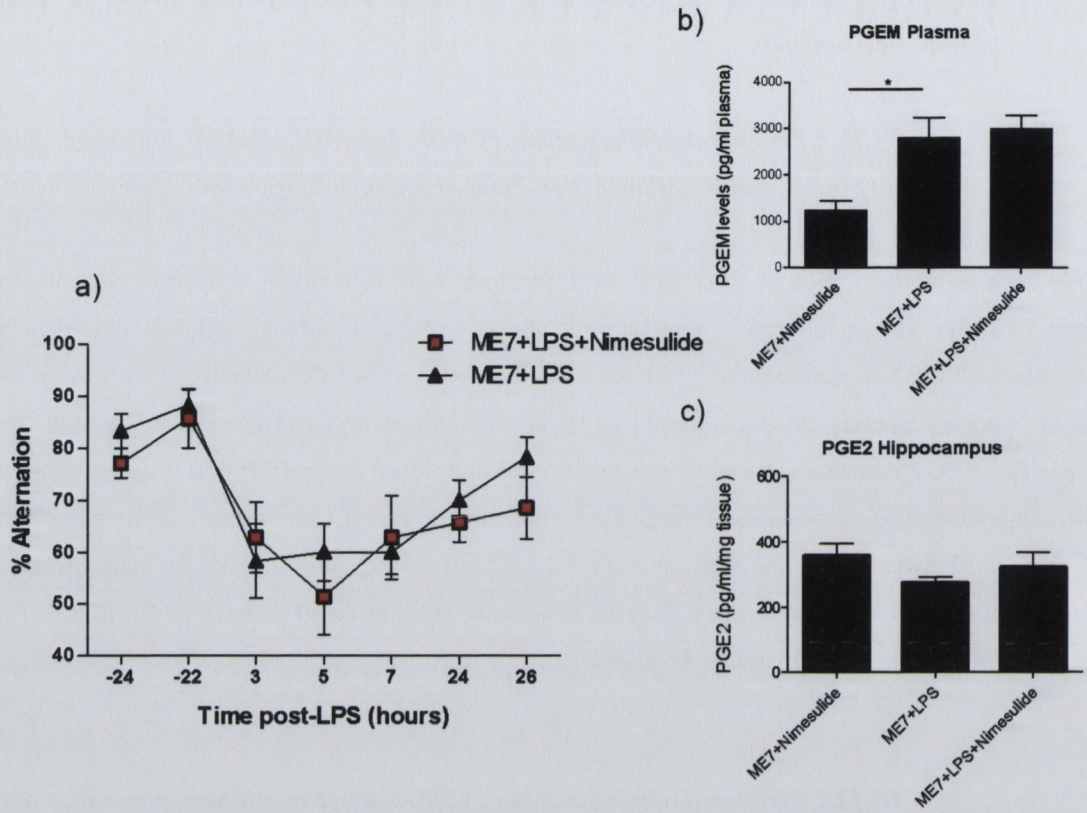

Figure 4.17 *COX-2 inhibition fails to protect diseased animals from LPS-induced working memory deficits and does not reduce PGE2 levels in plasma and brain.* a) Working memory was assessed by alternation in the paddling T-maze. ME7 and NBH animals were challenged with LPS (100 μg/kg) in the presence or absence of the COX-2-selective inhibitor nimesulide given at 10 mg/kg i.p. 60 minutes before LPS. Data were analysed by two-way ANOVA and Bonferroni post-hoc analyses followed significant main effects and are denoted by * $p < 0.05$. ME7 + LPS + nimesulide, n = 7; ME7 + LPS, n = 12. b) Plasma PGEM was also assessed, 2 hours post-challenge with saline or LPS (100 μg/kg), in the presence or absence of nimesulide (10 mg/kg) or vehicle (Tris-HCl), in ME7 animals, using ELISA assay. After a significant one-way ANOVA analysis, Bonferroni post-hoc tests revealed significant differences, as denoted by ** $p < 0.01$. ME7 + nimesulide, n = 4. Other groups n = 5 c) Hippocampal PGE2 was also assessed in the same animals, 2 hours post-challenge with saline or LPS (100 μg/kg), in the presence or absence of nimesulide (10 mg/kg) or vehicle (Tris-HCl), in ME7 animals, using ELISA assay. ME7 + nimesulide, n = 4. Other groups n = 5. All data have been presented as mean ± SEM.

4.3.12.3 Peripheral administration of COX-2-specific inhibitor nimesulide inhibits LPS-induced hypothermia

To demonstrate efficacy of nimesulide, at the dose and route used here, we assessed the ability of nimesulide pre-treatment to inhibit LPS-induced hypothermia, as COX-2-dependent prostaglandins are known to have a role in the temperature response to inflammation (Yamagata et al., 2001). A higher dose of LPS (250 μg/kg i.p.) was used in this experiment to elicit a robust temperature change against which the efficacy of nimesulide could be assessed. In naive animals, at 2 hours post-injection, LPS induced a robust hypothermic response, which nimesulide (10 mg/kg) pre-treatment significantly attenuated (Figure 4.18). Following a significant one-way ANOVA ($F = 13.81$, df 5, 32, $p = 0.0001$) a Bonferroni post-hoc test showed that LPS induced a significant decrease in temperature in vehicle + LPS treated animals with respect to LPS animals pre-treated with nimesulide at 2 hours ($p < 0.01$).

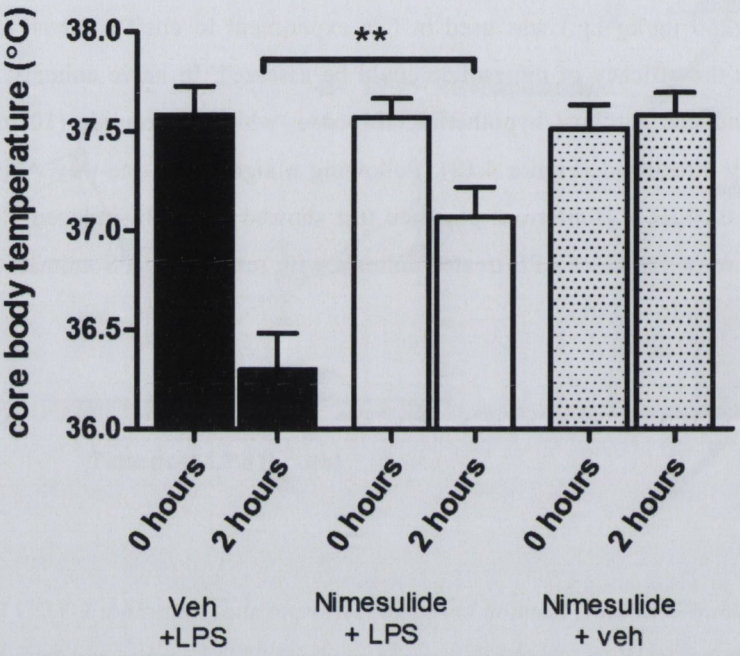

Figure 4.18 *Peripheral administration of COX-2-specific inhibitor nimesulide inhibits systemic LPS-induced hypothermia.* Core-body temperature at baseline and 2 hours in naïve animals treated with saline or LPS (250 μg/kg), pre-treated with vehicle (Tris-HCl) or nimesulide (10 mg/kg). ** denotes $p < 0.01$ by Bonferroni post-hoc test after a significant main effect of treatment by one-way ANOVA. n = 7 (nimesulide + LPS) n = 6 (vehicle + LPS, nimesulide + saline). All data have been presented as mean ± SEM.

4.3.13 Investigation of the contribution of COX-1-derived prostaglandins to acute cognitive dysfunction

4.3.13.1 Peripheral administration of the COX-1-selective inhibitor piroxicam protects against inflammation-induced cognitive dysfunction

Given the alterations observed in CNS COX-1 mRNA and protein expression, we assessed the effects of COX-1 inhibition on LPS-induced cognitive dysfunction. Diseased animals treated with the COX-1-selective inhibitor piroxicam (10 mg/kg) were afforded significant protection from LPS (100 μg/kg)-induced working memory deficits (Figure 4.19 a). Administration of piroxicam alone had no impact on alternation performance. There was no interaction between treatment and time ($F = 1.707$, df 12, 162, $p = 0.0695$) but there was a main effect of treatment ($F = 17.1$, df 2, 27, $p < 0.0001$) and a main effect of time ($F = 6.12$, df 6, 162, $p < 0.0001$). Bonferroni post-hoc tests revealed a significant difference between ME7 + LPS and ME7 + LPS + piroxicam 3 hours post-challenge ($p < 0.05$). Furthermore, repeated measures ANOVA comparison of the two LPS-treated groups revealed a main effect of treatment with piroxicam ($F = 17.9$, df 1, 21, $p < 0.001$).

4.3.13.2 Peripheral administration of the COX-1-selective inhibitor piroxicam inhibits PGE2 synthesis in plasma and brain

Since piroxicam was effective in reducing LPS-induced working memory deficits, we assessed its impact on PGE2 levels in the blood and in the hippocampus. At 2 hours post-LPS challenge (100 μg/kg) there was a significant increase in PGEM levels, which was completely blocked by piroxicam (10 mg/kg) pre-treatment (Figure 4.19 b). Bonferroni post-hoc tests after a significant one-way ANOVA ($F = 12.92$, df 4, 14, $p = 0.0001$) showed that LPS significantly induced PGEM ($p < 0.001$ vs. ME7 + saline) and that piroxicam significantly inhibited this increase ($p < 0.001$ vs. ME7 + LPS).

Hippocampal PGE2 levels were not significantly changed by systemic LPS (tresponse was variable in LPS-treated animals). However, peripheral administration of piroxicam completely abrogated hippocampal PGE2. A one-way ANOVA ($F = 6.807$, df 4, 24, $p = 0.0008$), followed by Bonferroni post-hoc tests showed that ME7 + LPS + piroxicam animals differed from ME7 + saline and ME7 + LPS animals ($p < 0.05$). Thus, piroxicam reduced central and peripheral pools of PGE2.

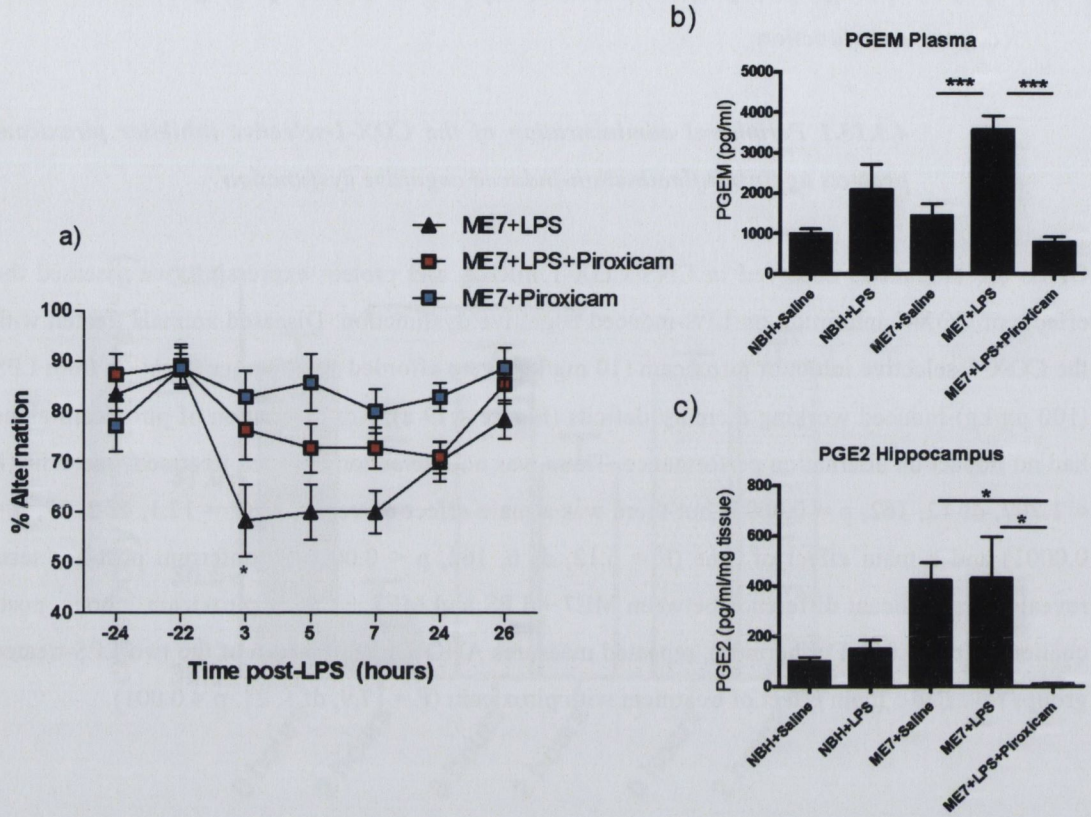

Figure 4.19 *The COX-1-selective inhibitor piroxicam protects against LPS-induced working memory dysfunction and inhibits PGE2 synthesis in plasma and brain of diseased animals.* Working memory was assessed by alternation in the paddling T-maze. ME7 and NBH animals were challenged with LPS in the presence or absence of the COX-1-selective inhibitor piroxicam, given at 10 mg/kg i.p. 60 minutes before LPS. Data were analysed by two-way ANOVA. Bonferroni post-hoc analyses showed that ME7 + LPS + piroxicam differed significantly from other groups at 3 hours, denoted by * $p < 0.05$. ME7 + piroxicam, n = 8; ME7 + LPS, n = 12 and ME7 + LPS + piroxicam, n = 11. b) Plasma PGEM and c) hippocampal PGE2 were also assessed, 2 hours post-challenge with LPS (100 µg/kg) or saline, in the presence or absence of piroxicam (2 mg/kg), using ELISA assays. After a significant one-way ANOVA analysis, Bonferroni post-hoc tests revealed significant differences, as denoted by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. NBH + saline, n = 3; NBH + LPS, n = 4; ME7 + LPS + piroxicam, n = 4; ME7 + saline, n = 4; ME7 + LPS, n = 4. All data have been presented as mean ± SEM.

4.3.13.3 Hippocampal PGE2 is elevated by disease but not acute systemic LPS challenge

Thus far, evidence of disease and challenge-induced elevations in elements of the prostaglandin synthesis system has been presented. PGE2 is the prostaglandin subtype most widely implicated in disease-induced and inflammation-induced changes in CNS function. However, we have not observed an LPS-induced elevation of hippocampal PGE2 in 16-week ME7 animals or NBH animals in the piroxicam or nimesulide experiments.

We directly analysed the effect of ME7 disease and systemic LPS (100 μg/kg, injected 2 hours pre-sacrifice) on hippocampal PGE2 levels (Figure 4.20). Mean PGE2 levels were elevated in diseased animals compared to normal animals but did not show any challenge-induced acute elevation in either diseased or normal animals. Two-way ANOVA revealed no interaction between disease and LPS treatment ($F = 0.04940$, df 1, 12, $p = 0.8728$) or main effect of treatment ($F = 0.1022$, df 1,12, $p = 0.7547$). However, a main effect of disease on hippocampal PGE2 levels was demonstrated ($F = 46.26$, df 1, 12, $p = 0.0073$).

Thus, COX-1 inhibition offers protection from LPS-induced impairment on a hippocampal-dependent T-maze task. There is, however, no acute elevation of hippocampal PGE2 after LPS challenge. A disease-related elevation of hippocampal PGE2 was observed, and appears to be COX-1-dependent, given that piroxicam administration almost completely eliminates synthesis.

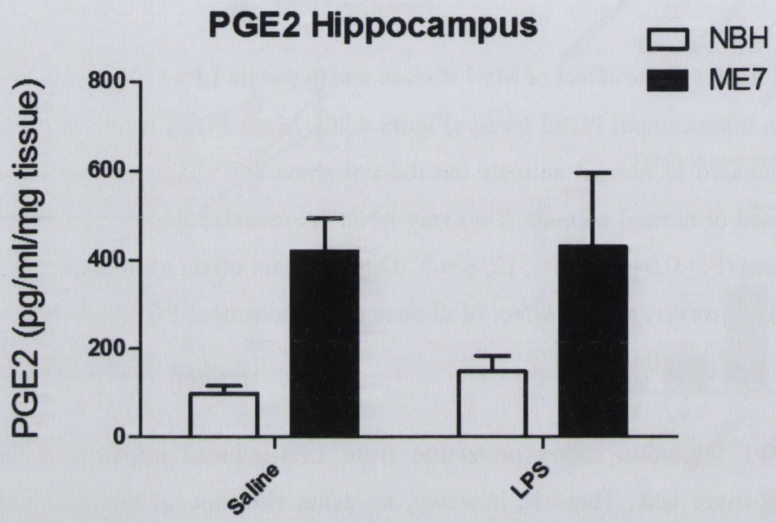

Figure 4.20 *Hippocampal PGE2 is elevated by disease but not acute LPS-treatment.* Hippocampal PGE2 levels were assessed in diseased and normal animals 16 weeks post-inoculation, 2 hours post-challenge with LPS (100 μg/kg) or saline by ELISA. Data were analysed by two-way ANOVA and main effects are described in the main text. NBH + saline, n = 3; NBH + LPS, n = 5; other groups n = 4. All data have been presented as mean ± SEM.

4.3.13.4 Peripheral administration of the COX-1-selective inhibitor piroxicam does not alter LPS-induced alterations in plasma and brain cytokine expression in diseased animals

Given the protection afforded by piroxicam from inflammation–induced cognitive impairment, its impact on systemic pro-inflammatory cytokine synthesis and hippocampal pro-inflammatory transcription post-LPS challenge was examined (figure 4.21).

Plasma IL-1β, TNF-α, IL-6 and CXCL1 levels were examined (Figure 4.21 a). Following a significant one-way ANOVA ($F = 122.1$, df 2, 11, $p = 0.0001$), Bonferroni post-hoc tests showed that IL-6 was elevated 2 hours post-LPS ($p < 0.001$ vs. ME7 + saline), but not significantly altered by piroxicam pre-treatment ($p > 0.05$ ME7 + LPS vs. ME7 + LPS + piroxicam). Treatment also significantly altered systemic TNF-α ($F = 6.683$, df 2, 12, $p = 0.0133$). If anything, the rise in TNF-α in LPS-treated ME7 animals was exacerbated by piroxicam. Piroxicam pre-treatment increased the levels of TNF-α but also the variability of the LPS-induced increase, such that the ME7 + LPS group was not different to ME7 + saline group. Bonferroni analysis showed that that ME7 + LPS + piroxicam, but not but ME7 + LPS, was different to ME7 + saline ($p < 0.01$). Systemic IL-1β was also altered by treatment ($F = 9.423$, df 2, 11, $p = 0.0062$). Bonferroni post-hoc analysis showed that the LPS-induced elevation in IL-1β ($p < 0.05$) was not reduced by piroxicam ($p > 0.05$). Likewise, following a one-way ANOVA ($F = 626.6$, df 2, 11, $p = 0.0001$), post-hoc tests revealed that CXCL1 was induced by LPS treatment ($p < 0.001$) and not affected by piroxicam ($p > 0.05$).

Q-PCR for a number of pro-inflammatory cytokines was examined in the hippocampus of LPS-treated ME7 animals, with or without piroxicam pre-treatment (Figure 4.21 b). One-way ANOVA analysis demonstrated robust LPS-induced alterations in hippocampal mRNA level for IL-1β ($F = 7.055$, df 2, 10, $p = 0.0171$), TNF-α ($F = 5.440$, df 2, 8, $p = 0.0322$), IL-6 ($F = 5.306$, df 2, 10, $p = 0.0341$) and CXCL1 ($F = 14.38$, df 2, 10, $p = 0.0022$). Piroxicam pre-treatment did not inhibit transcription of any of these cytokines. Bonferroni post-hoc tests revealed that the ME7 + LPS group was significantly increased with respect to the ME7 + saline group for IL-1β, IL-6 and CXCL1 (all $p < 0.01$). The LPS-induced TNF-α increase was rather variable and because TNF-α levels are also elevated in the ME7 model per se, this increase was not significant ($p > 0.05$). The ME7 + LPS + piroxicam group was not significantly different to ME7 + LPS group for any transcripts ($p > 0.05$). Thus, systemically administered piroxicam has no impact on systemic LPS induction of plasma pro-inflammatory cytokines or CNS pro-inflammatory cytokine transcripts.

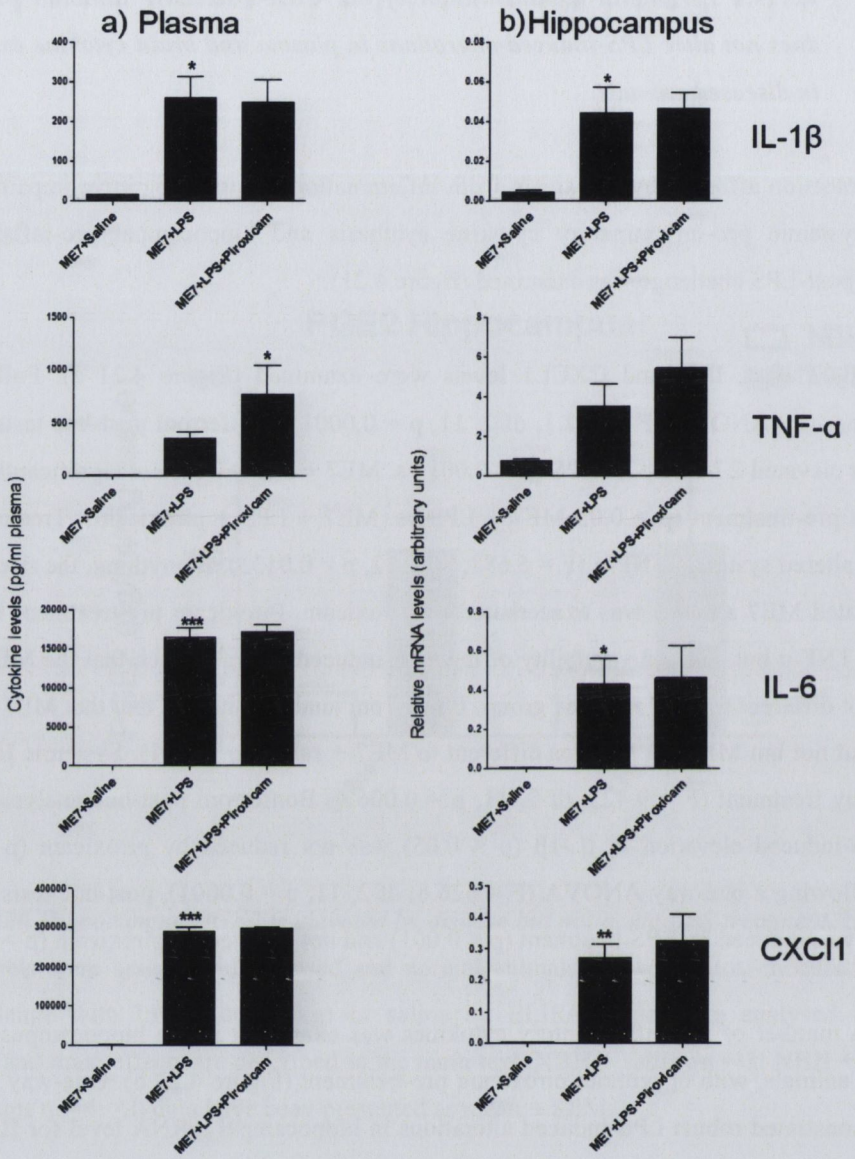

Figure 4.21 *The impact of COX-1-selective inhibitor piroxicam on plasma and brain cytokine expression in diseased animals after LPS treatment.* Plasma levels of pro-inflammatory cytokines, determined by ELISA, and brain transcription of pro-inflammatory cytokines, determined by Q-PCR, were examined in ME7 animals after systemic LPS administration, in the presence or absence of piroxicam. a) Expression of IL-1β, TNF-α, IL-6 and CXCL1 in plasma of ME7 animals 2 hours post-challenge with saline, LPS (100 μg/kg) or LPS + piroxicam (10 mg/kg). b) Hippocampal mRNA levels of ME7 animals challenged as above. Differences between ME7 + saline and ME7 + LPS by Bonferroni post-hoc test after one-way ANOVA are denoted by * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$. $n = 3$ for ME7 + LPS + piroxicam and $n = 4$ for all other groups. All data presented as mean ± SEM.

4.3.13.5 Peripheral administration of the COX-1-selective inhibitor piroxicam inhibits hypothalamic PGE2.

Given that we found no further acute elevation of PGE2 in diseased animals systemically treated with LPS, we investigated whether there was an LPS (100μg/kg)-induced increase in the hypothalamus, the key region for the induction of the hypothalamic pituitary (stress) axis, 2 hours post-challenge. Furthermore, we assessed the ability of systemic administration of piroxicam (10 mg/kg) to inhibit disease-induced elevation of PGE2 (Figure 4.22).

Hypothalamic PGE2 levels were increased by LPS in the hypothalamus of both NBH and ME7 animals but these increases were not significant when analysed by Bonferroni post-hoc tests ($p < 0.05$) after a significant one-way ANOVA ($F = 5.856$, df 4, 14, $p = 0.0055$). A Student t test comparing NBH + saline with NBH + LPS showed a significant increase in PGE2 ($t = 4.475$, df 5, $p = 0.0065$). The response to LPS in ME7 animals was more variable and t test comparison between ME7 animals treated with LPS and saline was not statistically significant ($t = 0.7075$, df 6, $p = 0.5058$).

Importantly, piroxicam completely abrogated disease-elevated hypothalamic PGE2. ME7 animals administered with piroxicam exhibited significantly lower PGE2 than ME7 animals treated with LPS ($p < 0.01$) and treated with saline ($p < 0.05$). These were demonstrated by Bonferroni post-hoc tests after the aforementioned one-way ANOVA. The results indicate that in disease, COX-1-derived prostaglandins form the majority of hypothalamic PGE2, in a similar manner to hippocampal PGE2.

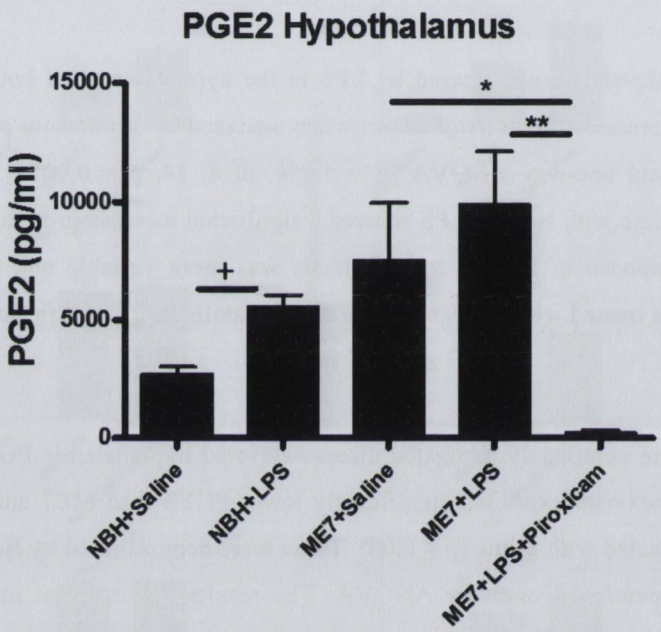

Figure 4.22 *The COX-1-selective inhibitor piroxicam eliminates PGE2 synthesis in the hypothalamus of diseased animals.* Hypothalamic PGE2 was assessed, 2 hours post-challenge with LPS (100 µg/kg) or saline, in the presence or absence of piroxicam (10 mg/kg), in ME7 and NBH animals, using ELISA assays. After a significant one-way ANOVA analysis, Bonferroni post-hoc tests revealed significant differences, as denoted by * $p < 0.05$ and ** $p < 0.01$. + indicates a significant difference by Student t test, $p = 0.0065$. NBH + saline, n = 3; NBH + LPS, n = 4; ME7 + LPS + piroxicam, n = 4; ME7 + saline, n = 4; ME7 + LPS, n = 4. All data have been presented as mean ± SEM.

4.3.13.6 Peripheral administration of the COX-1-specific inhibitor SC-560 protects from inflammation-induced cognitive dysfunction.

Since there is debate in the literature concerning the COX-1-selectivity of piroxicam (Blanco et al., 1999, Buharalioglu et al., 2011, Ogino et al., 1997, Warner et al., 1999), we investigated the ability of the COX-1-specific inhibitor SC-560 to inhibit LPS-induced acute cognitive dysfunction.

Animals treated with the COX-1-specific inhibitor SC-560 were afforded significant protection against the acute working memory deficits induced by LPS (Figure 4.23). SC-560 alone had no impact on alternation performance. There was an interaction between time and treatment ($F = 2.259$, df 12,204, $p = 0.0105$) and main effects of treatment ($F = 3.952$, df, 6,34, $p = 0.0286$) and time ($F = 7.955$, df, 6,204, $p < 0.0001$). Bonferroni post-hoc tests revealed a significant difference between ME7 + LPS and ME7 + LPS + SC-560 at 5 hours post-challenge ($p < 0.05$). Furthermore repeated measures ANOVA comparison of the two LPS-treated groups also revealed a significant main effect of treatment with SC-560 ($F = 3.952$, df, 6,34, $p = 0.0286$).

In the current results, we demonstrate that two COX-1 selective inhibitors, piroxicam and SC-560 but not the specific COX-2 inhibitor attenuated LPS-induced working memory deficits. Thus, it can be stated that COX-1-derived prostaglandins have a role in systemic inflammation-induced cognitive impairment in ME7 animals.

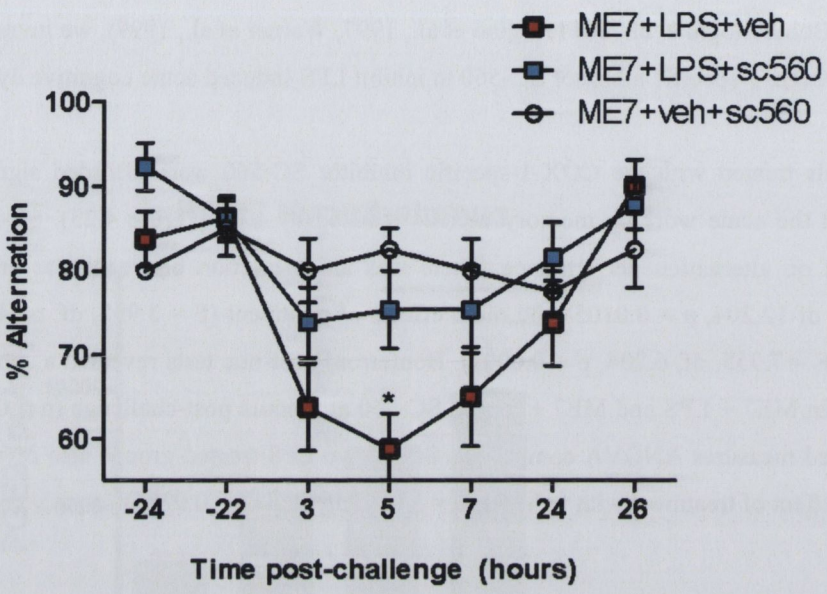

Figure 4.23 *Administration of the COX-1-specific inhibitor SC-560 protects against LPS-induced working memory dysfunction.* Working memory was assessed by alternation in the paddling T-maze. ME7 and NBH animals were challenged with LPS in the presence or absence of the COX-1-specific inhibitor SC-560, given at 30 mg/kg i.p. 60 minutes before LPS. Data were analysed by two-way ANOVA. Bonferroni post-hoc analyses showed that ME7 + LPS + SC-560 differed significantly from other groups at 5 hours, denoted by * $p < 0.05$. ME7 + LPS + SC-560, n = 13; ME7 + SC-560, n = 8; ME7 + LPS, n = 16. All data have been presented as mean ± SEM.

4.3.14 Systemic inflammation induces sickness behaviour and neurodegeneration in the absence of circulating systemic cytokines

4.3.14.1 Systemic administration of dexamethasone blocks LPS-induced hypothermia but not sickness behaviour

We were also interested in other CNS consequences of systemic inflammation during neurodegenerative disease, apart from cognitive changes. To this end, we assessed the ability of systemically administered dexamethasone-21-phosphate, a synthetic glucocorticoid that inhibits cytokine synthesis, to attenuate LPS-induced hypothermia, sickness behaviour and acute neurodegeneration in ME7 and NBH animals, 18 weeks post-inoculation.

Systemic injection of LPS (500 μg/kg) in normal animals induced a clear hypothermic response with respect to NBH + saline animals, while dexamethasone-21-phosphate alone had little effect (Figure 4.24a). Pre-treatment with dexamethasone-21-phosphate completely blocked this LPS-induced hypothermic response in normal animals ($p < 0.001$). The hypothermic response was significantly greater, or exaggerated, in LPS-challenged ME7 animals compared to LPS-challenged NBH animals ($p < 0.01$). It was apparent that ME7 + LPS animals, dexamethasone-21-phosphate was also capable of reversing the hypothermic response ($p < 0.001$). These effects were demonstrated by Bonferroni tests following a one-way ANOVA ($F = 29.35$, df 7, 50, $p < 0.0001$). In addition, subjective assessment of these mice indicated that dexamethasone-21-phosphate significantly inhibited the appearance of sickness signs, such as hunched posture and piloerection.

The number of rears was also very markedly affected by LPS treatment (Figure 4.24 b). LPS induced statistically significant decreases in both NBH and ME7 groups, when compared to NBH + saline ($p < 0.01$) and ME7 + saline groups ($p < 0.05$). Pre-treatment of animals with dexamethasone-21-phosphate before the LPS treatment did not prevent the marked decrease in rears in either diseased or normal animals. That is to say, NBH + LPS animals were not statistically different to NBH + LPS + dex animals, and ME7 + LPS animals were not significantly different to ME7 + LPS + dex animals ($p > 0.05$). Again, these effects were demonstrated by Bonferroni pairwise comparisons following a significant one-way ANOVA ($F = 6.087$, df 7, 30, $p < 0.0002$).

Thus, peripheral administration of dexamethasone-21-phosphate does not protect against decreased locomotor activity, despite protecting against the LPS-induced hypothermic response

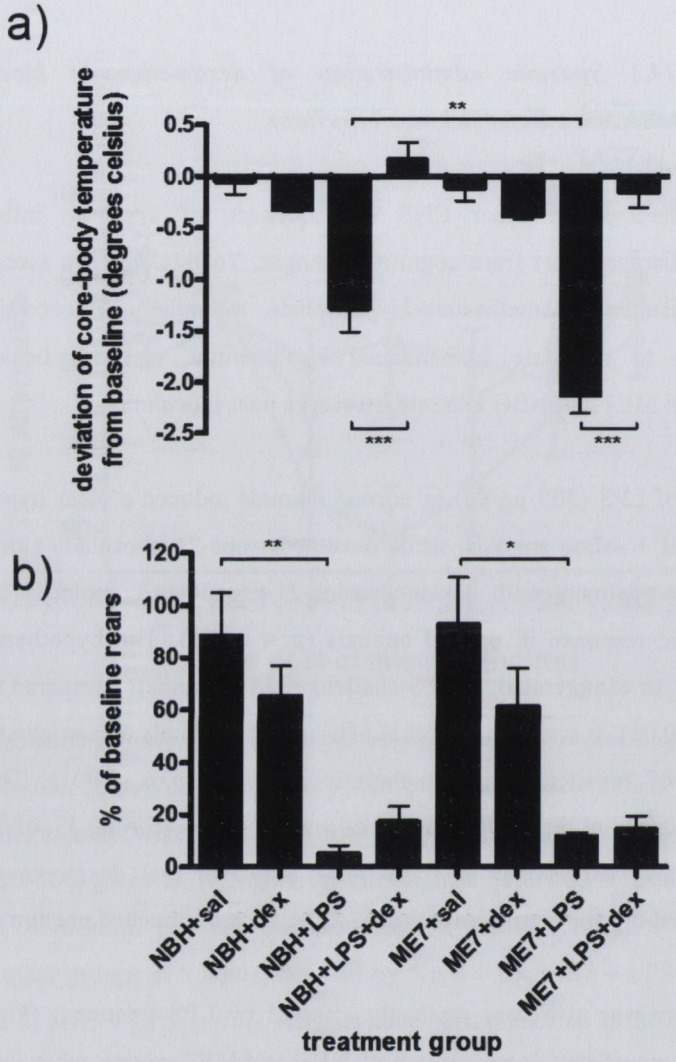

Figure 4.24 *Impact of dexamethasone-21-phosphate on LPS-induced core body temperature and activity changes.* a) The deviation of core body temperature, at 3.5 hours, from baseline (t = 0) in NBH or ME7 animals treated with saline or LPS (500 μg/kg), pre-treated with dexamethasone-21-phosphate (DEX; 2mg/kg) or sterile saline. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ denotes Bonferroni post-hoc test results after a significant one-way ANOVA. n = 6 NBH + LPS, n = 7 ME7 + LPS and n = 5 for all other groups. b) Rearing activity at 2 hours post-challenge with LPS expressed as a percentage of baseline rears (-24 hours). ** $p < 0.01$, *** $p < 0.001$ by Bonferroni post-hoc test after a significant one-way ANOVA. n = 4 for all groups except ME7 + LPS+ dex n=5. All data have been presented as mean ± SEM.

4.3.14.2 Peripherally administrated LPS is detectable in plasma

Ablation of the systemic cytokine response with dexamethasone did not impact on the sickness behaviour response, as measured by reduced rearing. Moreover, parallel experiments in the laboratory showed that CNS cytokines were induced at similar levels despite almost complete ablation of the systemic cytokine response by dexamethasone (Murray et al., 2011).

We wished to investigate the possibility that LPS reaches the blood at measureable concentrations, obviating the need for systemic cytokines to transduce inflammatory signals across the BBB. To this end, we hypothesised that we could label LPS and measure it in plasma after injection. Development of the assay is described in detail in the methods section (Chapter 2). In short, we biotinylated LPS and injected mice with a concentration of 100 μg LPS/kg and then developed a novel ELISA assay with which to detect this in plasma. This was achieved by coating 96-well plates with an anti-biotin antibody and then using streptavidin-HRP to bind directly to the bound biotin. A standard curve was constructed using a serial dilution of biotinylated LPS and the curve showed good linearity in the concentration range 16-1000 pg/ml. Biotinylated LPS was detected at 30, 45 and 60 minutes post-i.p. injection, demonstrating that LPS reaches the blood at this dose. Thus, it is in a position to activate the brain endothelium, independent of circulating cytokines.

Biotinylated LPS levels were not significantly different at the assessed time points (30, 45 and 60 minutes) and, thus, were combined for quantification purposes for an average level of 8.3 ± 1.8 ng LPS/ml of plasma. No signal was detected, using this ELISA, for non-biotinylated LPS or for PBS that was exposed to a biotinylation protocol. In animals treated with biotinylated LPS, we sought to detect this labelled LPS at the cerebral endothelium in the hippocampal area. However, we failed using the ABC method to directly bind peroxidase to biotin. We also attempted to detect it by using an anti-biotin antibody that would allow detection of this biotin followed by the amplification, facilitated by the addition of a secondary antibody, before conjugation of the peroxidase enzyme. Thus, if LPS is bound at the endothelium in these experiments, it is at a level below the detection limit of the methods employed. We also verified that biotinylated LPS is active, thus capable of binding to its receptor complex, by demonstrating induction of TNF-α in plasma in each sample in which LPS was detected in the blood. Average levels were 952 pg/ml, SEM was 746 pg/ml. This high variability stemmed from a particularly high TNF-α level in one animal. Hence, LPS injected intraperitoneally, reaches the blood at detectable levels. Thus, it is available to bind its receptors at the brain endothelium to facilitate signalling directly into the CNS.

4.3.14.3 Systemic administration of dexamethasone does not block LPS-induced apoptotic cell death

To investigate whether the absence of systemic cytokines would impact upon LPS-induced acute neurodegeneration in the hippocampus and thalamus of diseased animals, ME7 and NBH animals, 18 weeks post-inoculation, were challenged with LPS (i.p. 500 μg/kg).

Apoptotic cell death was quantified at 15 hours post-challenge with LPS by counting the number of TUNEL-positive cells in coronal sections (Figure 4.25 b). Evidence of nuclear condensation was also used to confirm apoptosis in cells that were TUNEL-positive (Figure 4.25 a). As previously described (Cunningham et al., 2005), there were very few if any TUNEL-positive cells detectable in NBH animals when compared to the relatively large numbers in ME7 animals at 18 weeks. Bonferroni post-hoc tests, following a significant one-way ANOVA ($F = 8.670$, df 4, 16, $p = 0.0006$) demonstrated that there was a statistically significant increase in TUNEL-positive cells at 15 hours post-LPS ($p < 0.01$) and that this increased apoptosis was not prevented by dexamethasone-21-phopshate pre-treatment ($p > 0.05$).

Thus, acute cell death occurs in the hippocampus and thalamus of diseased animals after systemic LPS challenge independent of systemic cytokine synthesis.

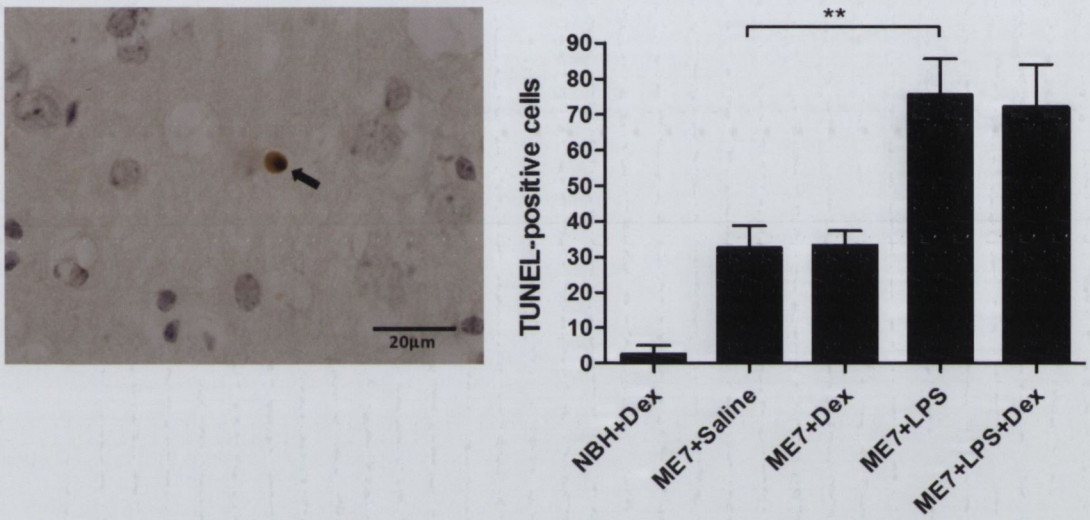

Figure 4.25 *TUNEL-positive apoptotic cell counts.* TUNEL-positive apoptotic cells were counted in the hippocampus and thalamus of 10µm coronal sections from ME7 animals, treated with or without LPS, in the presence or absence of dexamethasone-21-phosphate. NBH animals treated with dexamethasone were also included to demonstrate lower numbers of apoptotic cells in non-diseased animals. Two different coronal sections were counted and summed in each case. Data were analysed by Bonferroni post-hoc test after a significant one-way ANOVA and ** denotes $p < 0.01$. n=4 for ME7 + dex and NBH + dex and n = 5 for all other groups. All data have been presented as mean ± SEM.

4.4 Discussion

Episodes of systemic inflammation in the demented frequently elicit episodes of delirium. In the present study, we have refined and utilised a novel animal model of inflammation-induced delirium during dementia in an attempt to elucidate the inflammatory route to acute cognitive dysfunction in this vulnerable population. Progression of neurodegenerative disease was shown to increase the incidence, severity and duration of inflammation-induced cognitive dysfunction. This increasing vulnerability to acute disturbance of cognition is associated with progressive synaptic loss in the hippocampus. In parallel, there is significant up-regulation of COX-1 in microglia and perivascular macrophages and increased expression of prostaglandin synthases, mPGES-1 and HPGDS, and receptors, EP1 and EP4 as disease progresses. We also observed disease-induced elevation of hippocampal PGE2. Systemic challenge with LPS acutely increased systemic PGE2 but we did not find evidence of acute central elevation of this prostaglandin subtype. Systemic challenge with LPS elicited marked working memory deficits from which COX- 1 but not COX-2 inhibition offered protection. Both systemic and central PGE2 pools were significantly reduced by systemic administration of a COX-1 inhibitor. These data suggest a role for COX-1-dependent prostaglandins in acute cognitive dysfunction (See Table 4.1).

4.4.1 Delirium pathophysiology, cognitive reserve and cognitive decline

While there is a dearth of knowledge regarding the pathophysiology of delirium, it has been consistently shown that conditions with a systemic inflammatory component can trigger delirious episodes (Chang et al., 2008, Kat et al., 2008, Rudolph et al., 2009). Although direct CNS infection can cause delirium in many individuals (McCullers et al., 1999), relatively banal systemic inflammatory events can precipitate delirium in the demented (Inouye and Charpentier, 1996). Relatively mild urinary tract infections do not effect cognition in young healthy individuals but often trigger delirium in elderly (Eriksson et al., 2011) and demented individuals (Manepalli et al., 1990).

Table 4.1 Summary of study on role of prostaglandins in inflammation-induced cognitive dysfunction

Measure	Disease Progression	LPS
Cognitive decline	↑	
Synaptic density	↓	
Inflammation-induced cognitive dysfunction	↑	
Hippocampal PGE2	↑	
Hippocampal mRNA		
COX-1	↑	
COX-2	↑	↑
mPGES-1	↑	↑
mPGES-2		
cPGES		
EP1	↑	
EP2		
EP3		
EP4	↑	
HPGDS	↑	
LPGDS		
DP1		
DP2		↓
PGFS	↑	
PGIS		
Microglial Protein		
COX-1 (+perivascular)	↑	
COX-2 (+endothelial)	↑	↑
HPGDS	↑	

NSAID	LPS-induced dysfunction	Plasma PGE2	Hippocampal PGE2	Plasma Cytokines	Hippocampal Cytokine mRNA
COX-1 Piroxicam, SC-560	↓	↓	↓	–	–
COX-2 Nimesulide	–	–	–	–	–

A notable aspect of one clinical study linking delirium and systemic inflammatory markers is that the association between elevated plasma markers and acute cognitive dysfunction was lost when the data were adjusted for prior cognitive impairment (van Munster et al., 2010). Thus, while mild to moderate systemic inflammation is a common precipitating factor for delirium, it is unlikely to trigger such an event unless it occurs in an individual with some predisposition; putatively this may be pathology in CNS cognitive/attentional circuitry. The clearest empirical evidence that a combination of systemic inflammation and prior pathology induces acute cognitive dysfunction comes from animal studies reported by our laboratory. Murray et al., (2012) showed that systemic inflammation superimposed on neurodegenerative disease in mice elicits acute and reversible impairment of working memory/attention, which mirrors aspects of impairment fundamental to delirium. Saline-challenged diseased animals and LPS-challenged non-diseased animals retained intact performance. Other studies have reported increased vulnerability of aged and demented animals to inflammation-induced acute cognitive impairment. Barrientos and colleagues (2006) showed that an *E. Coli* infection impaired memory consolidation of aged animals, but not young animals, on the contextual fear conditioning (CFC) task. A recent study by this group also demonstrates that the combination of ageing and peripheral inflammation interact to interfere with LTP in the hippocampus (Chapman et al., 2010).

Other studies have shown that mental flexibility or working memory is more disrupted in immune-challenged aged animals, compared to young animals. One study, using the Morris water maze (MWM), demonstrated reduced ability of LPS-treated aged animals to learn the new location of a hidden platform, compared to unchallenged aged animals or LPS-treated younger animals (Chen et al., 2008). Our laboratory has demonstrated that ME7 animals show impairment on learning a Y-maze reference memory task, during systemic inflammation (Cunningham et al., 2009). Thus, when an animal with reduced CNS function (from ageing and neurodegeneration) experiences a systemic inflammatory event cognitive dysfunction can arise. Here, we demonstrate that animals 12 weeks post-inoculation with ME7 develop inflammation-induced cognitive dysfunction while normal animals challenged with LPS do not show working memory impairments. However, LPS-challenged animals 16 weeks into disease are more likely to develop acute cognitive dysfunction than 12 week animals, and the dysfunction that the 16 week animals show is of greater severity and duration. The implication of this finding is that progression of neurodegenerative disease further increases the susceptibility of an already vulnerable individual to acute inflammation-induced disruption of cognitive functioning.

We have previously shown that in neurodegenerative disease there is a more robust CNS inflammatory response to systemic inflammation, as a result of microglial priming (Cunningham et al., 2005). In the current study, amplification of systemic inflammation by the diseased or ageing brain could certainly account for a large component of the increased vulnerability to acute disruption of CNS function. However, normal animals are not prone to inflammation-induced acute cognitive dysfunction even when given an inflammatory challenge that is five times greater than that required to cause dysfunction in diseased animals (Cunningham et al., 2009, Murray et al., 2012). Thus, greater CNS inflammation alone may not be sufficient to induce robust acute cognitive dysfunction, as assessed by the T-maze alternation task. This suggests that additional risk factors in the CNS milieu, common to ageing and neurodegenerative disease, contribute to the increased susceptibility to acute impairment.

A loss of cognitive reserve may explain how the aged and elderly are increasingly vulnerable to disturbances of CNS function. This broad notion conceptualises how the mind, or cognition, is resistant to the effects of pathology or functional loss (Stern, 2009). The cognitive reserve theory attempts to explain why environmental factors, like degree of education and career, and physical factors, for example brain size, complexity, efficiency of function or availability of alternative processes, are associated with lower dementia rates (Valenzuela and Sachdev, 2006, Fotenos et al., 2008). It hypothesises that environmental factors can lead to differences in neural structure that explain oft-observed disparities between disease pathology and dementia symptoms (Satz et al., 2011, Stern, 2009). Synaptic terminals are clearly important in cognitive functioning and their loss represents a tangible decrease of brain reserve (Satz et al., 2011). A number of studies have shown that the magnitude of loss of synapse-associated proteins in the CNS correlates better with cognitive dysfunction in dementia than any other pathological feature, including plaque burden (Morris and Price, 2001, Sze et al., 1997, DeKosky and Scheff, 1990, Terry et al., 1991).

The change in susceptibility to inflammation-induced cognitive dysfunction observed in the current studies was associated with progressive synaptic loss, demonstrated by reduced density of expression of the synaptic protein synaptophysin. Thus, we show that disease-related loss of physical reserve, manifesting as loss of neural connectivity, associates with increased vulnerability to disturbance of cognition. It is worth noting that LPS-challenged ME7 animals do not show additional significant changes in synaptic density with respect to ME7 without LPS. Therefore, the inflammation-induced reversible acute impairments likely result from aberrant function, rather than physical deterioration of elements of the synapse. A small number of studies have discussed the

relationship between delirium and cognitive reserve but such an approach is in its infancy (Jones et al., 2010). A review of neuroimaging studies has reported that increased white matter disease (thus, reduced neuronal connectivity) confers a higher risk for delirium on an individual (Soiza et al., 2008). Educational attainment in hospitalised elderly patients has also been shown to decrease the risk of onset of delirium (Jones et al., 2006).

One implication of the current results is that the occurrence of delirium may be a harbinger of an undiagnosed underlying dementia. This is not to say that delirium will only occur in individuals with prodromal or symptomatic dementia. However, the current data is the clearest empirical data that progressing features of neurodegeneration increase the vulnerability to episodes of acute cognitive dysfunction, such as delirium. Thus, an ostensibly healthy individual who develops delirium during a relatively banal inflammatory event should be assessed subsequently for underlying dementia. Though the dysfunction has been triggered by systemic inflammation in the current study, one would predict that underlying pathology imbues a vulnerability to perturbation by diverse exogenous and endogenous stimuli; for example, changes in medication, stress etc. Importantly, this model generates testable hypotheses that can be assessed in clinical cohorts.

4.4.2 The inflammatory route to delirium

In the current study we sought to investigate key mediators of inflammation-induced cognitive dysfunction during neurodegeneration, utilising our novel animal model of delirium during dementia (Murray et al., 2012). We previously demonstrated that microglia are primed by neurodegenerative changes to show exaggerated pro-inflammatory responses to subsequent systemic inflammatory stimulation (Cunningham et al., 2005). A component of this exaggerated CNS inflammatory response to an acute immune challenge may be a key contributor to acute cognitive dysfunction of systemic inflammation in the aged and demented population. Given that COX-2 is readily induced by inflammatory stimuli in microglia and endothelium (Ek et al., 2001, Uchikado et al., 2004) it has often been the pharmacological target of choice in neuroinflammation research. Here we show that both COX-2 mRNA and protein are increased as a function of disease. Moreover, as has been previously observed (Yamagata et al., 2001, Murray et al., 2011), we show that COX-2 is robustly induced at the brain endothelium in response to systemic LPS challenge. As a result of these observations, we used the COX-2 specific inhibitor nimesulide, with a view to blocking LPS-induced effects on working memory. However; we found no evidence of protection

against LPS-induced cognitive deficits. If anything, COX-2 inhibitor-treated animals showed slightly prolonged impairment. Importantly, the same preparation of nimesulide did afford protection against LPS-induced hypothermia in a subsequent experiment, demonstrating that the drug is physiologically active at the dose and route used.

There are reasons to believe that COX-2 inhibition could in fact be deleterious to CNS function. COX-2 is expressed constitutively in neurons in the brain (Kaufmann et al., 1996, Chen et al., 2002). There is neurophysiological evidence that neuronal PGE2, released from post-synaptic dendritic spines can bind pre-synaptic prostaglandin receptors EP2 and/or EP4 and increase the amplitude of stimulus-evoked excitatory post-synaptic potentials (EPSPs) in the hippocampus (Sang et al., 2005). This work suggests that inhibition of COX-2 activity would be deleterious for memory function. Accordingly, specific inhibition of basal COX-2 activity has been shown to inhibit memory consolidation in Morris MWM experiments (Teather et al., 2002). Later experiments replicated this finding and showed that COX-1 inhibition does not impair memory function (Cowley et al., 2008). In this context, the number of clinical trials that have tested drugs selectively targeting COX-2 is surprising (Aisen et al., 2002, Aisen et al., 2003, Reines et al., 2004, Soininen et al., 2007, Aisen et al., 2008, Leoutsakos et al., 2011, Lyketsos et al., 2007). COX-2 activity appears to have a deleterious role in acute neuroinflammatory situations, such as cerebral ischemia, (Iadecola et al., 2001) and excitotoxic injury (Nakayama et al., 1998). However, we found no evidence for a COX-2 role in inflammation-induced memory impairment during neurodegenerative disease.

From study of the biochemical changes inherent in disease, we also had reason to investigate the role of COX-1 in systemic inflammation-related acute cognitive dysfunction during disease. A disease-related increase in COX-1 transcription was observed and this increased further between 12 and 16 weeks of disease. Expression of the protein was demonstrated on microglial cells, indicating that the enzyme could have a role in acute CNS inflammatory alterations. It has recently become clear that COX-1 is fundamental to the neuroinflammatory response to immune stimuli (Choi et al., 2009). Pharmacological inhibition, or genetic deletion, of COX-1 reduces LPS and β-amyloid-induced neuroinflammation, including reduced levels of NFκB-mediated pro-inflammatory cytokine synthesis and decreased histological evidence of microglial activation (Choi et al., 2008, Choi and Bosetti, 2009). There is also evidence to implicate the biosynthetic enzyme in chronic neuroinflammatory diseases. COX-1 expression is increased in microglia in AD (Hoozemans et al., 2001, Yermakova et al., 1999) and its inhibition has proved beneficial in recent studies of AD in

both mice and humans (Coma et al., 2010, Gomez-Isla et al., 2008). Moreover, the COX-1 inhibitor piroxicam protects against LPS-induced behavioural depression while the COX-2 specific nimesulide does not (Teeling et al., 2010). Our evidence of disease-induced changes in its expression and the aforementioned studies indicate that COX-1 has an important role in facilitating or conveying inflammatory signals during neuroinflammation.

The significant protection offered by the COX-1 inhibitors, piroxicam and SC-560, in the current study indicates that COX-1 has a causative role in LPS-induced cognitive deficits in diseased animals. Piroxicam is a COX-1-selective, rather than specific, drug and the literature is not definitive on its relative selectivity, since tissue preparation, assay method and species all affect its IC_{50} (Mitchell et al., 1995, Warner et al., 1999). However, there is ample evidence that piroxicam shows selectivity towards COX-1 (Blanco et al., 1999, Mitchell et al., 1995, Smith et al., 1994a, Smith et al., 1994b). To demonstrate that inhibition of the COX-1 isoform is sufficient to offer protection, we performed additional experiments with the COX-1-specific inhibitor SC-560, which is reported to be 700 times more active towards COX-1 than towards COX-2 (Smith et al., 1998). SC-560 also significantly attenuated LPS-induced acute cognitive dysfunction in ME7 animals. Taken together, the results from studies with two COX-1 inhibitors, strongly suggest that COX-1-dependent prostaglandins have a causative role in acute inflammation-induced cognitive dysfunction during progressive CNS disease, and these data have relevance for delirium during dementia.

Ostensibly, there are 4 ways in which COX-1 activity could be causing inflammation-induced cognitive impairment.

1. Central PGE2 is acutely elevated and elicits dysfunction
2. Peripheral PGE2 is elevated in plasma and this is sufficient for the effects
3. Central PGE2 is chronically elevated and facilitates dysfunction when other mediators, such as IL-1β are induced by the acute inflammation
4. Another prostanoid such as PGD2 is acutely generated by COX-1 activity to induce dysfunction.

PGE2 has also been implicated in a variety of CNS physiological and pathological processes (Andreasson, 2010). Accordingly, it may be the key prostaglandin subtype downstream of COX-1 activity which contributes to the acute CNS dysfunction demonstrated in the current study. Direct infusion of PGE2 into the brain can induce working memory deficits (Matsumoto et al., 2004). Moreover, IL-1β infused into the hippocampus of mice elicits prostaglandin-dependent impairment

on a fear conditioning task and infusion of PGE2 into the hippocampus is sufficient to induce the memory impairment (Hein et al., 2007). We found evidence of alterations of other key components of PGE2 synthesis and signalling in diseased animals. Increased transcription of mPGES-1, a PGE2 terminal synthase, at the mRNA level in disease and further elevation by LPS challenge was shown; though immunohistochemical analysis proved inconclusive. Typically mPGES-1 couples with COX-2 (Murakami et al., 2003) but there are examples in the literature of co-expression of COX-1 and mPGES-1 (Radilova et al., 2009, Schneider et al., 2004). While mPGES-1 showed some acute elevation, the remaining prostaglandin E synthases, cPGES and mPGES-2, which couple more readily with COX-1 (Murakami et al., 2003, Park et al., 2006), appear to be constitutively expressed in the hippocampus of diseased and normal animals.

We also found evidence of disease-induced elevated mRNA for the PGE2 receptors EP1 and EP4. Increased EP1 has been reported to mediate neurotoxic effects of ischemia and excitotoxicity, via elevation of intracellular Ca^{2+} (Kawano et al., 2006), and to induce vasoconstriction (Ahmad et al., 2006). Given the increased PGE2 and EP1 it is plausible that PGs may contribute to both acute dysfunction and long-term decline observed in animal models post-LPS, and in patients post-delirium (Cunningham et al., 2009, Fong et al., 2009, MacLullich et al., 2009). Consequently, blocking of inflammation-induced deleterious CNS signalling, via this receptor, may be a factor in the protection from dementia progression that is offered by peripheral NSAID administration (McGeer et al., 1996). EP4 receptor has been described to be involved in vasoconstriction (Maubach et al., 2009) and increased wakefulness (Huang et al., 2003). Notably, it also has been shown to have important anti-inflammatory effects after LPS treatment (Shi et al., 2010), and thus may have a role in the return of CNS homeostasis after acute insult.

The current results show that EP3 mRNA is not altered by disease but it is not necessary that a receptor is upregulated for it to be important to acute cognitive impairment. It is already clearly established that EP3 is important in the generation of fever (Ushikubi et al., 1998, Lazarus et al., 2007) but Hein and O'Banion have argued that EP3 could also have a role in acute cognitive dysfunction (2009). Obviously considerably more work needs to be done to directly implicate PGE2 and any of its receptors. Since specific antagonists exist for some of these, there is scope to further investigate these pathways.

While the current results do not allow us to directly implicate PGE2, we do show that hippocampal PGE2 was completely abrogated by peripheral administration of piroxicam, which also protects

against cognitive deficits (Ongoing work in our laboratory suggests that SC-560 also blocks hippocampal PGE2 synthesis). PGE2 was elevated in the hippocampus of ME7 animals compared to NBH but it was not further elevated by systemic LPS administration. LPS-challenged ME7 animals showed a variable response and further work is required to definitively assert whether there was indeed no further increase after acute challenge. As the data currently stand, we cannot find evidence that systemic inflammation induces acute hippocampal elevation of PGE2. It is notable that very few studies in the literature report acute hippocampal elevation of PGE2 after systemic LPS challenge. This may be a result of the short half-life of PGE2. However, we were able to demonstrate a robust disease-induced increase in PGE2 and if a further elevation occurred after secondary challenge, to the degree that it affected cognition, it should then have been quantifiable. One study that reported acute PGE2 elevation after systemic LPS and IL-1β administration used an *ex vivo* method to record hourly changes in hippocampal slices, and demonstrated that the peak levels after LPS and IL-1β challenge were at 6 hours (Weidenfeld et al., 1995). This time course is consistent with the time course of LPS-induced cognitive deficits. As such, future work must consider whether the time point we use to assess brain PGE2 levels (2 hours) is too early to capture later elevation. We must also consider whether our assay has sufficient sensitivity and specificity to capture smaller, more localised acute CNS PGE2 changes.

Chronic IL-1β elevation in the brain inhibits memory consolidation in a COX-1-dependent manner (Matousek et al., 2010). In that study, overexpression of IL-1β in the hippocampus induced increased COX-1 and PGE2, which the authors suggest arose from the increase in the number of microglial cells as result of the chronic inflammation. SC-560 completely abrogated central PGE2 in these animals and protects from impairment on the CFC task. That study, like the present one, does not directly implicate PGE2 as opposed to another COX-1-dependent prostaglandin. Use of EP receptor agonists would be the most elegant demonstration of a causative role for PGE2 signalling. Given the complexity and labour-intensive nature of our cognitive experiments, this was not feasible in the current thesis.

If PGE2 is the prostaglandin subtype which contributes to the working memory deficits, it is unlikely that acute LPS-induced elevation of hippocampal PGE2 is the direct trigger for deficits. One possibility is that cognition is impaired by the presence of disease–induced PGE2 and a second central mediator, synthesised in response to the acute systemic inflammatory event. In this scenario, disease-associated COX-1-mediated PGE2 is a facilitator of cognitive dysfunction rather than the direct trigger. It is of interest that we have shown protection against the acute working

memory deficits induced by LPS using piroxicam, a drug that blocks prostaglandin synthesis but has no effect on either blood or brain cytokine synthesis. Firstly, this indicates that piroxicam is likely acting to inhibit prostaglandin production specifically and not through 'off target' pathways often ascribed to NSAIDs. This suggests that central cytokines could be acting in tandem with COX-1-dependent PGE2. We have previously demonstrated that systemic LPS challenge elicits exaggerated hippocampal IL-1β production in ME7 animal (Cunningham et al., 2005). Thus, it may be that there is a synergistic relationship between acutely elevated central IL-1β and disease-elevated PGE2, which cause transient impairment of cognition. This hypothesis is in broad agreement with the work of Matousek and others (2010), who show that overexpression of hippocampal IL-1β elicits memory impairment in a COX-1-dependent manner. Interestingly, there is evidence that cytokines can modulate the signalling of prostaglandins in the fever response (Nilsberth et al., 2009). Central expression of other cytokines, namely CXCL1 (Campbell et al., 2010) and IL-6 (Sparkman et al., 2006), have also been implicated in inflammation-induced behavioural changes. It could be that centrally synthesised pro-inflammatory cytokines facilitate the signalling of disease-elevated COX-1-dependent prostaglandins to influence neuronal functioning. Conversely, the prostaglandins elevated by disease could alter the ligand-receptor binding of acutely-elevated cytokines, or alter the neuronal response to cytokine signalling. These possibilities are entirely speculative at this point.

A COX-1-positive perivascular macrophage (PVM) population has recently been described and the production of PGE2 by PVMs is thought to play a role in LPS-induced increases in adrenocorticotropin (ACTH) and corticosterone secretion (Elander et al., 2009, Garcia-Bueno et al., 2009). It may be significant that the EP1 receptor is important in the ACTH response to bacterial LPS (Matsuoka et al., 2003) and that it promotes increased dopaminergic activity leading to hyperlocomotion and impulsive behaviour under stress (Kitaoka et al., 2007, Matsuoka et al., 2005). Since HPA activation and hyperdopaminergia are believed to be key facets of delirium (Pearson et al., 2010, Robertsson et al., 2001a) this indicates that prostaglandin perturbations and the increased expression of EP1 observed here may have a role in acute cognitive dysfunction. The current data are consistent with the hypothesis that systemic LPS or IL-1β, acting via PVM COX-1, elicits hypothalamic PGE2, which in turn activates the HPA axis, to produce corticosteroids that interfere with cognitive function. We found a significant elevation of hypothalamic PGE2 in response to systemic LPS in NBH animals, although the response to LPS was more variable in ME7 animals and did not reach significance. However, other work from the laboratory shows that COX-1 inhibition of prostaglandins with SC-560 does not affect plasma corticosterone levels at 2

hours post-LPS. Thus, it seems unlikely that the COX-1-mediated cognitive deficits observed in the current study are mediated by PGE2 activation of the HPA axis. We also show that COX-1 positive PVM cells are present in the hippocampus. Thus it is possible that these cells mediate an interaction between cytokines generated in response to systemic inflammation and COX-1-dependent prostaglandins elevated by disease.

It is also plausible that systemically generated COX-1-mediated PGE2 could cross the blood-brain barrier and act directly upon the vulnerable hippocampus of ME7 animals to elicit dysfunction. We demonstrate that piroxicam treatment at the dose used in the cognitive experiments successfully blocks blood PGE2 elevations. In contrast, we show that nimesulide does not impact on LPS-induced systemic PGE2 levels. These findings are in agreement with previous published studies (Teeling et al., 2010). Subsequent experiments from our group demonstrate that SC-560 also inhibits circulating PGE2 in a similar manner to piroxicam. Together, these results indicate that elevation of systemic PGE2 after LPS challenge is mediated by COX-1. PGE2 generated in the periphery during systemic inflammation have been demonstrated to cross the BBB and signal centrally (Abul et al., 1997, Romanovsky et al., 1999). Another study showed, by careful assay, that peripheral PGE2 enters the brain, probably via the third ventricle (Davidson et al., 2001). However, if systemically generated PGE2 crossed into the brain at levels sufficient to contribute to the deficits, one might expect that these levels would have accumulated in the hippocampal PGE2 pool, after LPS administration and have been measureable in the hippocampal PGE2 assays. There is also the possibility that peripheral COX-1-dependent prostaglandins alter cerebral blood flow during systemic inflammation and impart dysfunction without crossing the BBB. Reversible cerebral vasoconstriction syndrome has recently been associated with transient cognitive impairment (Ducros et al., 2007) and hypoperfusion may be an important feature during delirium (Meagher et al., 2007). COX-1 plays a role in peripheral endothelial dysfunction and pathology (Vanhoutte, 2009) and has been shown to be vital in maintenance of cerebral vascular tone (Niwa et al., 2001). Additionally, the EP1 (Ahmad et al., 2006) and EP4 (Maubach et al., 2009) receptors have been implicated in deleterious cerebral vasoconstriction. Thus, peripheral COX-1-dependent PGE2 could plausibly affect cognition, via vasoconstriction, during systemic inflammation and cause dysfunction in the diseased brain in this manner. Such a possibility merits further consideration in future studies.

While COX-1-induced PGE2 may indeed act on EP receptors to elicit observed cognitive impairments, there are also receptors for PGD2, PGI2, and PGF2α in the CNS (Bosetti, 2007). Their roles in neuroinflammation have not been studied to any great degree. In the current model, blockade of their synthesis with COX-1 inhibition could contribute to protection from acute cognitive dysfunction observed. We show that HPGDS, argued to be important for inflammation-induced PGD2 (Rajakariar et al., 2007), is expressed in microglia. It is known that HPGDS can couple with COX-1 (Mohri et al., 2007) and this perhaps makes it a more plausible partner for the observed microglial COX-1 observed here. Consequently, cells capable of producing PGD2 are increasing in the diseased brain. We also show evidence of transcription of its two cognate receptors, DP1 and DP1, in both diseased and normal animals, although DP2 does decrease with LPS treatment. PGD2 is known to be the primary mediator of vasodilatation and permeability (Cheng et al., 2006). It is known to be the most common prostaglandin in the CNS and has been shown to play an important role in sleep (Hayaishi, 2000). However, there is a surprising lack of research on its other functions in the CNS. In recent years, it has been shown to have an important role in microglial activation and neuroinflammation (Mohri et al., 2006). In that study, microglial HPGDS and astrocytic DP1 were found to be upregulated in a genetic demyelination animal model. Elevated PGD2, produced by activated microglia was shown to induce astrogliosis and apoptosis in oligodendrocyte apoptosis. Prostaglandin D synthase and the receptor DP1 also show increased expression in AD brains and in the Tg2576 AD model (Mohri et al., 2007). Recent evidence also demonstrates a role for peripheral HPGDS-dependent PGD2 in LPS-generated fever (Gao et al., 2009). Thus, both peripheral and central PGD2 have been implicated in neuro-immune interactions and we provide evidence for disease-related elevation of HPGDS, which can couple readily with COX-1. Ongoing work in the laboratory is assessing whether PGD2 is elevated by either, or both, disease and acute systemic inflammatory challenge and whether it is affected by COX-1 inhibition.

4.4.3 The contribution of systemic cytokines to LPS-induced acute CNS responses in neurodegenerative disease.

It is important to stress the negative long-term outcomes for those suffering episodes of delirium. Risks for long-term cognitive decline, conversion to, and acceleration of, dementia increase and times to institutionalisation and death shorten (see (Witlox et al., 2010) for meta-analysis). While delirium impacts negatively on progression of dementia (Fong et al., 2009, MacLullich et al., 2009, Witlox et al., 2010), it has also been shown that systemic inflammation has deleterious consequences for decline even in the absence of delirium (Holmes et al., 2009). This suggests that an inflammatory insult is sufficient to trigger accelerated decline in those with underlying CNS

pathology. In the ME7 model, we have shown that repeated systemic inflammatory challenges, on a background of progressing neurodegenerative disease, induce acute functional impairments, which are more severe and irreversible as disease progresses (Field et al., 2010). It is apparent that understanding the factors in disease, which increase susceptibility to delirium and other acute exacerbations of dysfunction, might shed light on how systemic insults contribute to the pathological burden of neurodegenerative disease (Cunningham, 2011).

In the current chapter, we show that systemically administered dexamethasone-21-phopshate (2 mg/kg), which is sufficient to block over 80% of systemic LPS-induced IL-1β and IL-6 (demonstrated in experiments by others in the laboratory and published with the current data in (Murray et al., 2011)), can completely block LPS-(500 μg/kg) induced hypothermia in normal animals. Dexamethasone-21-phosphate also protects from the exaggerated LPS-induced hypothermic response in diseased animals. However, this dose cannot block the LPS-induced decrease in open field rearing behaviour. Moreover, it does not affect LPS-induced cell death occurring in the hippocampus of diseased animals. Thus, LPS elicits a deleterious inflammatory response in the brain, which is not inhibited by dexamethasone and is, thus, independent of circulating cytokines.

The synthetic glucocorticoid dexamethasone-21-phosphate shows the classical steroidal anti-inflammatory characteristics in blocking systemic cytokine production. The fact that it reduced systemic cytokines by over 80% clearly demonstrates that administered drug was converted to its active metabolite dexamethasone. Although the mechanisms of dexamethasone cytokine inhibition are still incompletely understood, it is thought to block NFκB-dependent gene transcription by direct transrepression (Adcock et al., 1999).

As has been previously described, hypothermia was exaggerated in ME7 animals with respect to NBH animals given the same LPS challenge (Combrinck et al., 2002). The marked suppression of NFκB-dependent systemic cytokines, with dexamethasone-21-phosphate, was sufficient to block the hypothermic response in both groups. The results are consistent with studies which demonstrate an important role for systemic cytokines in an inflammation-induced temperature response (Teeling et al., 2010, Chai et al., 1996, Wang et al., 1997). As discussed in detail in previous chapters (Chapters 1 and 3), IL-1β and IL-6 have been strongly associated with the induction of temperature changes in rodents, in response to systemic LPS challenge. Systemic IL-1β is thought to be important in the induction of hypothalamic IL-6 and the subsequent induction of temperature

alterations (Chai et al., 1996). In the previous chapter (chapter 3) we demonstrated that systemic IL-1β itself can induce a hypothermic response and elevated hippocampal IL-6 transcription. In that chapter, we failed to show that systemic IL-6 can induce hypothermia or CNS inflammatory transcription but it is strongly implicated in the facilitation of the effects of other inflammatory mediators to induce a temperature response (Harden et al., 2006, Nilsberth et al., 2009). Hence, our current results are consistent with other findings that demonstrate the role of systemic cytokine signalling in the temperature response to peripheral LPS challenge.

It is notable that there is dissociation between the hypothermic response and the behavioural depression after systemic LPS administration. Despite the robust effect of dexamethasone-21-phosphate on hypothermia, exploratory behaviour, as measured by rearing activity, was not protected by administration of this drug. CNS cytokines were also not inhibited, despite the effective blocking of systemic cytokine synthesis. These results indicate that systemic cytokine elevation is required for some systemic inflammation-induced alterations in CNS function but not others. In the current study, behavioural depression after LPS administration appears to be independent of systemic cytokines. These data are consistent with recent findings from our group and collaborators, which show that systemic administration of specific antibodies against the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α does not block LPS-induced inhibition of burrowing at low (1μg/kg) and moderate (100 μg/kg) doses of LPS (Teeling et al., 2007).

It is likely that dexamethasone did not penetrate the hippocampus at significant concentrations. The intact CNS transcription and synthesis of cytokine genes that are also NFκB-dependent (also demonstrated in experiments by others in our group (Murray et al., 2011) indicates that dexamethasone is either not effective in the CNS or does not reach the CNS at sufficient concentrations to exert effects. There is evidence from previous studies of CNS injury to show that dexamethasone cannot block CNS cytokine transcription (Bruccoleri et al., 1999). It is reported to penetrate the brain in much lower quantities than endogenous glucocorticoids (Ronald De Koloet, 1997). Thus, the behavioural change could be driven by central cytokine elevation. Central administration of cytokines is well documented to elicit sickness behaviour (Dantzer, 2004). However Teeling et al., (2010) report that administration of the non-selective NSAIDs indomethacin and ibuprofen to normal animals offers protection from LPS-induced reductions in open field activity and burrowing, suggesting a fundamental role for prostaglandins in behavioural depression. Accordingly, there is evidence to suggest that both central cytokines and prostaglandins are important in inflammation-induced behaviour responses in normal animals. It is interesting to

return to the evidence in this chapter which suggests that both central cytokines and prostaglandins are fundamental to systemic inflammation-induced cognitive impairment in diseased animals. This implication of central cytokines and prostaglandins supports the argument that inflammation-induced delirium during dementia represents a maladaptive or dysregulated sickness behaviour response. (Cunningham and Maclullich, In Press).

In the current studies, we also demonstrate that administration of systemic LPS induces acute cell death in the hippocampus of diseased animals. The numbers of apoptotic cells reported in the current study are in agreement with those originally observed by our group using this experimental paradigm. In that previous study, we demonstrated that the apoptotic cells were neuronal, by double labelling cells with antibodies against cleaved caspase-3 and neurofilament heavy chain (Cunningham et al., 2005). Thus, persistence of neuronal death, despite the administration of dexamethasone, suggests that systemic cytokines are not necessary for the degenerative effects. Given that central production of cytokines is intact in these animals, CNS IL-1β (Cunningham et al., 2005) could be eliciting neuronal death. Central IL-1β expression has been implicated in neuronal injury and death (Allan et al., 2005). It has also been shown to worsen underlying CNS pathology. In an animal model of Parkinson's disease centrally administered LPS exacerbated neuronal death in an IL-1-and iNOS-dependent fashion (Godoy et al., 2008). The authors show that a second central immune challenge in the degenerating substantia nigra induces increased neurodegeneration. Importantly it was demonstrated that central IL-1RA overexpression decreased neurodegeneration and IL-1β exerts its neurodegenerative effects in a manner that is partially dependent on nitric oxide expression. As noted previously, central IL-1β exerts cognitive dysfunction in a COX-1-dependent manner (Matousek et al., 2010). Future work should consider the important question as to whether central cytokines and prostaglandins also contribute to systemic inflammation-induced cell death during ongoing neurodegenerative disease. If one could show that acute inflammation-induced cognitive changes and neurodegeneration are driven by interaction of the same inflammatory mediators (central cytokines and disease-elevated COX-1 prostaglandins), it would represent a breakthrough of genuine importance for ongoing research on both delirium and dementia. Given that COX-2 activity appears to have a role in acute neurodegeneration in cerebral ischemia, (Iadecola et al., 2001) and excitotoxic injury (Nakayama et al., 1998), it may also have a role in the neurodegeneration observed.

Since we have successfully detected circulating biotinylated LPS, one might argue that the neuronal loss is occurring as a consequence of sepsis/toxic shock. It is well known that severe

sepsis has considerable consequences for CNS integrity (Semmler et al., 2008). However, the LPS doses used in those studies of sepsis in rodents are considerably higher (20-fold) than those in use here. Using this dose of 500 μg/kg LPS in a previous study, we did not observe infiltration of inflammatory cells to the brain parenchyma or any significant apoptosis in the normal brain of LPS-challenged animals (Cunningham et al., 2005).

There are other routes by which LPS could be inducing CNS inflammation, behavioural depression and neurodegeneration independent of systemic cytokines (such routes have been discussed in detail in chapter 1). Systemic LPS is capable of directly activating the vagal nerve (Hosoi et al., 2005). Though the nucleus tractus solitarius (NTS), the neuronal body to which vagal afferents signal, does not directly innervate the hippocampus, multisynaptic relays from the NTS to the hippocampus have been identified (Castle et al., 2005). However, it would seem unlikely that vagal afferents induce the CNS changes observed in the present study. Vagal afferents also project, via the NTS, to the hypothalamus (Ricardo and Tongju Koh, 1978), yet we see marked inhibition of hypothalamic activation in the presence of dexamethasone-21-phosphate. Thus, the vagal afferent signalling is not sufficient to induce hypothermic changes in the absence of systemic cytokines. Accordingly, it would be surprising if the vagal afferents were sufficient to elicit hippocampal inflammation and neurodegeneration.

It is possible that direct interaction between LPS and the brain endothelium might best explain the CNS cytokine transcription, behavioural changes and acute neurodegeneration, which we show are independent of systemic cytokine synthesis. Recent data indicate that LPS can interact directly with brain endothelial cells in vitro (Singh and Jiang, 2004). Work by Banks and Robinson, suggests that LPS minimally penetrates the brain but does interact with the CNS endothelium (2010). Chakravarty & Herkenham (2005) have demonstrated that deletion of the TLR4 gene in peripheral cells is not sufficient to block CNS transcription of inflammatory genes, indicating that TLR4 expression at the brain endothelium can effect blood to brain signalling transduction. Data published previously by our group also supports the argument that LPS signals at the brain endothelium. The pattern and time course of gene induction in our own studies (Murray et al., 2012) are more consistent with LPS signalling at the endothelium than the signalling actions of LPS-induced systemic cytokines, which first must be produced by tissue macrophages or circulating cells. Moreover, in the same study we reported central IFN-β transcription by systemic LPS. This most likely resulted from direct stimulation of the endothelial cell TLR4 MyD88-independent pathway by LPS (Kawai et al., 2001). However, proinflammatory cytokines do not

elicit IRF3 phosphorylation or IRF3-dependent gene transcription (Clark et al., 2009, Clark et al., 2011), which are fundamental to IFN-β gene induction (Colonna, 2007). Accordingly, in chapter 3 we demonstrated that systemic IL-1β and TNF-α do not elicit a peripheral or central type-1 IFN response.

Here we have labelled LPS using biotin and have clearly shown that LPS is present in the bloodstream. Given that it reaches the bloodstream, LPS could directly induce apoptotic signalling cascades in the CNS which could explain the cell death, independent of systemic cytokines. Moreover, we observed LPS as soon as 30 minutes after intraperitoneal injection, thus, it would be present sufficiently early to induce the central cytokine patterns and reduced locomotor activity observed. Attempts to locate biotinylated LPS at the brain endothelium proved unsuccessful, thus we failed to demonstrate LPS bound to cells of the brain vasculature. It may be tempting to speculate that excessive biotinylation might prevent ligand-receptor binding between LPS and the TLR4 receptor complex. However, we also found that animals injected with the biotinylated form of LPS produce an intact TNF-α response. Consequently, the biotinylated LPS is biologically active. The failure to locate LPS at the endothelium could instead be explained by the low level expression of TLR4 and LPS binding proteins at the brain endothelium, or indeed the relatively low levels of LPS in the circulation.

4.4.4 Summary

In summary, it is now apparent that systemic inflammation has significant deleterious effects on the progression of neurodegenerative disease and understanding the mechanisms by which this occurs is an important priority. To this point, we have shown that systemic cytokines and prostaglandins are important in neuro-immune interactions during systemic inflammation. However, with the current data we demonstrate that the presence of systemic bacterial endotoxins is sufficient to exert deleterious CNS actions in animals with neurodegeneration. In the current chapter, we also show that progression of disease increases the incidence, severity and duration of inflammation-induced cognitive dysfunction. This increased susceptibility to cognitive impairment is associated with synaptic loss and alterations in the CNS prostaglandin system. Systemic administration of a COX-1 but not COX-2 inhibitor offers protection from working memory deficits. Thus, COX-1-dependent prostaglandins have an important role in systemic inflammation-induced cognitive dysfunction during progressing neurodegenerative disease.

Chapter 5: Systemic IL-1β contributes to cognitive dysfunction in an animal model of delirium during dementia

The work contained in this chapter forms the majority of a manuscript currently under review at *Journal of Neuroscience*

5.1 Summary

Delirium, the acute and severe disruption of facets of cognition and behaviour, is a highly prevalent neuropsychiatric syndrome, particularly in dementia patients. While systemic infection is a widely accepted risk factor for delirium, sterile systemic inflammation, resulting from trauma or surgery, can also interact with chronic neurodegeneration to induce delirium. IL-1β is a fundamental mediator of both aseptic and septic inflammation. In the present study, we investigate the role of IL-1β in inflammation-induced acute cognitive dysfunction, assessed with a novel water-based T-maze task, in the progressive neurodegenerative ME7 murine model of prion disease. LPS (100 μg/kg i.p.) induces acute cognitive dysfunction in ME7 animals but not in normal animals. In comparison to LPS-challenged normal animals, LPS-challenged ME7 animals show elevated hippocampal IL-1β mRNA but equivalent systemic IL-1β levels. IL-1 Receptor 1 knockout (IL-1RI-/-) animals inoculated with ME7 disease are still prone to profound cognitive impairment after systemic LPS treatment, indicating that IL-1RI is not essential for LPS-induced cognitive dysfunction. Dexamethasone (2 mg/kg i.p.) administration offers significant protection to IL-1RI-/-ME7 animals, which demonstrates that circulating cytokines have an important role in LPS-elicited impairment in these animals. Simultaneous treatment of ME7 animals with IL-1RA (i.p. 10 mg/kg) offers significant protection from LPS-induced acute cognitive impairment. Systemic injection of IL-1β (i.p. 15 μg/kg) is sufficient to induce acute and transient cognitive impairment, and exaggerated hippocampal IL-1β mRNA expression, in ME7 but not normal animals. These results suggest that systemic IL-1β can mediate and elicit inflammation-induced acute episodes of cognitive dysfunction in animals with prior pathology, and have important implications for understanding the mechanisms by which both septic and aseptic inflammation can interact with and exacerbate dementia.

5.2 Introduction

Delirium, the profound and sudden onset of brain dysfunction, is associated with an increased risk of cognitive decline (Jackson et al., 2004, MacLullich et al., 2009), dementia (Fong et al., 2009), institutionalisation and death (Witlox et al., 2010). Despite these stark facts, tailored therapeutic interventions for vulnerable populations are lacking (Skrobik, 2010). Peripheral infections are known to trigger episodes of delirium, particularly in the elderly and demented (George et al., 1997, Elie et al., 1998, Chang et al., 2008). Sterile systemic inflammation, resulting from tissue trauma or surgery, can also induce delirium and associated cognitive decline (Kat et al., 2008, van Munster et al., 2008). While the mechanisms have not been elucidated, there are likely to be unifying mediators of infection- and sterile inflammation-induced delirium, which could offer novel effective pharmacological targets.

Cytokines are key mediators of septic and aseptic inflammation and their elevation in serum has been associated with delirium (Beloosesky et al., 2007) and cognitive decline (Yaffe et al., 2003), particularly in populations with prior cognitive impairment (van Munster et al., 2010, Holmes et al., 2009). IL-1β is a potent inflammatory mediator (Dinarello, 1996, Martinon et al., 2006) and systemic blockade of its activity abrogates symptoms in numerous acute inflammatory diseases (Dinarello, 2005, Church et al., 2008). Increased systemic IL-1β is associated with subsequent development of Alzheimer's disease (AD) (Tan et al., 2007) and acceleration of cognitive decline in AD patients (Holmes et al., 2003).

Maze and colleagues have implicated IL-1 in a model of postoperative cognitive dysfunction (POCD) using a fear conditioning task. IL-1RA-treated animals were protected from systemic LPS- and tibial fracture-induced inflammation and memory impairments (Terrando et al., 2010b, Cibelli et al., 2010). However, whether systemic IL-1β alone is sufficient to induce memory impairment was not examined in the above studies. These studies demonstrate that relatively severe surgical trauma impairs the ability of rodents to consolidate a new memory, but demonstrating the profound working memory dysfunction and attentional deficits fundamental to episodes of delirium (WHO, 1992, APA, 1994) requires cognitive assessment that is dynamic and ethologically valid.

Our group has previously developed a novel animal model of inflammation induced-delirium during dementia (Murray et al., 2012). The model recapitulates a frequent biological context within which delirium is seen by superimposing a mimetic of mild to moderate systemic infection (i.p.

LPS 100 μg/kg) on a background of CNS neurodegeneration (ME7 strain of prion disease). The T-maze alternation task utilised assesses the acute onset of impairments in facets of cognition, working memory and attention, across time after a systemic inflammatory challenge. While many learning and memory tasks are confounded by sickness behaviour and reduced motivation elicited by inflammatory challenge (Cunningham and Sanderson, 2008), the water-based T-maze task is also less sensitive to these confounding factors since it is based on correct and incorrect responses only. Using this task our laboratory has shown that LPS induces acute and reversible hippocampal mediated-cognitive dysfunction in animals at 12 weeks post-ME7 inoculation, but not in normal animals.

IL-1β elevation in the periphery elicits systemic and central inflammation, and sickness behaviour broadly similar to LPS (Dantzer et al., 2008, Gosselin and Rivest, 2008)(Chapter 3) and circulating IL-1β is elevated in LPS-challenged animals. Thus, we hypothesised that systemic IL-1 signalling contributes to LPS-induced cognitive impairment in animals with ongoing neurodegeneration. In the current study we establish that LPS-induced acute cognitive impairment in diseased animals is accompanied by systemic IL-1β elevation equivalent to that seen in normal animals but exaggerated hippocampal IL-1β transcription. To investigate the contribution of systemic IL-1β, T-maze performance was assessed after 1) ME7-inoculated IL-1RI-/- animals were challenged with systemic LPS and 2) ME7-inoculated animals were injected concomitantly with systemic IL-1RA (i.p. 10 mg/kg) and LPS (100 μg/kg). We also hypothesised that systemic challenge with IL-1β would induce delirium like-acute and reversible cognitive impairment in ME7 animals. To this end, the impact of systemic IL-1β (i.p.15 μg/kg) challenge on ME7 and NBH control animal's performance on the T-maze task was investigated.

Systemic LPS challenge elicits exaggerated sickness behaviour in ME7 animals (Combrinck et al., 2002, Cunningham et al., 2009) and systemic IL-1β replicates much of the sickness behaviour elicited by LPS in normal animals (Chapter 3) (Dantzer et al., 2008). In light of these findings, we also assess whether there are exaggerated sickness behaviour and hypothalamic inflammatory responses to systemic IL-1β in diseased animals. Peripheral LPS challenge also elicits normal systemic but exaggerated hippocampal inflammation in diseased animals (Cunningham et al., 2005). Moreover, hippocampal inflammation has been directly associated with cognitive dysfunction (Frank et al., 2010, Barrientos et al., 2009, Pugh et al., 1998). Whether systemic IL-1β challenge induces exaggerated hippocampal inflammation in ME7 animals is currently unclear.

Consequently, the acute systemic and hippocampal inflammatory responses to peripheral IL-1β were measured in normal animals and diseased animals.

In summary, this chapter aims to investigate the role of IL-1β in the inflammation-induced memory deficits in diseased animals previously shown in this thesis.

5.3 Results

5.3.1 LPS-induced acute cognitive dysfunction is accompanied by exaggerated hippocampal IL-β transcription in diseased animals compared to normal animals, despite equivalent circulating IL-β

As reported in the previous chapter, systemic challenge with LPS (100 μg/kg i.p.) produces episodes of greater acute cognitive dysfunction in 16-week ME7 mice than 12-week ME7 animals as shown using the T-maze alternation task. In the present experiment (Figure 5.1 a) ME7 animals and control animals, 16-weeks post-inoculation, were injected peripherally with LPS or saline and T-maze performance was assessed from 3 to 9 hours post-challenge. ME7 animals challenged with LPS showed an acute impairment in working memory in the blocks of 5 trials at 5 and 7 hours post-challenge compared to saline-treated ME7 animals and in the block of 5 at 5 hours compared to normal animals challenged with LPS. Repeated measures ANOVA showed no significant interaction between treatment and time ($F = 1.10$, df 18, 156, $p = 0.3554$), or main effect of treatment ($F = 1.36$, df 3, 26, $p = 0.2329$). There was, however, a significant main effect of time ($F = 5.95$, df 6, 156, $p = 0.0007$). Bonferroni post-hoc tests demonstrated a significant difference in errors made between ME7 + LPS and ME7 + saline at 5 hours ($p < 0.05$) and 7 hours ($p < 0.05$). Performance of ME7 + LPS animals also differed significantly from NBH+LPS animals at 5 hours ($p < 0.001$).

The impact of peripheral LPS and saline challenge (100 μg/kg) on systemic IL-1β synthesis and hippocampal IL-1β transcription in 16-week ME7 and NBH animals at 2 hours post challenge was examined. Circulating IL-1β was increased by systemic LPS administration but there was no difference between levels induced in NBH and ME7 animals (Figure 5.1 b). Two-way ANOVA revealed a main effect of LPS challenge on circulating IL-1β ($F = 60.06$, df 1, 12, $p < 0.0001$) but there was no interaction between treatment and disease ($F = 0.32$, df 1, 12, $p = 0.5830$) and no effect of disease ($F = 0.32$, df 1, 12, $p = 0.5830$). Bonferroni post-hoc tests showed that systemic LPS administration elicited a significant systemic increase in IL-1β in ME7 animals ($p < 0.001$) and NBH animals ($p < 0.001$) in comparison to their respective saline controls. LPS treatment did not induce a higher systemic IL-1Rβ response in ME7 animals compared to NBH animals ($p > 0.05$).

ME7 animals systemically challenged with LPS showed greater increases in hippocampal IL-1β mRNA than similarly challenged NBH animals (Figure 5.1 c). Two-way ANOVA demonstrated a significant interaction of treatment and disease ($F = 8.057$, df 1, 14, $p = 0.0131$) and main effects of treatment ($F = 20.34$, df 1, 14, $p = 0.0005$) and disease ($F = 19.96$, df 1, 14, $p = 0.0005$). Bonferroni post-hoc tests indicated that the interaction was driven by a robust induction of IL-1β in ME7 but not NBH animals after systemic LPS injection. LPS-treated ME7 animals differed significantly from saline-treated ME7 ($p < 0.001$) and LPS-treated NBH animals ($p < 0.001$). Therefore, systemic LPS challenge induces an amplified IL-1β mRNA response in ME7 animals compared to NBH.

Thus, the acute cognitive impairment observed in 16-week ME7 animals following systemic LPS challenge is accompanied by acute exaggerated hippocampal IL-1β transcription but equivalent plasma IL-1β levels compared to LPS-challenged NBH animals.

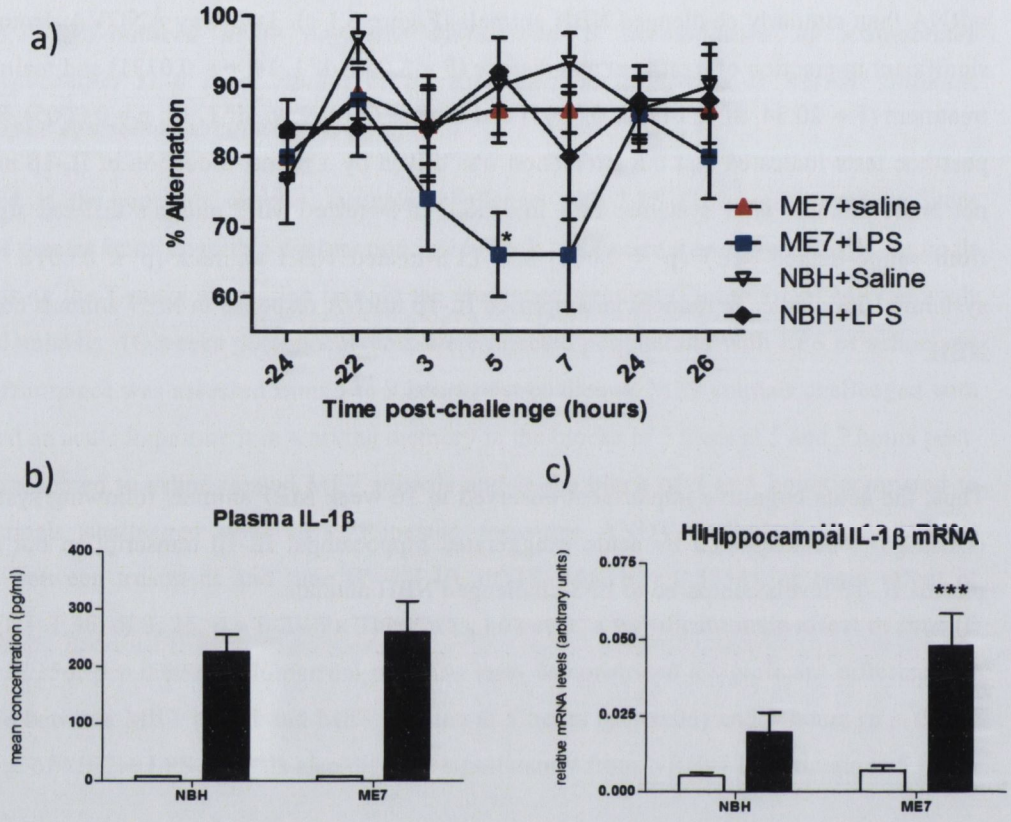

Figure 5.1 *LPS-induced acute cognitive dysfunction is accompanied by exaggerated hippocampal IL-1β transcription in diseased animals compared to normal animals, despite equivalent circulating IL-1β.* a) Working memory performance of NBH and ME7 animals 16 weeks post-ME7-inoculation, challenged with LPS (100 μg/kg i.p.) or saline, assessed by T-maze alternation for 10 trials 24 hours pre-challenge, 15 trials 3-9 hours post-challenge and 10 trials 24 hours post-challenge. Data was analysed with repeated measures ANOVA and significant Bonferroni post-hoc difference between ME7 + LPS and NBH + LPS is denoted by *$p < 0.05$. ME7 + LPS n = 10, ME7 + saline n = 9, NBH + LPS n = 6 and NBH + saline n = 5. b) Plasma levels of IL-1β, measured by ELISA, from 16-week ME7 and NBH animals 2 hours post-LPS treatment. Bonferroni post-hoc test, after two ANOVA, showed no significant difference between ME7 +LPS and NBH +LPS ($p > 0.05$). All groups n = 4. c) Hippocampal transcription of IL-1β in 16-week ME7 and NBH animals, 2 hours post-LPS treatment. *** denotes a significant Bonferroni post-hoc difference, following two-way ANOVA, between ME7 + LPS and NBH + LPS groups ($p < 0.001$). All groups n = 7 except NBH + LPS n = 5. All data have been presented as mean ± SEM.

5.3.2 IL-1R1 is not necessary for LPS-induced acute working memory deficits in ME7 animals but blockade of systemic cytokines affords protection

Experiment performed by Carol Murray. To investigate the contribution of IL-1 signalling to LPS-induced episodes of delirium during dementia, IL-1R1-/- animals, 16 weeks post–ME7 inoculation, in the presence or absence of systemic LPS (100 μg/kg i.p.), were compared with LPS-challenged wild type ME7 animals in terms of T-maze alternation performance (Figure 5.2 a). IL-1R1-/- ME7 animals were vulnerable to LPS-induced acute cognitive dysfunction and showed the same acute-onset working memory deficits after LPS challenge as wild type ME7 animals. A repeated measures two-way ANOVA showed an interaction between treatment and time ($F = 2.123$, df 12, 216, $p = 0.0162$) and main effects of treatment ($F = 16.61$, df 2, 36, $p < 0.0001$) and time ($F = 7.193$, df 6, 216, $p < 0.0001$). Bonferroni post-hoc tests demonstrated a significant difference between IL-1R1-/- ME7 + LPS animals and IL-1R1-/- ME7 + saline animals at 3 hours ($p < 0.01$) and 7 hours ($p < 0.05$). No significant differences between IL-1R1-/- ME7 + LPS animals and wild type ME7 + LPS animals were found. These results indicate that IL-1RI activation is not essential for LPS-induced acute working memory deficits in ME7-inoculated animals.

Since other pro-inflammatory cytokines are reported to compensate for loss of IL-1 signalling in IL-1RI-/- mice (Bluthe et al., 2000) we investigated whether systemic cytokine blockade using dexamethasone could protect from the above LPS-induced cognitive dysfunction. T-maze performance of IL-1R1-/- animals, 16 weeks post–ME7 inoculation, in the presence or absence of LPS (100 μg/kg i.p.) and dexamethasone-21-phosphate treatment (2 mg/kg) were compared (Figure 5.2 b). A repeated measures 2-way ANOVA demonstrated no significant interaction between treatment and time ($F = 1.76$, df 12, 174, $p = 0.0587$). Main effects of treatment ($F = 8.47$, df 2, 29, $p = 0.0013$) and time ($F = 5.38$, df 6, 174, $p = 0.0001$) were revealed. IL-1R1-/- ME7 animals showed acute onset cognitive dysfunction after systemic LPS challenge. Bonferroni post-hoc tests revealed no difference between IL-1R1-/- ME7 animals pre-treated with dexamethasone-21-phosphate 60 minutes prior to LPS challenge and animals challenged with LPS alone ($p > 0.05$), However, a two-way ANOVA comparing only these two groups revealed a significant main effect of treatment ($F = 7.271$, df 1, 17, $p < 0.0153$) indicating that dexamethasone-21-phosphate administration has a significant effect across the time course. It can be seen from the Figure 5.2b that any protection offered by steroid treatment had passed by 7-hours post-challenge. These results demonstrate that blockade of systemic cytokines confers on ME7-inoculated IL-1R1-/- animals time-dependent protection from LPS-induced acute cognitive impairment.

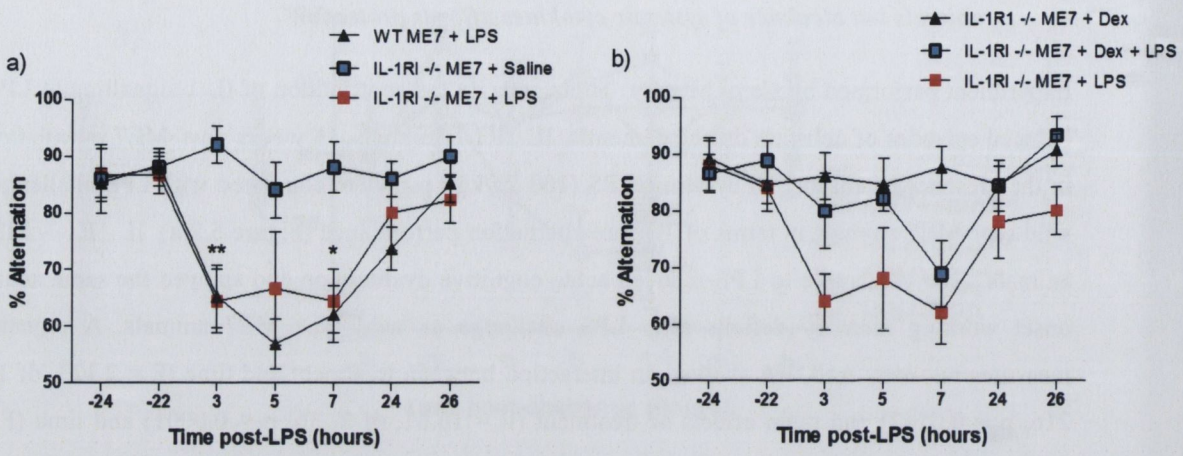

Figure 5.2 *IL-1R1 is not essential for LPS-induced acute working memory deficits in ME7 animals but blockade of systemic cytokines affords protection* (Experiment performed by Carol Murray). a) Working memory performance of IL-1RI-/- animals and wild type ME7 animals 16 weeks post-ME7 inoculation, in the presence or absence of systemic LPS challenge (100 μg/kg i.p.), was assessed by T-maze alternation. All animals were assessed for 10 trials 24 hours before acute challenge, 15 trials 3-9 hours post-challenge and 10 trials 24 hours after the challenge. A significant interaction between treatment and time and significant main effects of both treatment and time were demonstrated by repeated measures ANOVA ($p < 0.01$). Subsequent significant post-hoc differences between IL-1R1-/- ME7 + LPS and IL-1R1-/- ME7 + saline are denoted by $*p < 0.05$ and $**p < 0.01$. IL-1R1-/- ME7 + LPS n = 10, IL-1R1-/- ME7 + saline n = 10 and wild type ME7 + LPS n = 19. b) Working memory performance, assessed by T-maze alternation, of IL-1RI-/- animals 16 weeks post-ME7 inoculation in the presence or absence of systemic LPS (100 μg/kg i.p.) and dexamethasone-21-phosphate (2 mg/kg i.p.) challenges were compared. A significant main effect of treatment was demonstrated by a repeated measure ANOVA when IL-1R1-/- ME7 + LPS and IL-1R1-/- ME7 + DEX + LPS were compared. IL-1R1-/- ME7 + LPS n = 9, IL-1R1-/- ME7 + Dex + LPS n = 6 and IL-1R1-/- ME7 + Dex n = 4. All data have been presented as mean ± SEM.

5.3.3 Systemic IL-1RA administration offers protection from LPS-induced working memory deficits in diseased animals

To investigate whether IL-1 signalling contributes to the LPS-induced acute cognitive deficits observed in figure 5.1, NBH and ME7 animals 16 weeks post-inoculation were challenged with LPS (100 μg/kg i.p.) and IL-1RA (10 mg/kg i.p.) concurrently and assessed on T-maze alternation performance (Figure 5.3).

Systemic administration of IL-1RA afforded significant protection against the LPS-induced working memory impairments in ME7 animals. IL-1RA treated animals were protected from the deficits induced by LPS in the block of 5 trials 5 hours post-challenge. By 7 hours this protection was no longer apparent. Repeated measures ANOVA showed a significant interaction between treatment and time ($F = 2.509$, df 12, 196, $p = 0.0065$), and significant main effects of treatment ($F = 6.237$, df 2, 16, $p = 0.0099$) and time ($F = 4.13$, df 6, 96, $p = 0.001$). Bonferroni post-hoc tests demonstrated a significant difference between ME7+LPS and ME7+LPS+IL-1RA at 5 hours ($p < 0.01$). By 7 hours ME7 + LPS + IL-1RA animals were showing impairment compared to ME7 + IL-1RA animals ($p < 0.001$).

This indicates that IL-1 signalling has a significant role in LPS-induced episodes of acute cognitive dysfunction in ME7 animals and that injected IL-1RA offers significant but time-dependent protection from endotoxin-induced impairment.

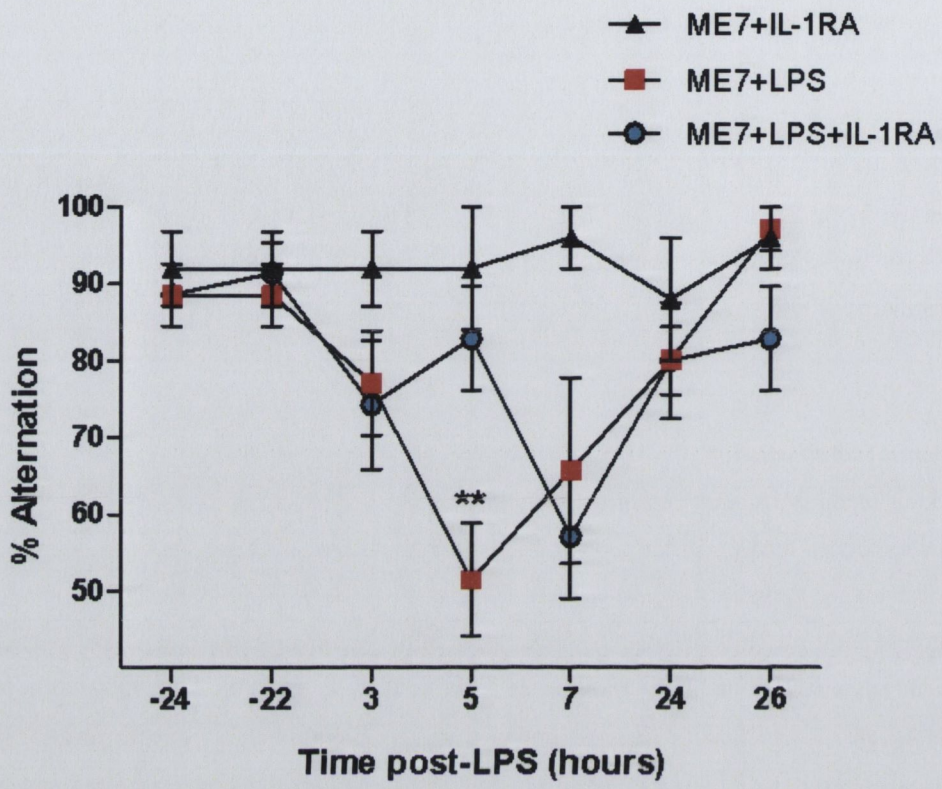

Figure 5.3 *Concurrently administered IL-1RA offers time-dependent protection from LPS-induced working memory deficits in diseased animals.* Working memory performance of ME7 animals, 16 weeks post-inoculation with ME7-infected homogenate, challenged with LPS (100 μg/kg i.p.) was assessed by T-maze alternation in the presence or absence of IL-1 receptor antagonist (IL-1RA), administered at 10 mg/kg i.p. immediately following LPS. All animals were assessed for 10 trials 24 hours before acute challenge, 15 trials 3-9 hours post-challenge and 10 trials 24 hours after the challenge. Significant Bonferroni post-hoc difference between ME7 + LPS and ME7 + LPS + IL-1RA, demonstrated after repeated measures ANOVA are denoted by $^{**}p < 0.01$. ME7 + LPS n = 7, ME7 + LPS + IL-1RA n = 7 and ME7 + IL-RA n = 5. All data have been presented as mean ± SEM.

5.3.4 Systemic IL-1β challenge induces acute and transient working memory deficits in animals 16 weeks post-ME7 inoculation

Given that systemic injection of IL-1RA offers protection from LPS-induced working memory impairments, the aim of the current experiment was to investigate whether administration of systemic IL-1β would be sufficient to elicit acute and transient cognitive dysfunction in animals 16 weeks post-ME7 inoculation.

The effect of systemic IL-1β challenge on cognitive functioning of ME7 and NBH animals 16 weeks after inoculation was assessed with the T-maze alternation task (Figure 5.4). Systemic challenge with IL-1β (15 μg/kg) did not induce memory impairments in NBH animals but the same challenge induced marked and transient working memory deficits in ME7 animals. Repeated measures ANOVA revealed a significant interaction between time and treatment ($F = 1.875$, df 18, 258, $p = 0.0404$) and a main effect of IL-1β treatment ($F = 5.587$, df 3, 43, $p = 0.0025$) but no effect of time ($F = 1.681$, df 6, 258, $p = 0.1564$). Bonferroni post-hoc tests revealed significant differences between ME7 + IL-1β and NBH + IL-1β groups at 1 hour ($p < 0.001$) and 3 hours ($p < 0.05$) post-challenge. Significant differences between ME7 + IL-1β and ME7 + saline groups at 1 hour ($p < 0.05$) and 3 hours ($p < 0.05$) post-challenge were also demonstrated. The deficits seen in ME7 animals following IL-1β challenge had returned to above 80%, which is regarded as intact performance, by 24 hours.

Thus, systemic challenge with IL-1β induces acute and transient working memory deficits in 16 week--ME7 but not NBH animals.

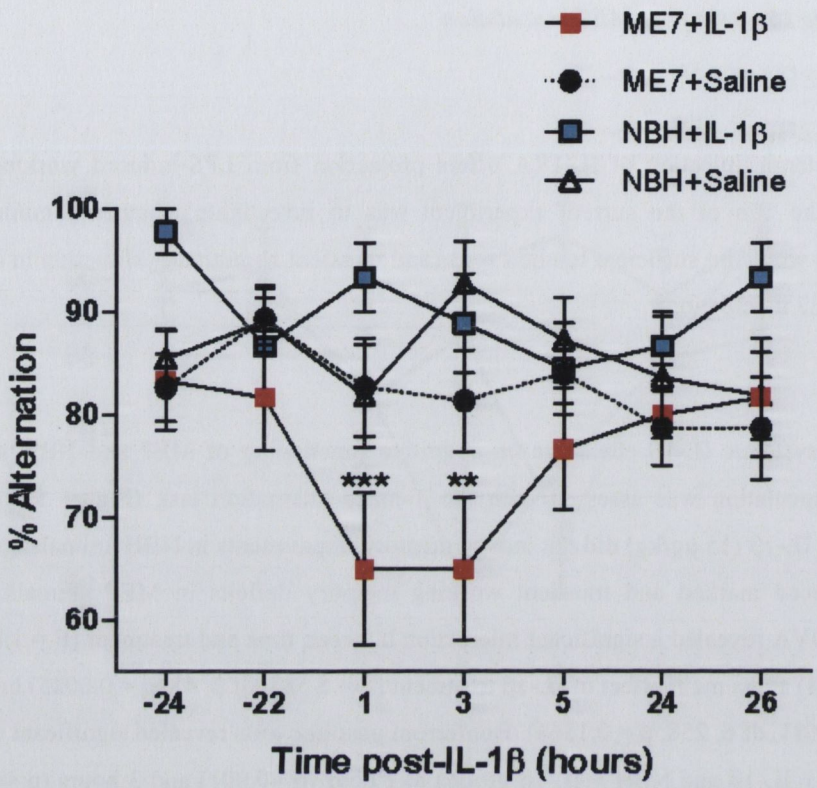

Figure 5.4 *Systemic IL-1β challenge induces acute and transient working memory deficits in animals 16 weeks post-ME7 inoculation.* Working memory performance of NBH and ME7 animals 16 weeks post-ME7-inoculation, challenged with IL-1β (15 μg/kg i.p) or saline, was assessed by T-maze alternation. All animals were assessed for 10 trials 24 hours before acute challenge, 15 trials 1-7 hours post-challenge and 10 trials 24 hours after the challenge. Following repeated measures ANOVA, significant differences by Bonferroni post-hoc analysis were demonstrated between ME7+IL-1β and NBH+IL-1β and are denoted by ** $p < 0.01$ and *** $p < 0.001$. ME7 + IL-1β n=12, ME7 + saline n = 15, NBH + IL-1β n = 9 and NBH + saline n = 11. All data have been presented as mean ± SEM.

5.3.5 Systemic IL-1β does not induce acute working memory deficits in animals 14 weeks post-ME7 inoculation

In the previous chapter (Chapter 4), it was shown that progression of disease affects the susceptibility of an animal to inflammation-induced cognitive impairment. In the present experiment, the ability of systemic IL-β challenge to induce acute and transient cognitive dysfunction in ME7 animals at 14 weeks post-inoculation (2 weeks prior to the above experiments) was assessed.

The ability of systemic IL-β challenge (15 μg/kg i.p.) to induce acute and transient cognitive dysfunction in ME7-diseased and normal animals 14 weeks after inoculation was investigated and utilised the water-based T-maze alternation task to assess working memory (Figure 5.5). Groups were compared by repeated measures ANOVA; no interaction between time and treatment ($F = 1.583$, df 12, 104, $p = 0.0868$) or main effects of time ($F = 0.824$, df 4, 104, $p = 0.4311$) or treatment ($F = 0.9881$, df 3, 26, $p = 0.4137$) were revealed. Systemic IL-1β challenge failed to induce impairments in the T-maze performance of normal and diseased animals.

Thus, ME7 animals 14 weeks post-inoculation do not display the acute cognitive dysfunction on the T-maze task after peripheral IL-1β challenge which are displayed by animals 16 weeks post-inoculation.

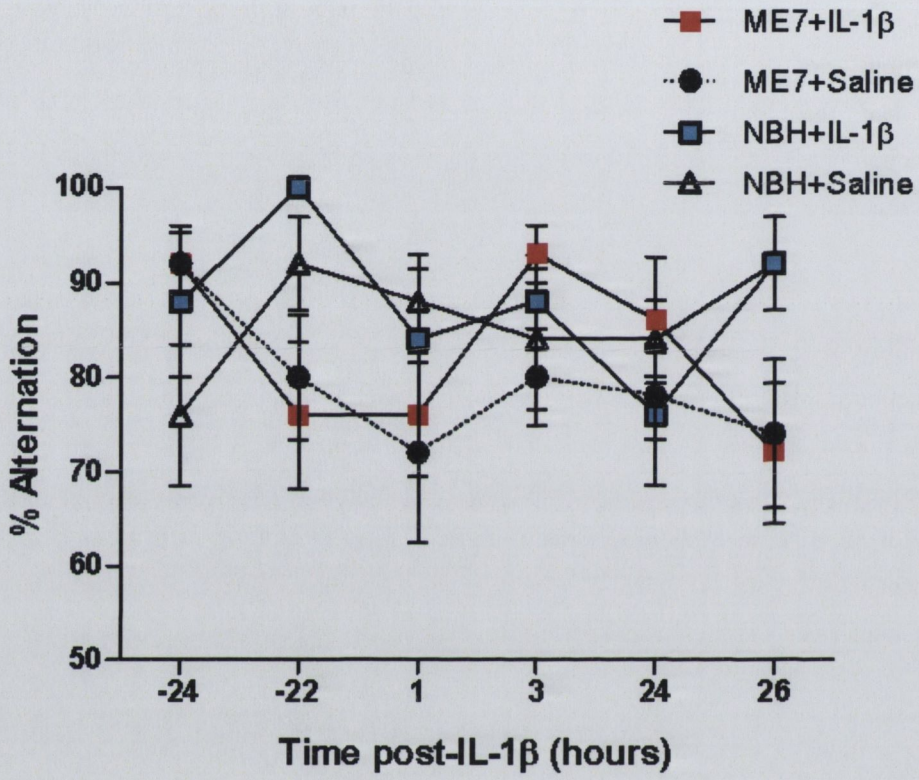

Figure 5.5 *Systemic IL-1β does not induce acute working memory deficits in animals 14 weeks post-ME7 inoculation.* Working memory performance of NBH and ME7 animals 14 weeks post-ME7-inoculation, challenged with IL-1β (15 μg/kg i.p) or saline, was assessed by T-maze alternation. All animals were assessed for 10 trials 24 hours before acute challenge, 10 trials 1-4 hours post-challenge and 10 trials 24 hours after the challenge. Groups were compared by repeated-measures ANOVA. ME7 + IL-1β n = 10, ME7 + saline n = 10, NBH + IL-1β n = 5 and NBH + saline n = 5. All data have been presented as mean ± SEM.

5.3.6 Equivalent systemic inflammatory response to systemic IL-1β challenge in normal and ME7 animals

Since systemic inflammation had differential effects on CNS function in normal and diseased animals, we assessed whether systemic inflammatory mediators were induced at equivalent or different levels in normal and diseased animals challenged with systemic IL-1β. The impact of peripheral IL-1β (15 μg/kg i.p.) and saline challenge on systemic inflammatory markers in ME7 and NBH animals, 16 weeks post-inoculation was examined at 2 hours (Figure 5.6).

As expected, plasma levels of IL-1β were elevated by intraperitoneal injection of IL-1β. The increase in plasma concentration levels post-challenge levels was equivalent in diseased and normal animals. Two-way ANOVA revealed no interaction between treatment and disease ($F = 0.004857$, df 1, 14, $p = 0.9454$) or main effect of disease ($F = 0.004857$, df 1, 14, $p = 0.9454$). However, a main effect of IL-1β challenge on circulating IL-1β levels was shown ($F = 19.07$, df 1, 14, $p < 0.0006$). Bonferroni post-hoc tests showed that IL-1β treatment did not induce a higher systemic IL-1β response in ME7 animals compared to NBH animals ($p > 0.05$).

Circulating IL-1RA was increased by systemic IL-1β administration but there was no difference between levels induced in NBH and ME7 animals. Two-way ANOVA revealed no interaction between treatment and disease ($F = 1.821$, df 1, 12, $p = 0.2021$) or main effect of disease ($F = 1.858$, df 1, 12, $p = 0.1979$). However, a main effect of IL-1β challenge on circulating IL-1RA was shown ($F = 29.87$, df 1, 12, $p < 0.0001$). Bonferroni post-hoc tests showed that IL-1β treatment did not induce a higher systemic IL-1RA response in ME7 animals compared to NBH animals ($p > 0.05$).

CXCL1 was induced by systemic IL-1β challenge but there was no difference in levels induced in NBH and ME7 animals. Two-way ANOVA revealed no interaction between treatment and disease ($F = 1.250$, df 1, 14, $p = 0.2824$) or main effect of disease ($F = 1.607$, df 1, 14, $p = 0.2824$). However, a main effect of IL-1β challenge on circulating CXCL1 ($F = 278.5$, df 1, 14, $p < 0.0001$)

was demonstrated. Bonferroni post-hoc tests showed that IL-1β administration did not induce greater CXCL1 levels in ME7 animals ($p > 0.05$).

PGEM, measured as a proxy for the rapidly metabolised plasma levels of PGE2, was robustly induced in both NBH and ME7 animals post-challenge. Two-way ANOVA revealed no interaction between treatment and disease ($F = 0.01772$, df 1, 28, $p = 0.8950$) or main effect of disease ($F = 0.1621$, df 1, 28, $p = 0.6903$). However, a main effect of IL-1β challenge on circulating PGEM ($F = 22.17$, df 1, 28, $p < 0.0001$) was demonstrated. Bonferroni post-hoc tests showed that systemic IL-1β did not induce a higher systemic PGEM response in ME7 animals compared to NBH animals ($p > 0.05$).

Equivalent increases in corticosterone were elicited in NBH and ME7 animals after systemic IL-1β challenge. Two-way ANOVA revealed no interaction between treatment and disease ($F = 0.2523$, df 1, 14, $p = 0.6233$) or main effect of disease ($F = 1.879$, df 1, 14, $p = 0.1920$). However, a main effect of IL-1β challenge on circulating corticosterone was demonstrated ($F = 44.57$, df 1, 14, $p < 0.0001$). Bonferroni post-hoc tests showed that IL-1β treatment did not induce a higher systemic corticosterone response in ME7 animals compared to NBH animals ($p > 0.05$).

Systemic TNF-α was assayed but no detectable levels were measured in any treatment group. Thus, systemic IL-1β administration does not induce systemic production of TNF-α in normal or diseased animals.

In summary, normal and ME7 animals display equivalent systemic inflammatory and stress responses to peripheral IL-1β administration.

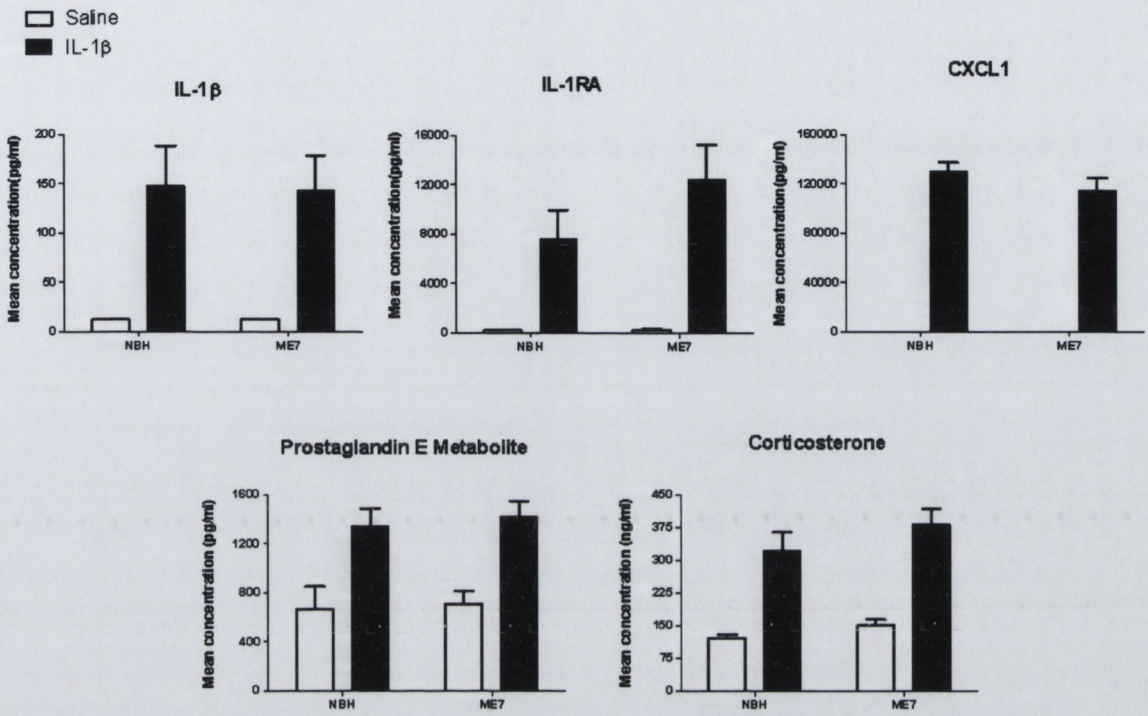

Figure 5.6 *Equivalent systemic inflammatory response to peripheral IL-1β challenge in normal and ME7 animals.* Plasma was prepared from whole blood of NBH and ME7 animals, 16 weeks post-inoculation, 2 hours post-treatment with IL-1β (15 μg/kg i.p.) or saline and analysed by ELISA. Circulating CXCL1 was not detected in saline challenged animals but set to detection limit so as to make statistical comparisons with detectable means in IL-1β groups. Following two-way ANOVA, Bonferroni post-hoc tests showed no significant difference between IL-β challenged ME7 and NBH animals ($p > 0.05$). ME7 + IL-1β n = 5, ME7 + saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4, except for IL-RA, ME7 + IL-1β n = 4, ME7 + saline n = 4, NBH + IL-1β n = 4 and NBH + saline n = 4 and PGEM ME7 + IL-1β n = 11, ME7 + saline n = 8, NBH + IL-1β n = 9 and NBH + saline n = 4 All data have been presented as mean ± SEM.

5.3.7 Hippocampal transcription levels of proinflammatory cytokines in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge

Since there were significant differing effects of systemic IL-1β on cognitive function but not on systemic inflammatory mediators between ME7 and NBH animals, we assessed whether there were differential inflammatory responses in the CNS. The transcription of pro-inflammatory cytokines 2 hours after systemic challenge with IL-1β (15 μg/kg) and saline in the hippocampus of 16-week ME7 and NBH animals was investigated (Figure 5.7).

ME7 animals systemically challenged with IL-1β showed greater increases in IL-1β mRNA than similarly challenged NBH animals. Two-way ANOVA demonstrated a significant interaction of treatment and disease ($F = 8.057$, 1, 14, $p = 0.0131$) and main effects of treatment ($F = 20.34$, df 1, 14, $p = 0.0005$) and disease ($F = 19.96$, df 1, 14, $p = 0.0005$). Bonferroni post-hoc tests indicated that the interaction was driven by a robust induction of IL-1β in ME7 but not NBH animals after systemic IL-1β injection. IL-1β-treated ME7 animals differed significantly from IL-1β-treated NBH animals ($p < 0.001$). Thus, systemic IL-1β challenge induces an amplified IL-1β mRNA response in ME7 animals compared to NBH.

CXCL-1 was induced by IL-1β challenge but there were no significant differences between ME7 and NBH animals. Two-way ANOVA demonstrated no significant interaction ($F = 1.037$, df 1, 14, $p = 0.3527$) or main effect of disease ($F = 1.053$, df 1, 14, $p = 0.3223$). A significant main effect of systemic IL-1β administration on CXCL1 ($F = 11.29$, df 1, 14, $p < 0.0047$) mRNA levels was shown. Bonferroni post-hoc tests demonstrated no difference in IL-1β induced-CXCL1 in ME7 compared to NBH ($p > 0.05$).

IL-6 mRNA was also elicited by IL-1β challenge but there were no significant differences between ME7 and NBH animals. Two-way ANOVA demonstrated no significant interaction ($F = 2.350$, df 1, 14, $p = 0.1476$) or main effect of disease ($F = 3.105$, df 1, 14, $p = 0.0999$). A significant main effect of systemic IL-1β administration on IL-6 ($F = 28.57$, df 1, 14, $p < 0.0001$) mRNA levels was shown. Bonferroni post-hoc tests demonstrated no difference in IL-1β-induced IL-6 in ME7

compared to NBH ($p > 0.05$) but there appears to be a trend towards higher IL-6 in ME7 + IL-1β animals compared to NBH + IL-1β animals.

Expression of TNF-α mRNA was higher in ME7 animals but there was no significant induction in ME7 animals after IL-1β challenge. Moreover, ME7+ IL-1β animals didn't differ significantly from NBH + IL-1β treated animals. Two-way ANOVA demonstrated no significant interaction between disease and treatment ($F = 0.4712$, 1, 13, $p < 0.0127$) but significant main effects of both IL-1β challenge ($F = 8.34$, df 1, 13, $p < 0.0127$) and disease ($F = 16.41$, df 1, 13, $p = 0.0014$). Bonferroni post-hoc test revealed no significant difference in IL-1β induced-TNF-α mRNA in ME7 compared to NBH ($p > 0.05$).

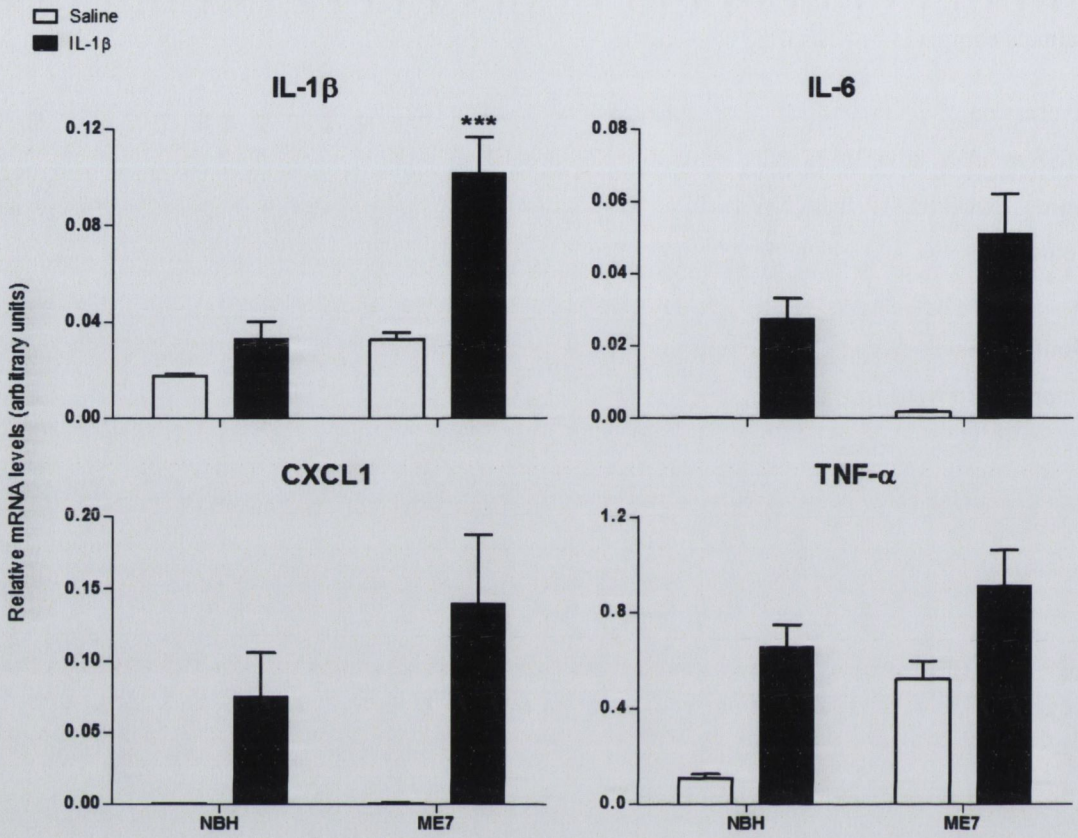

Figure 5.7 *Hippocampal transcription of proinflammatory cytokines in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge.* Expression of pro-inflammatory genes IL-1β, TNF-α, CXCL1 and IL-6 were examined in the hippocampus 2 hours after systemic challenge with IL-1β (15 μg/kg i.p.) in NBH and ME7 animals, 16 weeks post-inoculation. *** denotes a significant Bonferroni post-hoc difference between ME7 + IL-1β and NBH + IL-1β groups ($p < 0.001$) demonstrated after two-way ANOVA. ME7 + IL-1β n = 5, ME7 + saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.8 Hippocampal transcription levels of iNOS and PTX3 in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge

The impact of systemic IL-1β and saline challenges (15 μg/kg) on hippocampal mRNA levels of putative IL-1β-downstream molecules PTX3 and iNOS, 16 weeks post-inoculation, 2 hours post-injection were investigated in ME7 and NBH animals (Figure 5.8).

PTX3 transcripts were increased in the hippocampus of diseased animals but not further enhanced by IL-1β challenge. Two-way ANOVA revealed PTX3 mRNA levels were not affected by an interaction between disease and treatment ($F = 1.401$, df 1, 14, $p = 0.2563$) or treatment alone ($F = 0.5016$, df 1, 14, $p = 0.4904$). A main effect of ME7 disease on PTX3 levels was revealed ($F = 27.02$, df 1, 14, $p = 0.0001$).

iNOS mRNA levels were not significantly affected by an interaction of disease and treatment ($F = 0.09029$, df 1, 14, $p = 0.7662$) or by disease ($F = 0.03831$, df 1, 14, $p = 0.8475$) or treatment alone ($F = 0.9168$, df 1, 14, $p = 0.3546$). Thus, hippocampal iNOS mRNA levels were not affected by ME7 disease or acute IL-1β administration 2 hours post-challenge.

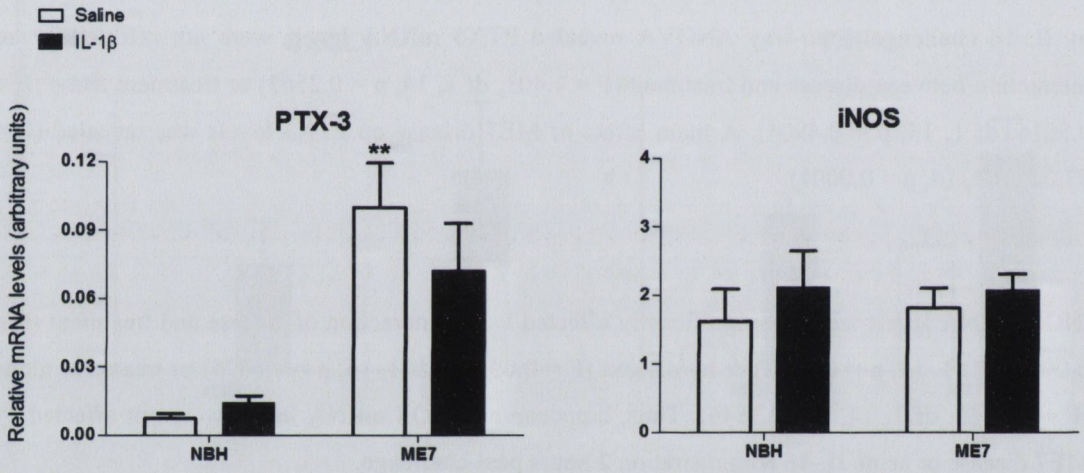

Figure 5.8 *Hippocampal transcription levels of inflammation-induced molecules iNOS and PTX3 in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge.* Gene expression of hippocampal PTX3 and iNOS 2 hours after systemic challenge with IL-1β (15 μg/kg i.p.) or saline in NBH and ME7 animals, 16 weeks post-inoculation was examined. A Bonferroni post-hoc test, following two-way ANOVA, demonstrated a significant difference between ME7 + saline and NBH + saline (denoted by ** $p < 0.01$). ME7+IL-1β n = 5, ME7+saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.9 Hippocampal transcription levels of IL-1 receptor antagonist and receptors type I and II in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge

The impact of systemic IL-1β and saline challenges (15 μg/kg) on hippocampal mRNA levels of IL-1RA, IL-1RI and IL-1RII in ME7 and NBH animals, 16 weeks post-inoculation, 2 hours post-injection was investigated (Figure 5.9).

Two-way ANOVA revealed IL-1RA mRNA levels were not affected by an interaction between disease and treatment ($F = 1.818$, df 1, 14, $p = 0.1989$). However, main effects of disease ($F = 36.16$, df 1, 14, $p < 0.0001$) and treatment ($F = 4.67$, df 1, 14, $p = 0.0484$) were demonstrated. A Bonferroni post-hoc test showed that IL-1RA mRNA was significantly higher in IL-1β-treated ME7 animals compared to IL-β-treated NBH animals ($p < 0.001$). Thus, IL-1RA is increased by disease and further increased by IL-1β challenge.

IL-1RI mRNA levels were not significantly affected by an interaction of disease and treatment ($F = 0.1761$, df 1, 14, $p = 0.6811$) or by disease ($F = 0.1500$, df 1, 14, $p = 0.7044$) or treatment alone ($F = 0.1601$, df 1, 14, $p = 0.6951$). Thus, IL-1R1 transcript levels were not altered by disease or systemic IL-1β administration.

IL-1RII mRNA levels were affected by both disease and IL-1β challenge. Two-way ANOVA showed no significant interaction between disease and treatment ($F = 0.5013$, df 1, 14, $p = 0.4905$). A main effect of disease ($F = 77.87$, df 1, 14, $p = 0.0001$) and treatment ($F = 7.75$, df 1, 14, $p = 0.0147$) was revealed. A Bonferroni post-hoc test showed that IL-1RII was significantly elevated in IL-1β-treated ME7 animals compared to IL-1β-treated NBH animals ($p < 0.001$).

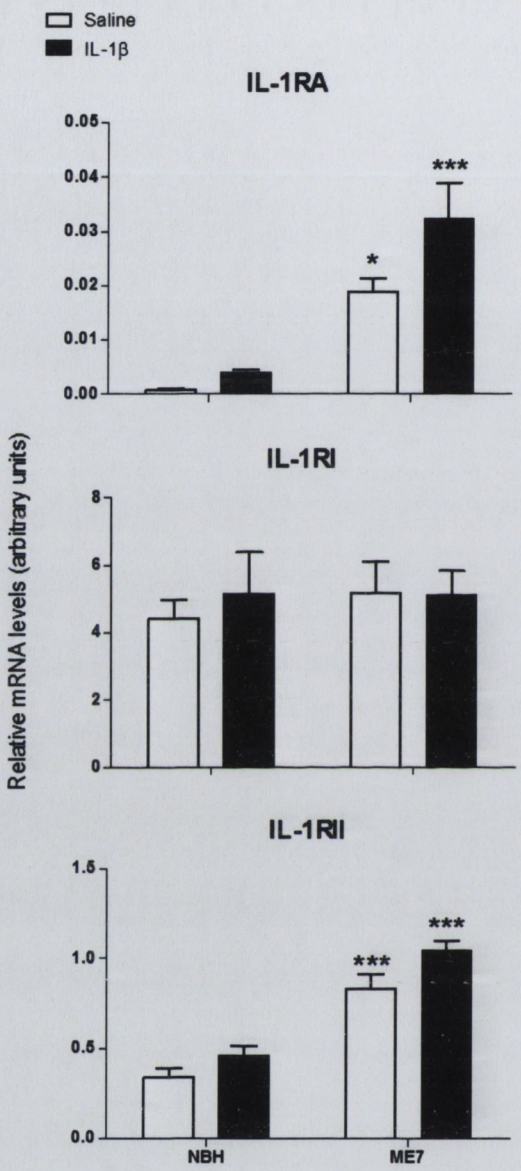

Figure 5.9 *Hippocampal transcription levels of IL-1 receptor antagonist and receptor type I and II in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge.* Gene expression of hippocampal IL-1RA, IL-1RI and IL-1RII 2 hours after systemic challenge with IL-1β (15 μg/kg i.p.) and saline in NBH and ME7 animals, 16 weeks post-inoculation was examined. Following two-way ANOVA, Bonferroni post-hoc tests showed ME7 + IL-1β significantly higher compared to NBH + IL-1β. * denotes $p < 0.05$, *** $p < 0.01$. ME7 + IL-1β n = 5, ME7 + saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.10 Hippocampal transcription levels of NLRP3 inflammasome components in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge

The NLRP3 inflammasome has been shown to cleave pro-IL-1β to its active mature form in some instances of sterile inflammation (Rock et al., 2010). Hippocampal transcription of NLRP3 inflammasome components, caspase-1 and NLRP3 itself, were examined 2 hours after systemic challenge with saline and IL-1β (15 μg/kg) in 16-week ME7 and NBH animals (Figure 5.10).

NLRP3 mRNA was increased by disease but not further induced by acute IL-1β challenge in ME7 or control animals. A two-way ANOVA showed no interaction between disease and treatment ($F = 0.5962$, df 1, 13, $p = 0.2951$) or main effect of treatment ($F = 4.070$, df 1, 13, $p = 0.0648$). Systemic IL-1β injection did not significantly increase NLRP3 mRNA across NBH or ME7 groups. A significant main effect of disease, however, was revealed ($F = 67.78$, df 1, 13, $p = 0.0001$). Bonferroni post-hoc tests showed that saline treated NBH and ME7 animals were significantly different to each other ($p < 0.001$); indicating that disease per se increases NLRP3 mRNA.

Caspase-1 mRNA was increased by disease but not acutely induced by peripheral IL-1β challenge in ME7 or control animals. A two-way ANOVA showed no interaction between disease and treatment ($F = 0.01290$, df 1, 13, $p = 0.9113$) or main effect of treatment ($F = 0.07718$, df 1, 13, $p = 0.7855$). A significant main effect of disease, however, was revealed ($F = 5.816$, df 1, 13, $p = 0.0314$). Hence, caspase-1 mRNA is higher in diseased animals, regardless of IL-1β challenge, when compared to NBH animals.

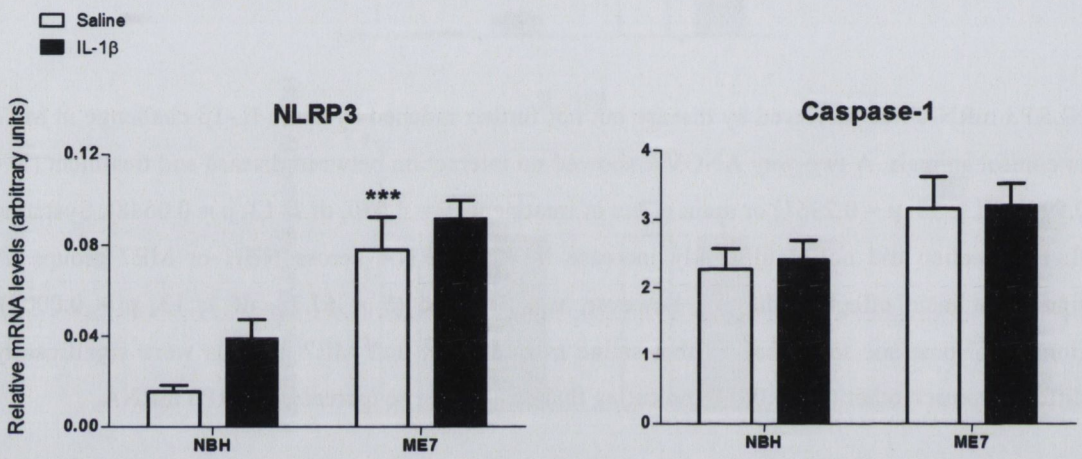

Figure 5.10 *Hippocampal transcription levels of NLRP3 inflammasome components in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge.* Gene expression of hippocampal NLRP3 and caspase-1, 2 hours after systemic challenge with saline and IL-1β (15 μg/kg i.p.) in NBH and ME7 animals, 16 weeks post-inoculation was examined. Two-way ANOVA was performed for both gene transcripts. ME7 + IL-1β n = 5, ME7 + saline n = 4, NBH + IL-1β n = 4 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.11 Sickness responses and hypothalamic cytokine levels in NBH and ME7-diseased animals following IL-1β challenge

Since ME7 animals displayed exaggerated cognitive effects and exaggerated hippocampal IL-1β transcription compared to normal animals after systemic IL-β challenge, the sickness responses and hypothalamic cytokine levels were also assessed 2 hours post-challenge.

5.3.11.1 Core body temperature

The impact of systemic IL-1β challenge on sickness responses in ME7 and NBH animals was examined. After 2 hours, systemic IL-1β (15μg/kg i.p.) had induced a hypothermic response in both normal and ME7-diseased mice, 16 weeks post-inoculation (Figure 5.11 a). Two-way ANOVA revealed no interaction between treatment and disease ($F = 0.4563$, df 1, 14, $p = 0.5104$) or main effect of disease ($F = 3.14$, df 1, 14, $p = 0.0981$). However, a main effect of treatment was shown ($F = 26.62$, df 1, 14, $p < 0.0001$). Bonferroni post-hoc tests showed that both NBH ($p < 0.05$) and ME7 ($p < 0.01$) groups had significantly reduced core-body temperature following systemic IL-1β. However, importantly there was no difference between ME7 and NBH groups in the reduction of temperature ($p > 0.05$).

5.3.11.2 Locomotor activity

IL-1β-treated ME7 and normal animals also showed marked reductions in locomotor activity 2 hours after challenge (Figure 5.11 b). Two-way ANOVA revealed no interaction between treatment and disease ($F = 0.0821$, df 1, 14, $p = 0.7786$) or main effect of disease ($F = 0.4634$, df 1, 14, $p = 0.5071$). A main effect of treatment was demonstrated ($F = 29.60$, df 1, 14, $p < 0.0001$). Bonferroni post-hoc tests showed that there was no significant difference between ME7 and NBH groups in the reduction of activity, in terms of distance travelled, following systemic IL-1β ($p > 0.05$).

Thus, ME7-inoculated and normal animals showed equivalent reductions in locomotor activity and body temperature following systemic IL-1β injection.

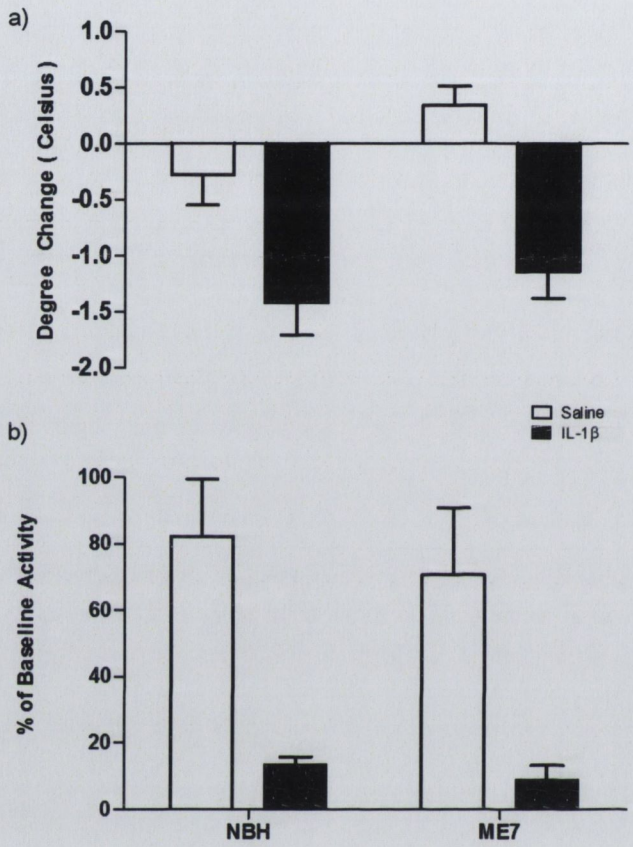

Figure 5.11 *Core body temperature and locomotor activity changes in NBH and ME7-diseased animals following IL-1β challenge.* The deviation of core body temperature (a) and locomotor activity (b) from baseline in NBH and ME7 animals, 16 weeks post-inoculation, 2 hours post-IL-1β (15 μg/kg i.p.) and saline challenge was assessed. Locomotor activity changes are presented as a percentage of baseline 2 hours after systemic IL-1β treatment. Following two-way ANOVA, Bonferroni post-hoc tests showed no significant difference between IL-1β-challenged ME7 and NBH animals. ME7 + IL-1β n = 5, ME7 + saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.11.3 Hypothalamic proinflammatory cytokine transcription

Expression of IL-1β, TNF-α and IL-6 mRNA was examined in the hypothalamus 2 hours post-challenge with IL-1β (15 μg/kg i.p.) and saline in NBH and ME7 animals 16 weeks post-inoculation (Figure 5.12). This was to assess whether there was exaggerated hypothalamic cytokine transcription in ME7 animals compared to NBH animals after peripheral administration of IL-1β.

IL-1β mRNA levels were increased by both disease and treatment. Two-way ANOVA revealed no interaction between treatment and disease ($F = 0.03526$, df 1, 14, $p = 0.2021$) but main effects of disease ($F = 5.83$, df 1, 14, $p = 0.0300$) and treatment ($F = 21.48$, df 1, 14, $p = 0.0004$). Bonferroni post-hoc tests showed that IL-1β challenge did not induce greater IL-1β levels in ME7 animals compared to NBH animals but that levels were significantly increased in IL-1β- animals compared to those challenged with saline ($p < 0.05$).

TNF-α mRNA levels were significantly elevated by ME7-disease and induced to a greater degree by IL-1β challenge in ME7 animals compared to NBH. Two-way ANOVA demonstrated no interaction between disease and treatment ($F = 0.22$, df 1, 13, $p = 0.6465$) but main effects of both IL-1β challenge ($F = 21.51$, df 1, 13, $p < 0.0005$) and disease ($F = 15.48$, df 1, 13, $p = 0.0017$). Bonferroni post-hoc tests revealed that there was an increase in levels in both ME7 ($p < 0.05$) animals and NBH animals ($p < 0.01$) post-challenge and expression was higher in challenged ME7 animals compared to challenged NBH animals ($p < 0.05$).

In terms of IL-6 mRNA expression, two-way ANOVA demonstrated no interaction between treatment and disease ($F = 3.395$, df 1, 14, $p = 0.0867$) or main effect of disease ($F = 3.937$, df 1, 14, $p = 0.0672$). However, a main effect of treatment ($F = 41.83$, df 1, 14, $p < 0.0001$) was revealed. Bonferroni post-hoc tests show the systemic IL-1β challenge increased IL-6 to a higher level in ME7 animals compared to NBH ($p < 0.05$). In summary, an exaggerated increase in IL-1β transcription was not observed in ME7 animals compared to NBH animals after systemic IL-1β challenge. TNF-α and IL-6 mRNA levels were shown to be higher in ME7 animals than NBH animals after peripheral administration of IL-1β.

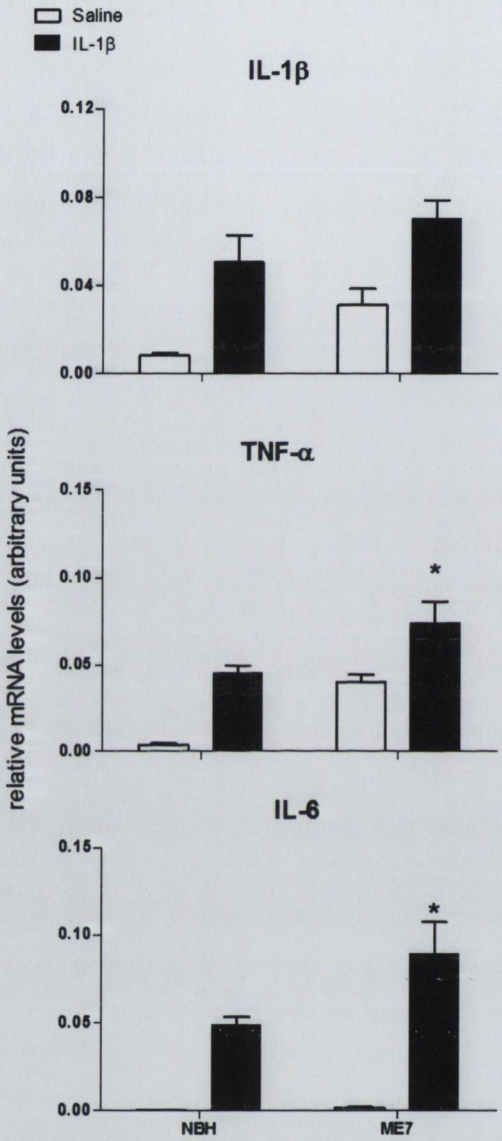

Figure 5.12 *Impact of systemic IL-1β treatment on hypothalamic proinflammatory cytokine transcription.* Expression of proinflammatory genes IL-1β, TNF-α and IL-6 was examined in the hypothalamus 2 hours after systemic challenge with IL-1β (15 μg/kg i.p.) and saline in NBH and ME7 animals, 16 weeks post-inoculation. After two-way ANOVA, Bonferroni post-hoc tests demonstrated that IL-1β treatment induced higher TNF-α and IL-6 but not IL-1β mRNA transcripts in ME7 animals compared to NBH. * denotes $p > 0.05$. ME7 + IL-1β n = 5 ME7 + saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.12 Hippocampal transcription levels of COX-1 and COX-2 in NBH and ME7 animals in the presence and absence of systemic IL-1β challenge

In the previous chapter, prostaglandins were implicated in LPS-induced cognitive impairment. Thus, we assessed inducible components of the prostaglandin system after systemic IL-1β challenge. To this end, the impact of peripheral IL-1β (15 μg/kg) and saline on hippocampal mRNA expression of COX-1 and COX-2 in ME7 and NBH animals, 16 weeks post-inoculation, 2 hours post-challenge was compared (Figure 5.13).

COX-1 mRNA expression was markedly increased in diseased animals but not affected by IL-1β challenge. A two-way ANOVA showed no interaction between disease and treatment ($F = 0.7383$, df 1, 14, $p = 0.4047$) or main effect of treatment ($F = 0.02181$, df 1, 14, $p = 0.8847$). A significant main effect of disease was revealed ($F = 40.75$, df 1, 14, $p = 0.0001$).

COX-2 mRNA levels were not significantly affected by an interaction of disease and treatment ($F = 0.4972$, df 1, 14, $p = 0.4923$) or by disease ($F = 0.2097$, df 1, 14, $p = 0.1696$) or treatment alone ($F = 1.096$, df 1, 14, $p = 0.3130$). Hence, COX-2 mRNA was not increased in disease or by IL-1β treatment.

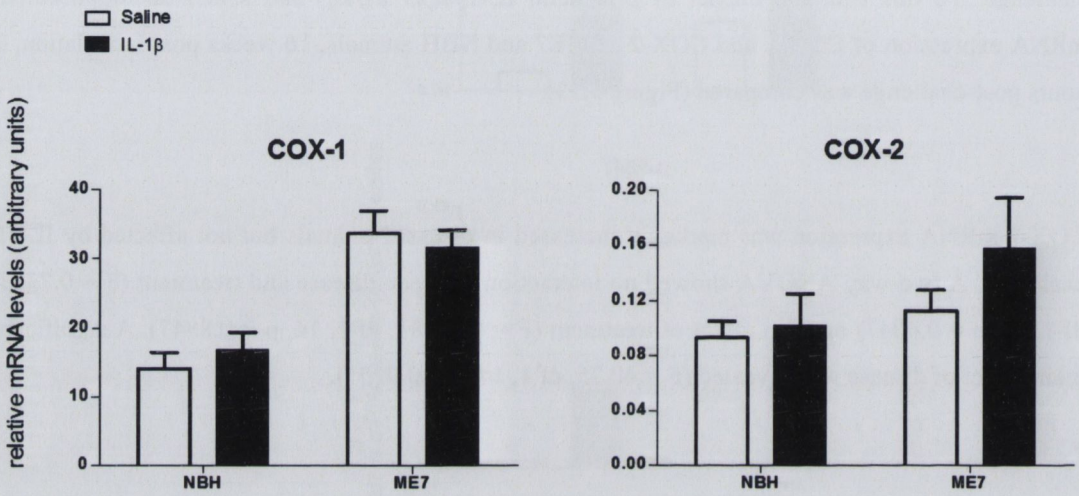

Figure 5.13 *Hippocampal expression of COX-1 and COX-2 mRNA in ME7 and NBH animals after systemic IL-1β or saline challenge.* Hippocampal COX-1 and COX-2 transcription in NBH and ME7 animals 16 weeks post-inoculation 2 hours post systemic IL-1β (15 μg/kg i.p.) or saline injection were assessed. Two-way ANOVA was performed for both gene transcripts. ME7 + IL-1β n = 5, ME7 + saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.13 Hippocampal transcription levels of prostaglandin synthase in ME7 and NBH animals, in the presence or absence of systemic IL-1β challenge

5.3.13.1 Prostaglandin E synthase isozymes

The transcription levels of the prostaglandin E synthase isozymes, mPGES-1, mPGES-2 and cPGES in ME7 and NBH animals, 16 weeks post-inoculation, 2 hours after systemic IL-1β and saline challenge were examined (Figure 5.14).

mPGES-1 mRNA was elevated by ME7 disease and induced to a greater degree by IL-1β challenge in ME7 animals when compared to NBH animals. A two-way ANOVA did not show an interaction between disease and treatment ($F = 0.9266$, df 1, 14, $p = 0.3521$). Significant main effects of disease ($F = 36.61$, df 1, 14, $p < 0.0001$) and treatment ($F = 26.25$, df 1, 14, $p = 0.0002$) were demonstrated. Bonferroni post-hoc tests showed IL-1β-treated ME7 animals differed significantly from IL-1β-treated NBH animals ($p < 0.001$)..

mPGES-2 mRNA was affected by an interaction of disease and treatment, as shown by a two-way ANOVA ($F = 5.863$, df 1, 14, $p = 0.0296$). No main effects of disease ($F = 0.1928$, df 1, 14, $p = 0.6673$) or treatment were demonstrated ($F = 0.9003$, df 1, 14, $p = 0.3588$). Thus, mPGES-2 mRNA was affected by IL-1β administration differentially, dependent on disease state. In NBH animals, IL-1β treatment resulted in a slight decrease in mPGES-2, whereas in ME7 animals, administration resulted in a minimal increase in mPGE2 expression levels.

cPGES mRNA levels were not affected by an interaction of disease and treatment ($F = 0.05329$, df 1, 14, $p = 0.8208$) or by disease ($F = 0.8272$, df 1, 14, $p = 0.3785$) or treatment alone ($F = 0.04046$, df 1, 14, $p = 0.84356$). Hence, cPGES mRNA was not increased in by disease or IL-1β treatment.

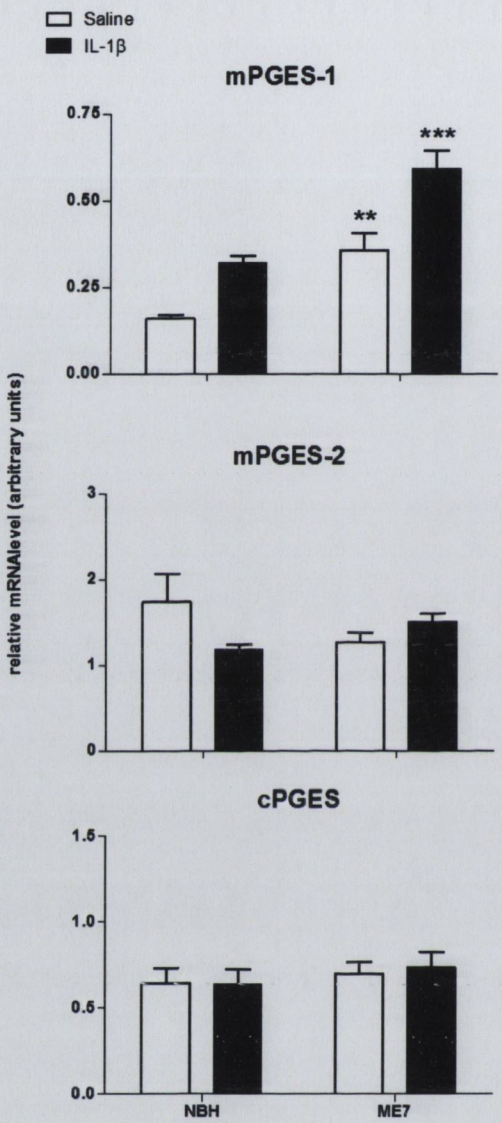

Figure 5.14 *Hippocampal Prostaglandin E synthase isozyme mRNA expression in ME7 and NBH animals after systemic IL-1β or saline challenge.* Hippocampal expression of mPGES-1, mPGES-2 and cPGES mRNA in NBH and ME7 animals 16 weeks post-inoculation following systemic saline and IL-1β treatment (15 μg/kg i.p.) was assessed. Bonferroni post-hoc tests, following two-way ANOVA demonstrated that basal mPGES-1 transcripts were higher in ME7 animals and that IL-1β treatment induced higher mPGES-1 mRNA transcripts in ME7 animals. ** denotes $p < 0.01$, *** $p < 0.001$. ME7 + IL-1β n = 5, ME7 + saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.13.2 Prostaglandin D, I and F synthases

Transcription levels of the prostaglandin D synthase isozymes, HPGDS and LPGDS, prostaglandin I/prostacyclin synthase PGIS and prostamide/prostaglandin F synthase in ME7 and NBH animals, 16 weeks post-inoculation, 2 hours post-systemic IL-1β and saline challenge were examined (Figure 5.15).

HPGDS mRNA expression was markedly increased in disease but not affected by IL-1β challenge. A two-way ANOVA showed no interaction between disease and treatment ($F = 0.007301$, df 1, 14, $p = 0.9331$) or main effect of treatment ($F = 0.06544$, df 1, 14, $p = 0.8018$). A significant main effect of disease was revealed ($F = 185.6$, df 1, 14, $p < 0.0001$).

LPGDS mRNA levels were affected by an interaction between IL-1β treatment and ME7 disease ($F = 8.053$, df 1, 14, $p = 0.0132$) as assessed by two-way ANOVA. Disease ($F = 7.740$, df 1, 14, $p < 0.0147$) but not treatment ($F = 2.375$, df 1, 14, $p = 0.1456$) had a main effect. Bonferroni post-hoc tests indicated that IL-1β-treated ME7 animals differed from saline-treated ME7 ($p < 0.05$) and IL-1β-treated NBH animals ($p < 0.01$). Thus, hippocampal LPGDS mRNA is induced after peripheral IL-1β challenge in diseased but not normal animals.

PGIS mRNA levels were not affected by an interaction of disease and treatment ($F = 1.616$, df 1, 14, $p = 0.2243$) or by disease ($F = 0.03900$, df 1, 14, $p = 0.8463$). A main effect of treatment was demonstrated ($F = 7.361$, df 1, 14, $p = 0.0168$). ME7 and NBH animals challenged with IL-1β showed reduced PGIS mRNA levels but Bonferroni post-hoc test only showed significant drop in IL-1β-treated NBH animals ($p < 0.05$).

Prostamide/prostaglandin F synthase mRNA levels were increased in diseased animals but there was no alteration after IL-1β challenge. A two-way ANOVA showed no interaction between disease and treatment ($F = 0.035$, df 1, 13, $p = 0.5618$) or main effect of treatment ($F = 2.52$, df 1, 13, $p = 0.1364$). A main effect of disease was revealed ($F = 5.47$, df 1, 13, $p < 0.0360$).

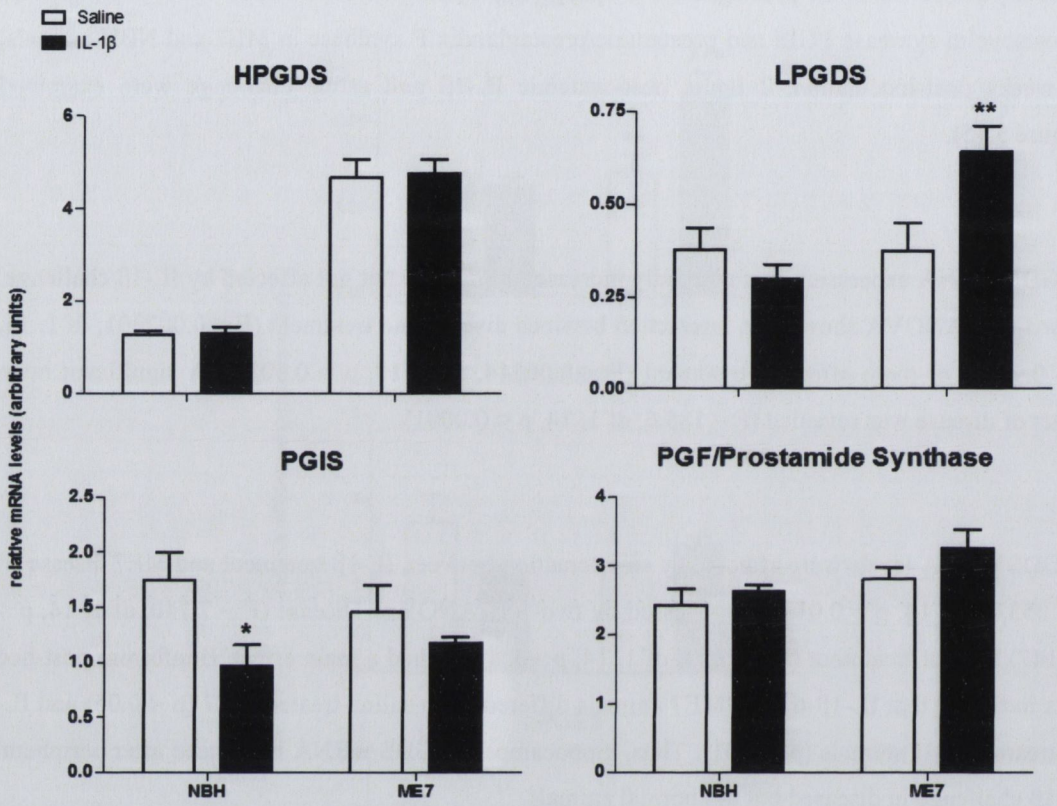

Figure 5.15 *Hippocampal Prostaglandin D and I synthase mRNA expression in ME7 and NBH animals after systemic IL-1β or saline challenge.* Hippocampal transcription of HPGDS, LPGDS, PGIS and prostamide/prostaglandin F synthase in NBH and ME7 animals 16 weeks post-inoculation following systemic IL-1β (15 μg/kg i.p.) and saline treatment was assessed. Following two-way ANOVA, Bonferroni post-hoc tests showed that LPGDS was significantly elevated in IL-1β-challenged compared to saline challenged ME7 animals. ** denotes $p < 0.01$ and * denotes significant IL-1β-induced decrease in PGIS in NBH animal $p < 0.05$. ME7+IL-1β n = 5, ME7+saline n = 4, NBH + IL-1β n = 5 and NBH + saline n = 4. All data have been presented as mean ± SEM.

5.3.14 Hippocampal Prostaglandin E2 levels in ME7 and NBH animals, in the presence or absence of systemic IL-1β challenge

The impact of systemic IL-1β (15 μg/kg) and saline treatment on prostaglandin E2 levels in the hippocampus of normal and diseased animals, 16 weeks post-inoculation, 2 hours post-challenge was assessed (Figure 5.16).

As was observed in the previous chapter, there is a disease-related elevation of PGE2. There was, however, no evidence of IL-1β-induced synthesis of PGE2 in either diseased or normal animals. Two-way ANOVA revealed no interaction between disease and LPS treatment ($F = 2.335$, df 1, 14, $p = 0.1488$. 8728) or main effect of treatment ($F = 0.1029$, df 1,14, $p = 0.7531$). However, a main effect of disease on hippocampal PGE2 levels was demonstrated ($F = 6.397$, df 1, 14, $p = 0.0241$).

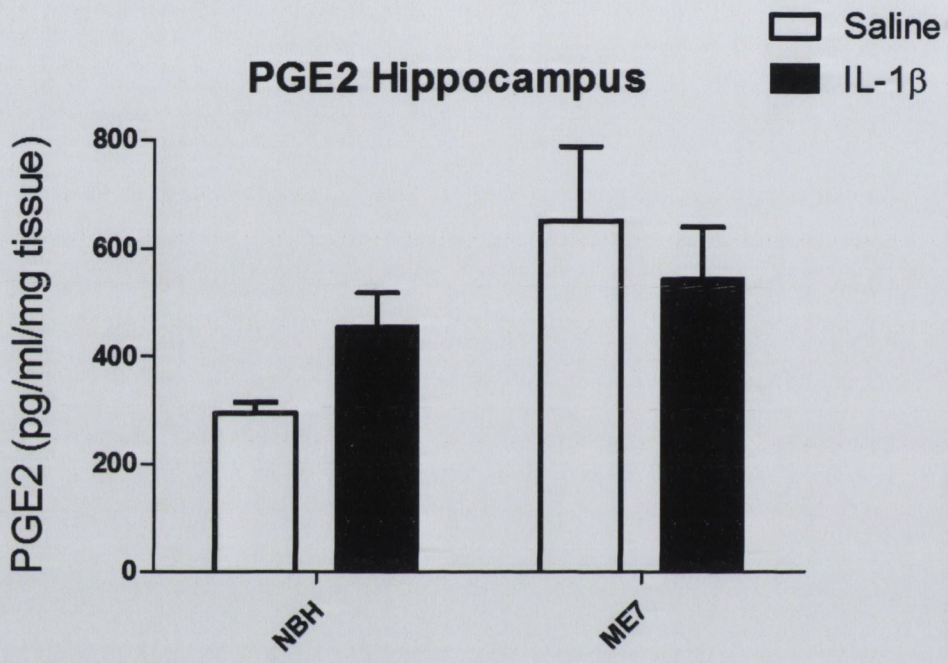

Figure 5.16 *Hippocampal Prostaglandin E2 levels in ME7 and NBH animals, in the presence or absence of systemic IL-1β challenge.* Hippocampal PGE2 levels were assessed in NBH and ME7 animals, 16 weeks post-inoculation, 2 hours after systemic IL-1β (15 μg/kg i.p.) and saline challenge. Two-way ANOVA was performed on the data. ME7 + IL-1β n = 8, ME7 + saline n = 6, NBH + IL-1β n = 5 and NBH + saline n = 4). All data have been presented as mean ± SEM.

5.4 Discussion

Despite the enormous medical and economic imperatives to understand delirium (Leslie et al., 2008), its pathophysiology is unclear (Skrobik, 2010, Maclullich et al., 2008). Studies show that inflammatory events, brought about by infection, tissue trauma or surgery, can induce episodes of delirium, especially in the elderly and demented (Cerejeira et al., 2010). IL-1β is a potent cytokine elevated in both septic and aseptic inflammation (Dinarello, 1996, Chen and Nuñez, 2010). It is produced in response to local tissue damage (Ortiz et al., 2007), surgery (Haeffner-Cavaillon et al., 1989) and infection (Sansonetti et al., 2000). This study assessed its role in mediating systemic inflammation-induced acute cognitive dysfunction and neuroinflammation when superimposed on a model of dementia. The data provide evidence that systemic IL-1 signalling has a role in endotoxin-induced acute cognitive dysfunction in animals with ongoing neurodegeneration. The results also demonstrate that peripheral challenge with IL-1β alone can elicit acute-onset cognitive dysfunction. IL-1β challenge induces equivalent systemic inflammation in ME7 and normal animals but exaggerated hippocampal transcription of IL-1β and prostaglandin synthases in ME7 animals.

5.4.1 IL-1β mediated cognitive dysfunction

In the present study, systemic LPS induced episodes of acute cognitive impairment in ME7 animals at 16 weeks, supporting findings of a similar nature in ME7 animals at 12 weeks (Murray et al., 2012) and 16 weeks (Chapter 4). IL-1β appears to be important in transducing systemic inflammation into the CNS; many of the central effects of systemically administered LPS, such as sickness behaviour and increases in markers of inflammation in the hippocampus, are also elicited by systemic IL-1β challenge (Chapter 3) (Dantzer et al., 2008). We demonstrate in the present study that simultaneous peripheral administration of IL-1RA offers significant initial protection to ME7 animals from LPS-induced acute cognitive impairment. These results implicate IL-1 signalling in acute endotoxin-induced cognitive dysfunction in this animal model of delirium during dementia. In addition, peripheral administration of IL-1β is shown to be sufficient to elicit acute and transient working memory dysfunction in diseased animals. The findings suggest that systemic IL-1β may be a common mediator of episodes of cognitive impairment in both septic and aseptic inflammation during neurodegeneration.

Table 5.1 Summary of behaviour experiments for study of the role of IL-1β in inflammation-induced cognitive dysfunction

- In diseased animals during LPS-induced cognitive dysfunction IL-1β is induced at equivalent levels to NBH animals in plasma. However, there is **exaggerated hippocampal IL-1β** mRNA expression
- **IL-1R1** is not essential for LPS-induced cognitive deficits in diseased animals
- **IL-1RA** offers protection from LPS-induced cognitive impairment in diseased animals
- **Systemic IL-1β** is sufficient to induce cognitive impairment in diseased animals

It is difficult to confirm the compartment in which IL-1RA exerted its protective effects against LPS-induced cognitive dysfunction; it may have been peripheral or central. However, it was shown that systemic administration of IL-1β is sufficient to elicit cognitive dysfunction and that systemic LPS challenge results in elevated systemic IL-1β. Thus, it is likely that blockade of systemic IL-1 signalling with IL-1RA offered some, if not all, of the observed protection from LPS-mediated impairment. Another data set presented here also indicates that cytokines in the systemic compartment contribute to cognitive impairment. That experiment demonstrated that systemic treatment of ME7-inoculated IL-1R1-/- with dexamethasone offers significant protection from LPS-induced cognitive deficits. We have previously shown that dexamethasone, under the same dosing regimen, successfully blocks systemic LPS-induced cytokine synthesis but does not prevent CNS induction of IL-1β (Chapter 4 and (Murray et al., 2011)). Accordingly, the protection offered by dexamethasone in the current study also demonstrates that systemic cytokine signalling contributes to systemic inflammation-induced cognitive impairments in diseased animals. Consequently, there is considerable evidence to support the argument that IL-1RA affords protection from endotoxin-induced cognitive impairment by blocking the action of systemic IL-1 signalling. It should be acknowledged, however, that IL-1RA treatment blocks IL-1α signalling along with IL-1β signalling. IL-1α is elevated by endotoxin challenge (Fantuzzi et al., 1996) and systemic administration has similar central effects to IL-1β (Anforth et al., 1998). As such, it can be stated that systemic IL-1β administration is sufficient to elicit the cognitive impairment and that blockade of systemic IL-1 signalling offers protection from endotoxin-induced impairment.

Table 5.2 Summary of the effects of systemic IL-1β injection on indicators of inflammation and sickness behaviour

Measure	ME7	ME7 + IL-1β
Plasma		
IL-1β		↑
IL-1RA		↑
PGEM		↑
CXCL1		↑
Corticosterone		↑
Hippocampal mRNA		
IL-1β	↑	↑↑↑
IL-6		↑
CXCL1		↑
TNF-α	↑	↑
PTX-3	↑	
iNOS		
IL-1RA	↑	↑
IL-1R1		
Il-1RII	↑	↑
NLRP3	↑	
Caspase-1		
COX-1	↑	
COX-2		
mPGES-1	↑	↑
mPGES-2		
cPGES		
HPGDS	↑	
LPGDS		↑
PGFS		
PGIS		↓
Hippocampal PGE2	↑	
Sickness Behaviour		
Locomotor Activity		↓
Temperature		↓
Hypothalamic mRNA		
IL-1β		↑
TNF-α	↑	↑
IL-6		↑

Despite the aforementioned evidence, it is possible that the protection offered by IL-1RA could be via direct CNS inhibition of signalling. Centrally administered IL-1RA has been shown to offer protection from peripheral infection mediated-deficits in memory consolidation in aged animals (Frank et al., 2010). Despite its large size (17 kDa), peripherally administered IL-1RA can cross the blood brain barrier (Banks and Kastin, 1991, Gutierrez et al., 1994), which may become more permeable in disease. This has been demonstrated *in vitro* to be via IL-1 receptors (Skinner et al., 2009). A study using PET imaging has shown a very low penetration of IL-1RA into the rat CNS when injected intravenously (Cawthorne et al., 2011). Indeed, only 0.01% of intravenously injected IL-1RA reaches the CSF in rats and humans after ischemia, but this dose was sufficient to be therapeutically effective (Clark et al., 2007). Whether a physiologically relevant dose might have entered the CNS to mitigate impairment in the present study is unclear, but we present robust evidence that the presence of systemic IL-1β can replicate the cognitive impairment.

There are two ostensibly plausible reasons for the time-dependent nature of IL-1RA protection. The lack of protection on the final block of trials may have resulted from the propagation of central mediators which are sufficient to inhibit cognitive functioning independent of IL-1 signalling. Direct activation, by systemic LPS, of blood-brain-barrier components, perivascular cells and endothelial cells, has been elegantly demonstrated (Chakravarty and Herkenham, 2005, Gosselin and Rivest, 2008, Serrats et al., 2010). Moreover, work from our laboratory has shown that systemic LPS results in CNS inflammatory gene transcription and IL-1β protein production despite abrogation of systemic cytokines with dexamethasone (Murray et al., 2011). Alternatively, the rapid renal metabolism of the IL-1RA protein (Cawthorne et al., 2011) may mean that levels fell below therapeutic efficacy during the T-maze experiment. IL-1RA has been reported to have a half-life between 6 (Dinarello, 1996) and 21 minutes (Granowitz et al., 1992, Cawthorne et al., 2011), which would mean that levels of IL-1RA were a tiny fraction of their original concentration by the 7th hour when deficits reappeared. The study by Clark and colleagues (2007), which demonstrated the protection from neuronal injury offered by systemic administration of IL-1RA, utilised a regime of intravenous bolus followed by infusion to maintain therapeutic doses. Interestingly, the incomplete protection offered by dexamethasone-administration in IL-1R1-/- animals was also absent by 7 hours post-challenge. If a higher dose or second administration of IL-1RA was shown to offer longer lasting protection than that observed in the current study it would indicate that systemic IL-1 signalling remains important beyond the duration demonstrated here.

Given the protection from LPS-mediated working memory impairment offered by IL-1RA administration and the ability of systemic IL-1β to induce cognitive dysfunction, it might have been expected that IL-1R1-/- animals would be less sensitive to LPS-induced cognitive impairments. However, IL-1R1-/- ME7-inoculated animals challenged with LPS showed robust impairment, which matched that experienced by wild type ME7 animals. However, it is clear that in LPS-induced sickness behaviour in IL-1R1-/- animals, TNF-α compensates for the absence of IL-1 signalling and replaces the fundamental role played by IL-1. Indeed, others have reported that IL-1RI-/- animals show the full spectrum of responses to systemic LPS, despite their lack of responsiveness to IL-1 (Bluthe et al., 2000). Given the importance of IL-1 signalling in immune responses (Dinarello, 1996), this compensation may be necessarily initiated at an early stage of development. It seems likely that in the current study one or multiple cytokines compensated for the absence of IL-1 activity in IL-1R1-/- animals. Unfortunately, Maze and colleagues, in their studies of IL-1-dependence of post-operative cognitive dysfunction, did not report whether IL-1R1-/- animals, like wild type animals treated with IL-1RA, were protected from memory dysfunction post-surgery (Cibelli et al., 2010). While the other behavioural results presented demonstrate that systemic IL-1 elevation is sufficient to induce cognitive dysfunction in ME7 animals, this particular result demonstrates that it is not essential.

Maze and others have recently investigated peripheral inflammation-induced memory impairment (Terrando et al., 2010b, Cibelli et al., 2010, Terrando et al., 2010a). These studies attempt to understand post-operative cognitive dysfunction and implicate systemic IL-1 signalling in impairment of memory consolidation using a trace fear conditioning task. They demonstrate LPS-induced deficits in consolidation at a single time point of acquisition in young healthy animals and show that IL-1RA administration protected from these consolidation deficits (Terrando et al., 2010b), Again utilising the same fear task, they have reported a role for IL-1β in memory dysfunction in a model of orthopaedic surgery. Systemic IL-1RA administration attenuated the inflammation induced by the tibial fracture and protected memory consolidation (Cibelli et al., 2010). These results are in line with previous work which has shown that peripheral and central IL-1β administration can impair memory consolidation (Pugh et al., 1998, Barrientos et al., 2002, Goshen et al., 2007). While other work has reported the ability of IL-1β to induce impairments of other facets of cognition (Oitzl et al., 1993, Gibertini et al., 1995), these studies are confounded by sickness behaviour responses induced by IL-1β (Cunningham and Sanderson, 2008). In contrast to

the studies assessing deficits in memory acquisition, the task utilised in the present study measures ongoing working memory impairment and demonstrates the ability of systemic IL-1β to induce acute impairment in animals with neurodegeneration but not normal animals.

5.4.2 Implications for Delirium and Dementia

Systemic inflammatory events can trigger episodes of delirium (Maclullich et al., 2008). Infection and sepsis in particular are well documented risk factors (George et al., 1997, Ebersoldt et al., 2007, van Gool et al., 2010). However, sterile inflammatory events elicited by surgery (Rogers et al., 1989, Rolfson et al., 1999, Kalisvaart et al., 2006, de Jonghe et al., 2007) and tissue trauma (Inouye and Charpentier, 1996, Dolan et al., 2000, Beloosesky et al., 2007, van Munster et al., 2008) can also result in delirium. Cytokines and IL-1β in particular play a fundamental role in the acute inflammatory response in both sterile and septic inflammation (Dinarello, 2000), yet the role of IL-1β in inflammation-induced delirium has not been addressed.

Murray et al (2012) describe a novel animal model for delirium during dementia. That study aimed to mimic a frequent biological context within which delirium is observed clinically; patients with neurodegenerative disease experiencing a systemic inflammatory event. A moderate dose of the bacterial endotoxin LPS (100 μg/kg), injected peripherally, was shown to elicit acute and transient cognitive dysfunction in ME7-inoculated animals. The present results validate this model's utility for delineation of the inflammatory mechanisms by which delirium can be elicited. By positing a significant role for IL-1 signalling in both septic and aseptic inflammation-induced acute cognitive dysfunction, the results identify a common signalling system, which can be investigated, and perhaps ultimately targeted therapeutically.

Normal animals, without CNS pathology, did not show cognitive impairment following systemic IL-1β challenge. This finding is in line with that of Murray et.al (2012), who showed that normal animals' T-maze performances were also unaffected by LPS-induced inflammation. Additionally in the present study, 14-week ME7-mice did not display cognitive impairment after systemic IL-1β challenge, but the same challenge in animals with 2 weeks further development of neurodegeneration elicited acute working memory impairment. Importantly, this increased

vulnerability in ME7 animals was not a result of a greater systemic inflammatory response compared to normal animals. Plasma IL-1β, IL-1RA, CXCL1, PGEM and corticosterone levels were equivalent in ME7 animals and normal animals challenged with IL-1β. Clinically, systemic IL-6, IL-8 and CRP have been associated with episodes of delirium (van Munster et al., 2010, de Rooij et al., 2007, Beloosesky et al., 2007). However, prior cognitive impairment is a greater risk factor for development of delirium and associations between systemic cytokines and delirium are not sufficiently robust to withstand adjustment for prior cognitive impairment (van Munster et al., 2010). The current study offers evidence to support the theory that ongoing CNS pathology and accompanying loss of brain reserve (Satz et al., 2011) sensitizes the brain to disruption of cognitive function upon environmental stressors (Chapter 4). As such, the results demonstrate how underlying CNS pathology is an important mediator of the relationship between systemic cytokines and delirium. This implies that symptoms of delirium after falls, surgery or infection in the elderly should not be viewed as a mere epiphenomenon of systemic tissue-damage or surgery-related inflammation, rather a potential indicator of previously masked neurodegenerative pathology.

Serum IL-1β is often undetectable in systemic auto-inflammatory diseases, yet blockage of IL-1 signalling has engendered rapid and sustained disease resolution in many of these conditions (Dinarello, 2005). This raises the possibility that problematic measurement of low circulating levels may conceal a role for IL-1β in delirium, the exacerbation of neurodegeneration and long-term cognitive decline. Systemic inflammation independent of delirium increases cognitive decline in Alzheimer's disease (Holmes et al., 2009). Systemic IL-1β levels have been associated with subsequent cognitive decline in the same dementia cohort (Holmes et al., 2003). A study of cognitively intact participants has shown that those whose peripheral mononuclear cells show higher spontaneous IL-1 production are at increased risk of subsequent Alzheimer's disease diagnosis (Tan et al., 2007). Hence, it seems that systemic inflammation, and IL-1β production in particular, might negatively impact upon a vulnerable CNS. Therefore, even relatively mundane systemic inflammation in the elderly should be carefully managed, regardless of whether delirium is present or absent.

Here systemic IL-1β has been shown to be capable of inducing acute impairment of cognition in animals with CNS pathology. As such, it models the interaction between sterile systemic inflammation and neurodegeneration. However, recent evidence has demonstrated critical sequential roles for IL-1α and IL-1β in the inflammatory response to necrotic cell death (Chen et

al., 2007, Rider et al., 2011). IL-1α from the dying cells initiates the inflammatory response and triggers neutrophil recruitment, while later IL-1β is essential for macrophage recruitment and propagation of the inflammatory response (Rider et al., 2011). This *in vitro* work suggests that IL-1β expression only becomes important days after the initial hypoxic damage. Delirium, however, can be present in hip fracture patients on admission (Dolan et al., 2000) and hence can develop acutely following tissue trauma. To further elucidate mechanisms underlying sterile inflammation-associated delirium it would be of interest to investigate the consequences of elevated systemic IL-1α in the model of delirium during dementia.

5.4.3 Acute neuroinflammation in ME7-diseased animals after systemic IL-1β challenge

An exaggerated neuroinflammatory response to systemic immune challenge has been demonstrated in diverse CNS disease models; Wallerian degeneration (Palin et al., 2008), transgenic models of Alzheimer's disease (Lee et al., 2002, Sly et al., 2001), amyotrophic lateral sclerosis (Nguyen et al., 2004) and Parkinson's disease (Villaran et al., 2010). Systemic elevation of IL-1β has been shown to alter microglial activation and induce markers of increased neuroinflammation in a 6-OHDA model of Parkinson's disease (Godoy et al., 2008). In a transgenic model of Alzheimer's disease, intra-articular over-expression of IL-1β, to induce osteoarthritis in the knees and temporomandibular joints, elicits exacerbated neuroinflammation. The elevated peripheral expression of IL-1β results in elevated central astrocyte and microglial activation in addition to elevated IL-1β and TNF-α transcription (Kyrkanides et al., 2011). Microglia, in particular, have been shown to be 'primed' in ME7-disease to produce a more robust response to subsequent inflammation. Systemic LPS administration generates detectable microglial IL-1β protein synthesis in the hippocampus of ME7-diseased animals but not in normal animals (Cunningham et al., 2005).

In the current study a primed hippocampal inflammatory response in ME7 animals was demonstrated. Systemic IL-1β challenge elicited exaggerated hippocampal IL-1β transcription in ME7 animals compared to normal animals at 2 hours post-challenge. Given the time point of exaggerated expression (2 hours) and the evidence that microglia in the ME7-dieased hippocampus are further activated by systemic LPS, it seems likely that microglia were the source of the 'primed' transcript, but this has yet to be demonstrated. While the study only assessed the acute CNS effects

of peripheral IL-1β elevation at one time point, it demonstrated exaggerated production of hippocampal IL-1β transcripts and a large body of evidence shows that elevated central IL-1β has deleterious and neurotoxic consequences (Allan et al., 2005). Increased hippocampal pro- or mature IL-1β levels cannot be inferred from transcript measures. However, given immunohistochemical evidence that exaggerated IL-1β mRNA levels after LPS challenge translates to increased protein production in the ME7-diseased hippocampus (Cunningham et al., 2005), it is reasonable to suggest that there would be increased IL-1β protein production accompanying the transcript increase after systemic IL-1β challenge. Other cytokines and inflammatory mediators were elevated in the hippocampus of both normal and diseased animals after challenge and can also exert damage (Block et al., 2007). In recent years, preclinical and clinical findings have suggested that systemic inflammation and IL-1β specifically can induce acceleration of CNS disease progression and cognitive decline. A single LPS dose results in more prolonged hippocampal inflammation than in normal animals (Murray et al., 2012), induces neuronal apoptosis (Cunningham et al., 2005) and accelerates progression of neurological impairment in ME7 disease (Cunningham et al., 2009). In the previously described model of Alzheimer's disease with osteoarthritis, amyloid plaques are increased in size and number by over-expression of IL-1β in the periphery (Kyrkanides et al., 2011). Systemic IL-1β also accelerates motor symptoms in a 6-OHDA model of Parkinson's disease (Godoy et al., 2008).

As expected, peripheral IL-1β induces sickness behaviour, in terms of reduced open field activity and drop in temperature. Somewhat surprisingly there is no exaggerated sickness behaviour or temperature drop in ME7 animals, given that previous studies show exaggerated decreases in open field movement and core body temperature in ME7 animals compared to normal animals after LPS challenge (Combrinck et al., 2002, Cunningham et al., 2009). The IL-1β challenge elicited exaggerated hypothalamic inflammation in terms of TNF-α and IL-6 but not IL-1β mRNA in ME7 animals. It can only be speculated that disease, which spreads from the area of injection in the hippocampus, had not yet affected the hypothalamus sufficiently to be more responsive to peripheral IL-1β challenge so as to induce exaggerated sickness behaviour and hypothermia. Interestingly, LPS challenge did not provoke a distinct response in ME7 animals at 8 weeks but did at 12 weeks (Combrinck et al., 2002, Cunningham et al., 2009). It may be that 2 further weeks of disease progression would result in an exaggerated response to systemic IL-1β challenge. The lack of exaggerated hypothalamic IL-1β transcription after systemic IL-1β challenge contrasts the exaggerated hippocampal IL-1β transcription. This may account for the failure to observe the exaggerated responses. Additionally, the single 2 hour time point at which the post-challenge

temperature response was assessed could have been too early; the LPS-induced exaggerated temperature reduction was observed 5 hours post-challenge (Cunningham et al., 2009).

If indeed active IL-1 is indeed acutely induced, the cytokine and disease-induced elevation of COX-1 may interact to induce the observed cognitive dysfunction. There is considerable evidence for a relationship between elevated hippocampal IL-1β, prostaglandins and cognitive impairment (Hein and O'Banion, 2009, Cunningham and Skelly, 2011). Elevation of hippocampal IL-1β is associated with impairment of cognition (Yirmiya and Goshen, 2011, Hein et al., 2007) and sustained elevation leads to contextual fear memory impairments in a COX-1-mediated manner (Matousek et al., 2010). Moreover, utilising the same test of cognition, in the previous chapter (Chapter 4) it was demonstrated that piroxicam, a COX-1 selective NSAID, offers protection from LPS-induced cognitive impairments in ME7 animals. COX isozymes are rate limiting enzymes but their product, prostaglandin G2 does not have a cognate receptor (Hein and O'Banion, 2009) and a range of prostaglandin synthases catalyse the conversion of PGG2 to various bioactive prostanoids. Observed increases in transcription of some of these synthases, mPGES-1 and LPGDS, after systemic IL-1β challenge and alterations in HPGDS in ME7-disease may be indicative of a role for prostaglandin E2 or D2 in the acute impairment.

The failure to show an IL-1β-related elevation in hippocampal PGE2 and the dearth of evidence in the literature for coupling between acutely induced mPGES-1 and COX-1 (Ueno et al., 2005) might indicate that PGE2 is not the key prostaglandin type in the acute episodes of cognitive impairment. However, mPGES-1-dependent prostaglandins have been demonstrated to have a role in mediating the central anorexic effects of peripherally administered IL-1β (Pecchi et al., 2006, Elander et al., 2007). Moreover, hippocampal administration of PGE2 induces memory impairment (Matsumoto et al., 2004). It may be that disease-elevated PGE2 may actually facilitate the signalling action of acutely elevated central cytokines (see chapter 4 and 6 for further discussion).

PGD2 is common in the CNS and has been shown to play an important role in sleep (Hayaishi, 2000). It acts on two receptors, DP1 and DP2 (Nagata et al., 1991) and is synthesised by HPGDS and LPGDS (Urade and Hayaishi, 2000). Here HPGDS is greatly increased in ME7-induced disease; it has been shown to be expressed on microglia surrounding senile plaques in models of Alzheimer's disease and the disease itself, and can couple with COX-1 (Mohri et al., 2007).

Furthermore, LPGDS (or β-trace) transcription was acutely induced in the hippocampus after systemic IL-1β administration in ME7-diseased animals in the present study. It is one of the most commonly expressed proteins in CSF (Hoffmann et al., 1993) and is expressed on perivascular and microglial cells (Nagasaka et al., 2004, Lee et al., 2012). While it is known to be induced by IL-1β elevation (Muraki et al., 2004), its coupling with the COX isozymes is as yet unclear (Ueno et al., 2005, Mathurin et al., 2011). These results suggest that central PGD2 may contribute to IL-1β-induced cognitive impairment. Overall, there is a need to further investigate the prostaglandin signalling system, and PGD2 and PGE2 in particular, in ME7 disease.

The potency of IL-1 signalling is evidenced by how IL-1β is uniquely regulated at a transcriptional, translational and maturation level (Dinarello, 2009). Here, both NLRP3 and caspase-1 transcripts are elevated in the hippocampus by disease, while hippocampal IL-1β mRNA is increased by peripheral acute challenge. Much of the cleavage of pro-IL-1β is mediated by caspase-1 (Thornberry et al., 1992) and the NLRP3-Caspase-1 inflammasome complex has been implicated in sterile inflammatory increases in mature IL-1β in general (Chen and Nuñez, 2010, Rock et al., 2010) and specifically in the CNS (Jha et al., 2010, Halle et al., 2008). Current framing of the mechanism for cleavage of pro-IL-1β from this inflammasome suggests that an NFκB-dependent formation of the complex precedes the sterile inflammatory signal, which triggers inflammasome activation and thus cleavage of pro-IL-1β (Rock et al., 2010). Whether the NLRP3 inflammasome complex ultimately results in the production of mature IL-1β in disease model used in the current study, where the activating (NFκB) and forming (a sterile factor of ME7-induced neurodegeneration) signals are reversed, warrants further investigation. It is important to establish whether there is increased protein synthesis of NLRP3 and caspase-1 in disease, since any secondary induction of pro-Il-1β protein, via systemic inflammation, could then be more readily processed to the active form.

As has been shown previously, PTX3 transcripts were elevated in the hippocampus of ME7-diseased animals (Murray et al., 2012). In that study, Murray and colleagues shown that both PTX3 and iNOS were elevated at 2 hours post-LPS challenge. Our group has evidence that PTX3 is downstream of IL-1β protein (Field et al., 2010, Murray et al., 2012) and iNOS appears to be NFκB-dependent (Xie et al., 1994). The failure to measure their increases in the current study could

plausibly indicate that systemic IL-1β challenge has not yet activated IL-1β protein synthesis and NFκB-dependent gene transcription and or that their acute elevation was missed. In chapter 3 systemic challenge with IL-1β elicited elevated hippocampal PTX3 and iNOS at 2 hours in normal animals yet in the current study neither gene was elevated by systemic IL-1β challenge in normal animals. Thus, these results run contrary to what would be expected and should be repeated with greater numbers in each group and further time points.

It has been argued that microglia are inherently anti-inflammatory under basal conditions but prone to exaggerated proinflammatory activation by additional immune challenge in many neurodegenerative diseases (Cunningham in press). The present study demonstrates disease-specific elevations of IL-1RA and IL-RII transcripts in the hippocampus which both mitigate IL-1β activity. These results may offer evidence of pathology-related mechanisms by which microglia attempt to dampen IL-1β activity. The active receptor IL-1R1 is not altered by disease pathology, although previous studies at 12 weeks post- ME7 inoculation have demonstrated an increase in IL-1RI transcription (Murray et al., 2012). It is of interest to know whether there are disease stage-specific changes in IL-1RI expression.

5.3.4 Summary

IL-1β, a mediator of both septic and aseptic inflammation plays an important role in acute systemic inflammation-induced cognitive impairment during neurodegenerative disease. Simultaneous treatment of ME7 animals with IL-1RA (10 mg/kg) offered significant protection from LPS (100 μg/kg-)-induced acute cognitive impairment. However, IL-1RI -/- animals inoculated with ME7 disease were still prone to profound cognitive impairment after LPS treatment. Peripheral administration of IL-1β (15 μg/kg) resulted in acute and transient cognitive impairment, and exaggerated hippocampal IL-1β mRNA transcription, in ME7 but not normal animals. Systemic IL-1β is sufficient to elicit, but not essential in, both sterile and endotoxin inflammation-induced acute episodes of cognitive dysfunction in animals with CNS pathology.

References

ABBAS, A. K., LICHTMAN, A. H. & BAKER, D. L. 2004. *Basic immunology: functions and disorders of the immune system*, Saunders.

ABUL, H. T., DAVIDSON, J., MILTON, A. S. & ROTONDO, D. 1997. Prostaglandin E2 Enters the Brain following Stimulation of the Acute Phase Immune Responsea. *Annals of the New York Academy of Sciences,* 813**,** 287-295.

ADCOCK, I. M., NASUHARA, Y., STEVENS, D. A. & BARNES, P. J. 1999. Ligand-induced differentiation of glucocorticoid receptor (GR) trans-repression and transactivation: preferential targetting of NF-κB and lack of I-κB involvement. *Br J Pharmacol,* 127**,** 1003-1011.

AHMAD, A. S., SALEEM, S., AHMAD, M. & DORE, S. 2006a. Prostaglandin EP1 receptor contributes to excitotoxicity and focal ischemic brain damage. *Toxicol Sci,* 89**,** 265-70.

AHMAD, A. S., ZHUANG, H., ECHEVERRIA, V. & DORE, S. 2006b. Stimulation of prostaglandin EP2 receptors prevents NMDA-induced excitotoxicity. *J Neurotrauma,* 23**,** 1895-903.

AISEN, P. S., SCHAFER, K. A., GRUNDMAN, M., PFEIFFER, E., SANO, M., DAVIS, K. L., FARLOW, M. R., JIN, S., THOMAS, R. G. & THAL, L. J. 2003. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. *JAMA,* 289**,** 2819-26.

AISEN, P. S., SCHMEIDLER, J. & PASINETTI, G. M. 2002. Randomized pilot study of nimesulide treatment in Alzheimer's disease. *Neurology,* 58**,** 1050-4.

AISEN, P. S., THAL, L. J., FERRIS, S. H., ASSAID, C., NESSLY, M. L., GIULIANI, M. J., LINES, C. R., NORMAN, B. A. & POTTER, W. Z. 2008. Rofecoxib in patients with mild cognitive impairment: further analyses of data from a randomized, double-blind, trial. *Curr Alzheimer Res,* 5**,** 73-82.

ALLAN, S. M., TYRRELL, P. J. & ROTHWELL, N. J. 2005. Interleukin-1 and neuronal injury. *Nat Rev Immunol,* 5**,** 629-640.

ANDREASSON, K. 2010. Emerging roles of PGE2 receptors in models of neurological disease. *Prostaglandins Other Lipid Mediat,* 91**,** 104-12.

ANFORTH, H. R., BLUTHE, R. M., BRISTOW, A., HOPKINS, S., LENCZOWSKI, M. J., LUHESHI, G., LUNDKVIST, J., MICHAUD, B., MISTRY, Y., VAN DAM, A. M., ZHEN, C., DANTZER, R., POOLE, S., ROTHWELL, N. J., TILDERS, F. J. & WOLLMAN, E. E. 1998. Biological activity and brain actions of recombinant rat interleukin-1alpha and interleukin-1beta. *Eur Cytokine Netw,* 9**,** 279-88.

APA 1994. *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV),* Washington, DC, American Psychiatry Association.

BAKER, B. J., AKHTAR, L. N. & BENVENISTE, E. N. 2009. SOCS1 and SOCS3 in the control of CNS immunity. *Trends Immunol,* 30**,** 392-400.

BAMJI, S. X., MAJDAN, M., POZNIAK, C. D., BELLIVEAU, D. J., ALOYZ, R., KOHN, J., CAUSING, C. G. & MILLER, F. D. 1998. The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *The Journal of cell biology,* 140**,** 911-923.

BANKS, W. A. & KASTIN, A. J. 1991. Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sci,* 48**,** PL117-21.

BANKS, W. A. & ROBINSON, S. M. 2010. Minimal penetration of lipopolysaccharide across the murine blood–brain barrier. *Brain Behav Immun,* 24**,** 102-109.

BARNES, P. J. 1998. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clinical Science,* 94**,** 557-572.

BARRETT, A., BURKE, H., CRONIN, H., HICKEY, A., KAMIYA, Y., KENNY, R. A., LAYTE, R., MATY, S., MCGEE, H. & MORGAN, K. 2011. Fifty Plus in Ireland 2011: First Results from the Irish Longitudinal Study on Ageing (TILDA).

BARRIENTOS, R. M., FRANK, M. G., HEIN, A. M., HIGGINS, E. A., WATKINS, L. R., RUDY, J. W. & MAIER, S. F. 2009. Time course of hippocampal IL-1 beta and memory consolidation impairments in aging rats following peripheral infection. *Brain Behav Immun,* 23**,** 46-54.

BARRIENTOS, R. M., HIGGINS, E. A., BIEDENKAPP, J. C., SPRUNGER, D. B., WRIGHT-HARDESTY, K. J., WATKINS, L. R., RUDY, J. W. & MAIER, S. F. 2006. Peripheral infection and aging interact to impair hippocampal memory consolidation. *Neurobiology of Aging,* 27**,** 723-732.

BARRIENTOS, R. M., HIGGINS, E. A., SPRUNGER, D. B., WATKINS, L. R., RUDY, J. W. & MAIER, S. F. 2002. Memory for context is impaired by a post context exposure injection of interleukin-1 beta into dorsal hippocampus. *Behav Brain Res,* 134**,** 291-8.

BAUER, J., STRAUSS, S., SCHREITER-GASSER, U., GANTER, U., SCHLEGEL, P., WITT, I., YOLK, B. & BERGER, M. 1991. Interleukin-6 and alpha-2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. *FEBS letters,* 285**,** 111.

BELLELLI, G. & TRABUCCHI, M. 2010. Diagnosing delirium. *JAMA,* 304**,** 2124-5; author reply 2126-7.
BELLINGER, F. P., MADAMBA, S. & SIGGINS, G. R. 1993. Interleukin 1 [beta] inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Research,* 628**,** 227-234.
BELLINGER, F. P., MADAMBA, S. G., CAMPBELL, I. L. & SIGGINS, G. R. 1995. Reduced long-term potentiation in the dentate gyrus of transgenic mice with cerebral overexpression of interleukin-6. *Neurosci Lett,* 198**,** 95-8.
BELOOSESKY, Y., HENDEL, D., WEISS, A., HERSHKOVITZ, A., GRINBLAT, J., PIROTSKY, A. & BARAK, V. 2007. Cytokines and C-reactive protein production in hip-fracture-operated elderly patients. *J Gerontol A Biol Sci Med Sci,* 62**,** 420-6.
BESEDOVSKY, H., DEL REY, A., SORKIN, E. & DINARELLO, C. A. 1986. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science,* 233**,** 652-654.
BETMOUNI, S. & PERRY, V. 1999. The acute inflammatory response in CNS following injection of prion brain homogenate or normal brain homogenate. *Neuropathology and applied neurobiology,* 25**,** 20-28.
BLACK, R. A., RAUCH, C. T., KOZLOSKY, C. J., PESCHON, J. J., SLACK, J. L., WOLFSON, M. F., CASTNER, B. J., STOCKING, K. L., REDDY, P. & SRINIVASAN, S. 1997. A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells.
BLANCO, F. J., GUITIAN, R., MORENO, J., DE TORO, F. J. & GALDO, F. 1999. Effect of antiinflammatory drugs on COX-1 and COX-2 activity in human articular chondrocytes. *The Journal of rheumatology,* 26**,** 1366-73.
BLATTEIS, C. M., LI, S., LI, Z., PERLIK, V. & FELEDER, C. 2004. Signaling the brain in systemic inflammation: the role of complement. *Frontiers in Bioscience,* 9.
BLOCK, M. L., ZECCA, L. & HONG, J. S. 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci,* 8**,** 57-69.
BLUM-DEGENA, D., MÜLLER, T., KUHN, W., GERLACH, M., PRZUNTEK, H. & RIEDERER, P. 1995. Interleukin-1 [beta] and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. *Neuroscience Letters,* 202**,** 17-20.
BLUTHE, R. M., LAYE, S., MICHAUD, B., COMBE, C., DANTZER, R. & PARNET, P. 2000a. Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. *Eur J Neurosci,* 12**,** 4447-56.
BLUTHE, R. M., MICHAUD, B., POLI, V. & DANTZER, R. 2000b. Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. *Physiol Behav,* 70**,** 367-73.
BLUTHÉ, R. M., PAWLOWSKI, M., SUAREZ, S., PARNET, P., PITTMAN, Q., KELLEY, K. W. & DANTZER, R. 1994. Synergy between tumor necrosis factor α and interleukin-1 in the induction of sickness behavior in mice. *Psychoneuroendocrinology,* 19**,** 197-207.
BOETTGER, S., PASSIK, S. & BREITBART, W. 2011. Treatment characteristics of delirium superimposed on dementia. *International psychogeriatrics,* 1**,** 1-6.
BOSETTI, F. 2007. Arachidonic acid metabolism in brain physiology and pathology: lessons from genetically altered mouse models. *J Neurochem,* 102**,** 577-86.
BOYLE, P., WILSON, R., AGGARWAL, N., TANG, Y. & BENNETT, D. 2006. Mild cognitive impairment. *Neurology,* 67**,** 441-445.
BOZZA, F. A., GARTEISER, P., OLIVEIRA, M. F., DOBLAS, S., CRANFORD, R., SAUNDERS, D., JONES, I., TOWNER, R. A. & CASTRO-FARIA-NETO, H. C. 2009. Sepsis-associated encephalopathy: a magnetic resonance imaging and spectroscopy study. *Journal of Cerebral Blood Flow & Metabolism,* 30**,** 440-448.
BRAAK, H. & BRAAK, E. 1997. Diagnostic criteria for neuropathologic assessment of Alzheimer's disease. *Neurobiol Aging,* 18**,** S85-8.
BREITBART, W., GIBSON, C. & TREMBLAY, A. 2002. The delirium experience: delirium recall and delirium-related distress in hospitalized patients with cancer, their spouses/caregivers, and their nurses. *Psychosomatics,* 43**,** 183-194.
BROWN, L. J. E., FERNER, H. S., ROBERTSON, J., MILLS, N. L., PESSOTTO, R., DEARY, I. J. & MACLULLICH, A. M. J. 2011a. Differential effects of delirium on fluid and crystallized cognitive abilities. *Archives of gerontology and geriatrics,* 52**,** 153-158.
BROWN, L. J. E., FORDYCE, C., ZAGHDANI, H., STARR, J. M. & MACLULLICH, A. M. J. 2011b. Detecting deficits of sustained visual attention in delirium. *Journal of Neurology, Neurosurgery & Psychiatry,* 82**,** 1334-1340.

BRUCCOLERI, A., PENNYPACKER, K. R. & HARRY, G. J. 1999. Effect of dexamethasone on elevated cytokine mRNA levels in chemical-induced hippocampal injury. *Journal of neuroscience research,* 57, 916-926.

BRUGG, B., DUBREUIL, Y. L., HUBER, G., WOLLMAN, E. E., DELHAYE-BOUCHAUD, N. & MARIANI, J. 1995. Inflammatory processes induce beta-amyloid precursor protein changes in mouse brain. *Proc Natl Acad Sci U S A,* 92, 3032-5.

BUHARALIOGLU, C. K., KORKMAZ, B., CUEZ, T., SAHAN-FIRAT, S., SARI, A. N., MALIK, K. U. & TUNCTAN, B. 2011. Piroxicam Reverses Endotoxin-Induced Hypotension in Rats: Contribution of Vasoactive Eicosanoids and Nitric Oxide. *Basic Clin Pharmacol Toxicol.*

BUSHNELL, P. & STRUPP, B. 2009. Assessing Attention in Rodents.

CACABELOS, R., ALVAREZ, X., FERNANDEZ-NOVOA, L., FRANCO, A., MANGUES, R., PELLICER, A. & NISHIMURA, T. 1994. Brain interleukin-1 beta in Alzheimer's disease and vascular dementia. *Methods and findings in experimental and clinical pharmacology,* 16, 141.

CACABELOS, R., BARQUERO, M., GARCIA, P., ALVAREZ, X. & VARELA, S. E. 1991. Cerebrospinal fluid interleukin-1 beta (IL-1 beta) in Alzheimer's disease and neurological disorders. *Methods and findings in experimental and clinical pharmacology,* 13, 455.

CAMPBELL, I. L., ABRAHAM, C. R., MASLIAH, E., KEMPER, P., INGLIS, J. D., OLDSTONE, M. & MUCKE, L. 1993. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proceedings of the National Academy of Sciences,* 90, 10061.

CAMPBELL, S. J., MEIER, U., MARDIGUIAN, S., JIANG, Y., LITTLETON, E. T., BRISTOW, A., RELTON, J., CONNOR, T. J. & ANTHONY, D. C. 2010. Sickness behaviour is induced by a peripheral CXC-chemokine also expressed in multiple sclerosis and EAE. *Brain Behav Immun,* 24, 738-46.

CARSWELL, E., OLD, L. J., KASSEL, R. L., GREEN, S., FIORE, N. & WILLIAMSON, B. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proceedings of the National Academy of Sciences,* 72, 3666.

CASTLE, M., COMOLI, E. & LOEWY, A. 2005. Autonomic brainstem nuclei are linked to the hippocampus. *Neuroscience,* 134, 657-669.

CAWTHORNE, C., PRENANT, C., SMIGOVA, A., JULYAN, P., MAROY, R., HERHOLZ, K., ROTHWELL, N. & BOUTIN, H. 2011. Biodistribution, pharmacokinetics and metabolism of interleukin-1 receptor antagonist (IL-1RA) using [18F]-IL1RA and PET imaging in rats. *Br J Pharmacol,* 162, 659-672.

CECILIANI, F., GIORDANO, A. & SPAGNOLO, V. 2002. The systemic reaction during inflammation: the acute-phase proteins. *Protein Pept Lett,* 9, 211-23.

CEREJEIRA, J., FIRMINO, H., VAZ-SERRA, A. & MUKAETOVA-LADINSKA, E. B. 2010. The neuroinflammatory hypothesis of delirium. *Acta Neuropathol,* 119, 737-54.

CHAI, Z., GATTI, S., TONIATTI, C., POLI, V. & BARTFAI, T. 1996. Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J Exp Med,* 183, 311-316.

CHAKRAVARTY, S. & HERKENHAM, M. 2005. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. *J Neurosci,* 25, 1788-96.

CHANG, Y.-L., TSAI, Y.-F., LIN, P.-J., CHEN, M.-C. & LIU, C.-Y. 2008. Prevalence and Risk Factors for Postoperative Delirium in a Cardiovascular Intensive Care Unit. *American Journal of Critical Care,* 17, 567-575.

CHAPMAN, T. R., BARRIENTOS, R. M., AHRENDSEN, J. T., MAIER, S. F. & PATTERSON, S. L. 2010. Synaptic Correlates of Increased Cognitive Vulnerability with Aging: Peripheral Immune Challenge and Aging Interact to Disrupt Theta-Burst Late-Phase Long-Term Potentiation in Hippocampal Area CA1. *The Journal of Neuroscience,* 30, 7598-7603.

CHAUDHRY, U., ZHUANG, H. & DORÉ, S. 2010. Microsomal prostaglandin E synthase-2: Cellular distribution and expression in Alzheimer's disease. *Experimental neurology,* 223, 359-365.

CHAUDHRY, U. A. & DORE, S. 2009. Cytosolic Prostaglandin E Synthase: Expression Patterns in Control and Alzheimer's Disease Brains. *American journal of Alzheimer's disease and other dementias,* 24, 46-51.

CHAUDHRY, U. A., ZHUANG, H., CRAIN, B. J. & DORÉ, S. 2008. Elevated microsomal prostaglandin-E synthase–1 in Alzheimer's disease. *Alzheimer's and Dementia,* 4, 6-13.

CHEN, C.-J., KONO, H., GOLENBOCK, D., REED, G., AKIRA, S. & ROCK, K. L. 2007. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med,* 13, 851-856.

CHEN, C., MAGEE, J. C. & BAZAN, N. G. 2002. Cyclooxygenase-2 Regulates Prostaglandin E2 Signaling in Hippocampal Long-Term Synaptic Plasticity. *J Neurophysiol,* 87, 2851-2857.

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

CHEN, G. Y. & NUÑEZ, G. 2010. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol,* 10, 826-837.

CHEN, H., O'REILLY, E. J., SCHWARZSCHILD, M. A. & ASCHERIO, A. 2008a. Peripheral Inflammatory Biomarkers and Risk of Parkinson's Disease. *American Journal of Epidemiology,* 167, 90-95.

CHEN, J., BUCHANAN, J. B., SPARKMAN, N. L., GODBOUT, J. P., FREUND, G. G. & JOHNSON, R. W. 2008b. Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system. *Brain Behav Immun,* 22, 301-11.

CHEN, L.-C., CHEN, C.-C., LIANG, Y., TSANG, N.-M., CHANG, Y.-S. & HSUEH, C. 2011. A novel role for TNFAIP2: its correlation with invasion and metastasis in nasopharyngeal carcinoma. *Mod Pathol,* 24, 175-184.

CHENG, K., WU, T. J., WU, K. K., STURINO, C., METTERS, K., GOTTESDIENER, K., WRIGHT, S. D., WANG, Z., O'NEILL, G. & LAI, E. 2006. Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. *Proceedings of the National Academy of Sciences,* 103, 6682-6687.

CHOI, S. H., AID, S. & BOSETTI, F. 2009. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. *Trends Pharmacol Sci,* 30, 174-81.

CHOI, S. H. & BOSETTI, F. 2009. Cyclooxygenase-1 null mice show reduced neuroinflammation in response to beta-amyloid. *Aging (Albany NY),* 1, 234-44.

CHOI, S. H., LANGENBACH, R. & BOSETTI, F. 2006. Cyclooxygenase-1 and -2 enzymes differentially regulate the brain upstream NF-kappa B pathway and downstream enzymes involved in prostaglandin biosynthesis. *J Neurochem,* 98, 801-11.

CHOI, S. H., LANGENBACH, R. & BOSETTI, F. 2008. Genetic deletion or pharmacological inhibition of cyclooxygenase-1 attenuate lipopolysaccharide-induced inflammatory response and brain injury. *FASEB J,* 22, 1491-501.

CHURCH, L. D., COOK, G. P. & MCDERMOTT, M. F. 2008. Primer: inflammasomes and interleukin 1beta in inflammatory disorders. *Nat Clin Pract Rheumatol,* 4, 34-42.

CIBELLI, M., FIDALGO, A. R., TERRANDO, N., MA, D., MONACO, C., FELDMANN, M., TAKATA, M., LEVER, I. J., NANCHAHAL, J., FANSELOW, M. S. & MAZE, M. 2010. Role of interleukin-1beta in postoperative cognitive dysfunction. *Ann Neurol,* 68, 360-8.

CLARK, K., PEGGIE, M., PLATER, L., SORCEK, R. J., YOUNG, E. R., MADWED, J. B., HOUGH, J., MCIVER, E. G. & COHEN, P. 2011. Novel cross-talk within the IKK family controls innate immunity. *Biochem J,* 434, 93-104.

CLARK, K., PLATER, L., PEGGIE, M. & COHEN, P. 2009. Use of the Pharmacological Inhibitor BX795 to Study the Regulation and Physiological Roles of TBK1 and IκB Kinase ϵ. *Journal of Biological Chemistry,* 284, 14136-14146.

CLARK, S. R., MCMAHON, C. J., GUEORGUIEVA, I., ROWLAND, M., SCARTH, S., GEORGIOU, R., TYRRELL, P. J., HOPKINS, S. J. & ROTHWELL, N. J. 2007. Interleukin-1 receptor antagonist penetrates human brain at experimentally therapeutic concentrations. *J Cereb Blood Flow Metab,* 28, 387-394.

CLEGG, A., WESTBY, M. & YOUNG, J. B. 2011. Under-reporting of delirium in the NHS. *Age Ageing,* 40, 283-6.

COLE, M. G. 2004. Delirium in elderly patients. *Am J Geriatr Psychiatry,* 12, 7-21.

COLE, M. G., CIAMPI, A., BELZILE, E. & ZHONG, L. 2009. Persistent delirium in older hospital patients: a systematic review of frequency and prognosis. *Age Ageing,* 38, 19-26.

COLLINS, O., DILLON, S., FINUCANE, C., LAWLOR, B. & KENNY, R. A. 2011. Parasympathetic autonomic dysfunction is common in mild cognitive impairment. *Neurobiology of Aging*.

COLONNA, M. 2007. TLR pathways and IFN-regulatory factors: to each its own. *Eur J Immunol,* 37, 306-9.

COMA, M., SERENO, L., DA ROCHA-SOUTO, B., SCOTTON, T. C., ESPANA, J., SANCHEZ, M. B., RODRIGUEZ, M., AGULLO, J., GUARDIA-LAGUARTA, C., GARCIA-ALLOZA, M., BORRELLI, L. A., CLARIMON, J., LLEO, A., BACSKAI, B. J., SAURA, C. A., HYMAN, B. T. & GOMEZ-ISLA, T. 2010. Triflusal reduces dense-core plaque load, associated axonal alterations and inflammatory changes, and rescues cognition in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis,* 38, 482-91.

COMBRINCK, M., WILLIAMS, J., DE BERARDINIS, M. A., WARDEN, D., PUOPOLO, M., SMITH, A. D. & MINGHETTI, L. 2006. Levels of CSF prostaglandin E2, cognitive decline, and survival in Alzheimer's disease. *J Neurol Neurosurg Psychiatry,* 77, 85-8.

COMBRINCK, M. I., PERRY, V. H. & CUNNINGHAM, C. 2002. Peripheral infection evokes exaggerated sickness behaviour in pre-clinical murine prion disease. *Neuroscience,* 112, 7-11.

COWLEY, T. R., FAHEY, B. & O'MARA, S. M. 2008. COX-2, but not COX-1, activity is necessary for the induction of perforant path long-term potentiation and spatial learning in vivo. *Eur J Neurosci,* 27, 2999-3008.

CROFFORD, L. 1997. COX-1 and COX-2 tissue expression: implications and predictions. *The Journal of rheumatology. Supplement,* 49, 15.

CROKER, B. A., KIU, H., PELLEGRINI, M., TOE, J., PRESTON, S., METCALF, D., O'DONNELL, J. A., CENGIA, L. H., MCARTHUR, K., NICOLA, N. A., ALEXANDER, W. S. & ROBERTS, A. W. 2012. IL-6 promotes acute and chronic inflammatory disease in the absence of SOCS3. *Immunol Cell Biol,* 90, 124-129.

CUNNINGHAM, A. J., MURRAY, C. A., O'NEILL, L. A. J., LYNCH, M. A. & O'CONNOR, J. J. 1996. Interleukin-1β (IL-1β) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. *Neuroscience Letters,* 203, 17-20.

CUNNINGHAM, C. 2011. Systemic inflammation and delirium - important co-factors in the progression of dementia. *Biochemical Society Transactions*.

CUNNINGHAM, C. In Press. Microglia and neurodegeneration: the role of systemic inflammation. *Glia*.

CUNNINGHAM, C., CAMPION, S., LUNNON, K., MURRAY, C. L., WOODS, J. F., DEACON, R. M., RAWLINS, J. N. & PERRY, V. H. 2009a. Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. *Biol Psychiatry,* 65, 304-12.

CUNNINGHAM, C., DEACON, R., WELLS, H., BOCHE, D., WATERS, S., DINIZ, C. P., SCOTT, H., RAWLINS, J. N. & PERRY, V. H. 2003. Synaptic changes characterize early behavioural signs in the ME7 model of murine prion disease. *Eur J Neurosci,* 17, 2147-55.

CUNNINGHAM, C. & MACLULLICH, A. M. In Press. At the extreme end of the psychoneuroimmunological spectrum: Delirium as a a maladaptive sickness behaviour response. *Brain Behav Immun*.

CUNNINGHAM, C. & SANDERSON, D. J. 2008. Malaise in the water maze: untangling the effects of LPS and IL-1beta on learning and memory. *Brain Behav Immun,* 22, 1117-27.

CUNNINGHAM, C. & SKELLY, D. T. 2011. Non-Steroidal Anti-Inflammatory Drugs and Cognitive Function: Are Prostaglandins at the Heart of Cognitive Impairment in Dementia and Delirium ? *J Neuroimmune Pharmacol*.

CUNNINGHAM, C., WILCOCKSON, D. C., CAMPION, S., LUNNON, K. & PERRY, V. H. 2005. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci,* 25, 9275-84.

CUNNINGHAM, O., CAMPION, S., PERRY, V. H., MURRAY, C., SIDENIUS, N., DOCAGNE, F. & CUNNINGHAM, C. 2009b. Microglia and the urokinase plasminogen activator receptor/uPA system in innate brain inflammation. *Glia,* 57, 1802-14.

DANTZER, R. 2001a. Cytokine-induced sickness behavior: where do we stand? *Brain Behav Immun,* 15, 7-24.

DANTZER, R. 2001b. Cytokine-Induced Sickness Behavior: Mechanisms and Implications. *Annals of the New York Academy of Sciences,* 933, 222-234.

DANTZER, R. 2004. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol,* 500, 399-411.

DANTZER, R. 2009. Cytokine, Sickness Behavior, and Depression. *Immunology and Allergy Clinics of North America,* 29, 247-264.

DANTZER, R. & KELLEY, K. W. 2007. Twenty years of research on cytokine-induced sickness behavior. *Brain Behav Immun,* 21, 153-160.

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

DANTZER, R., O'CONNOR, J. C., LAWSON, M. A. & KELLEY, K. W. 2011. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology,* 36, 426-36.

DAVIDSON, J., ABUL, H., MILTON, A. & ROTONDO, D. 2001. Cytokines and cytokine inducers stimulate prostaglandin E 2 entry into the brain. *Pflügers Archiv European Journal of Physiology,* 442, 526-533.

DAVIES, P. & MALONEY, A. J. 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet,* 2, 1403.

DE JONGHE, J. F., KALISVAART, K. J., DIJKSTRA, M., VAN DIS, H., VREESWIJK, R., KAT, M. G., EIKELENBOOM, P., VAN DER PLOEG, T. & VAN GOOL, W. A. 2007. Early symptoms in the prodromal phase of delirium: a prospective cohort study in elderly patients undergoing hip surgery. *Am J Geriatr Psychiatry,* 15, 112-21.

DE ROOIJ, S. E., VAN MUNSTER, B. C., KOREVAAR, J. C. & LEVI, M. 2007a. Cytokines and acute phase response in delirium. *J Psychosom Res,* 62, 521-5.

DE ROOIJ, S. E., VAN MUNSTER, B. C., KOREVAAR, J. C. & LEVI, M. 2007b. Cytokines and acute phase response in delirium. *Journal of Psychosomatic Research,* 62, 521-525.

DEININGER, M. H., BEKURE-NEMARIAM, K., TRAUTMANN, K., MORGALLA, M., MEYERMANN, R. & SCHLUESENER, H. J. 2003. Cyclooxygenase-1 and -2 in brains of patients who died with sporadic Creutzfeldt-Jakob disease. *J Mol Neurosci,* 20, 25-30.

DEKOSKY, S. T. & SCHEFF, S. W. 1990. Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Ann Neurol,* 27, 457-464.

DESFORGES, J. F. & LIPOWSKI, Z. J. 1989. Delirium in the elderly patient. *New England Journal of Medicine,* 320, 578-582.

DICKSON, D. W., LEE, S. C., MATTIACE, L. A., YEN, S. H. C. & BROSNAN, C. 1993. Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. *Glia,* 7, 75-83.

DIK, M. G., JONKER, C., HACK, C. E., SMIT, J. H., COMIJS, H. C. & EIKELENBOOM, P. 2005. Serum inflammatory proteins and cognitive decline in older persons. *Neurology,* 64, 1371-7.

DINARELLO, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood,* 87, 2095-147.

DINARELLO, C. A. 2000. Proinflammatory Cytokines*. *Chest,* 118, 503-508.

DINARELLO, C. A. 2005. Blocking IL-1 in systemic inflammation. *J Exp Med,* 201, 1355-1359.

DINARELLO, C. A. 2009. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol,* 27, 519-50.

DOLAN, M. M., HAWKES, W. G., ZIMMERMAN, S. I., MORRISON, R. S., GRUBER-BALDINI, A. L., HEBEL, J. R. & MAGAZINER, J. 2000. Delirium on Hospital Admission in Aged Hip Fracture Patients. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 55, M527-M534.

DOWLATI, Y., HERRMANN, N., SWARDFAGER, W., LIU, H., SHAM, L., REIM, E. K. & LANCTOT, K. L. 2010. A meta-analysis of cytokines in major depression. *Biol Psychiatry,* 67, 446-57.

DUBOIS, B., FELDMAN, H. H., JACOVA, C., CUMMINGS, J. L., DEKOSKY, S. T., BARBERGER-GATEAU, P., DELACOURTE, A., FRISONI, G., FOX, N. C. & GALASKO, D. 2010. Revising the definition of Alzheimer's disease: a new lexicon. *The Lancet Neurology,* 9, 1118-1127.

DUCROS, A., BOUKOBZA, M., PORCHER, R., SAROV, M., VALADE, D. & BOUSSER, M.-G. 2007. The clinical and radiological spectrum of reversible cerebral vasoconstriction syndrome. A prospective series of 67 patients. *Brain,* 130, 3091-3101.

DUNKIN, J. J. & ANDERSON-HANLEY, C. 1998. Dementia caregiver burden. *Neurology,* 51, S53-S60.

DYER, C. B., ASHTON, C. M. & TEASDALE, T. A. 1995. Postoperative delirium: a review of 80 primary data-collection studies. *Archives of Internal Medicine,* 155, 461.

EBERSOLDT, M., SHARSHAR, T. & ANNANE, D. 2007. Sepsis-associated delirium. *Intensive Care Med,* 33, 941-50.

EGUCHI, N., KANEKO, T., URADE, Y., HAYASHI, H. & HAYAISHI, O. 1992. Permeability of brain structures and other peripheral tissues to prostaglandins D2, E2 and F2 alpha in rats. *Journal of Pharmacology and Experimental Therapeutics,* 262, 1110-1120.

EK, M., ENGBLOM, D., SAHA, S., BLOMQVIST, A., JAKOBSSON, P. J. & ERICSSON-DAHLSTRAND, A. 2001. Inflammatory response: pathway across the blood-brain barrier. *Nature,* 410, 430-1.

EK, M., KUROSAWA, M., LUNDEBERG, T. & ERICSSON, A. 1998. Activation of Vagal Afferents after Intravenous Injection of Interleukin-1β: Role of Endogenous Prostaglandins. *The Journal of Neuroscience,* 18, 9471-9479.

ELANDER, L., ENGSTROM, L., HALLBECK, M. & BLOMQVIST, A. 2007. IL-1beta and LPS induce anorexia by distinct mechanisms differentially dependent on microsomal prostaglandin E synthase-1. *Am J Physiol Regul Integr Comp Physiol,* 292, R258-267.

ELANDER, L., ENGSTROM, L., RUUD, J., MACKERLOVA, L., JAKOBSSON, P. J., ENGBLOM, D., NILSBERTH, C. & BLOMQVIST, A. 2009a. Inducible prostaglandin E2 synthesis interacts in a temporally supplementary sequence with constitutive prostaglandin-synthesizing enzymes in creating the hypothalamic-pituitary-adrenal axis response to immune challenge. *J Neurosci,* 29, 1404-13.

ELANDER, L., ENGSTRÖM, L., RUUD, J., MACKERLOVA, L., JAKOBSSON, P. J., ENGBLOM, D., NILSBERTH, C. & BLOMQVIST, A. 2009b. Inducible Prostaglandin E2 Synthesis Interacts in a Temporally Supplementary Sequence with Constitutive Prostaglandin-Synthesizing Enzymes in Creating the Hypothalamic–Pituitary–Adrenal Axis Response to Immune Challenge. *The Journal of Neuroscience,* 29, 1404-1413.

ELANDER, L., RUUD, J., KOROTKOVA, M., JAKOBSSON, P. J. & BLOMQVIST, A. 2010. Cyclooxygenase-1 mediates the immediate corticosterone response to peripheral immune challenge induced by lipopolysaccharide. *Neuroscience Letters,* 470, 10-12.

ELDER, G. A., GAMA SOSA, M. A. & DE GASPERI, R. 2010. Transgenic Mouse Models of Alzheimer's Disease. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine,* 77, 69-81.

ELENKOV, I. J., WILDER, R. L., CHROUSOS, G. P. & VIZI, E. S. 2000. The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacological reviews,* 52, 595-638.

ELIE, M., COLE, M. G., PRIMEAU, F. J. & BELLAVANCE, F. 1998. Delirium Risk Factors in Elderly Hospitalized Patients. *Journal of General Internal Medicine,* 13, 204-212.

ELY, E. W., SHINTANI, A., TRUMAN, B., SPEROFF, T., GORDON, S. M., HARRELL, F. E., INOUYE, S. K., BERNARD, G. R. & DITTUS, R. S. 2004. Delirium as a predictor of mortality in mechanically ventilated patients in the intensive care unit. *JAMA,* 291, 1753 - 1762.

ENGELHART, M. J., GEERLINGS, M. I., MEIJER, J., KILIAAN, A., RUITENBERG, A., VAN SWIETEN, J. C., STIJNEN, T., HOFMAN, A., WITTEMAN, J. C. & BRETELER, M. M. 2004. Inflammatory proteins in plasma and the risk of dementia: the rotterdam study. *Arch Neurol,* 61, 668-72.

ENGELMANN, M., LANDGRAF, R. & WOTJAK, C. T. 2004. The hypothalamic–neurohypophysial system regulates the hypothalamic–pituitary–adrenal axis under stress: an old concept revisited. *Frontiers in neuroendocrinology,* 25, 132-149.

ERICSSON, A., ARIAS, C. & SAWCHENKO, P. 1997. Evidence for an intramedullary prostaglandin-dependent mechanism in the activation of stress-related neuroendocrine circuitry by intravenous interleukin-1. *The Journal of Neuroscience,* 17, 7166-7179.

ERICSSON, A., KOVACS, K. & SAWCHENKO, P. 1994. A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *The Journal of Neuroscience,* 14, 897-913.

ERIKSSON, I., GUSTAFSON, Y., FAGERSTRÖM, L. & OLOFSSON, B. 2011. Urinary tract infection in very old women is associated with delirium. *International psychogeriatrics,* 23, 496-502.

ETMINAN, M., GILL, S. & SAMII, A. 2003. Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. *BMJ,* 327, 128.

FANTUZZI, G. & DINARELLO, C. 1996. The inflammatory response in interleukin-1 beta-deficient mice: comparison with other cytokine-related knock-out mice. *Journal of Leukocyte Biology,* 59, 489-493.

FANTUZZI, G., ZHENG, H., FAGGIONI, R., BENIGNI, F., GHEZZI, P., SIPE, J., SHAW, A. & DINARELLO, C. 1996. Effect of endotoxin in IL-1 beta-deficient mice. *The Journal of Immunology,* 157, 291-296.

FARRALL, A. J. & WARDLAW, J. M. 2009. Blood–brain barrier: Ageing and microvascular disease–systematic review and meta-analysis. *Neurobiology of Aging,* 30, 337-352.

FEGHALI, C. A. & WRIGHT, T. M. 1997. Cytokines in acute and chronic inflammation. *Front Biosci,* 2, d12-26.

FELDMANN, M. & MAINI, R. N. 2003. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat Med,* 9, 1245-1250.

FERNANDO, A. B. P. & ROBBINS, T. W. 2011. Animal Models of Neuropsychiatric Disorders. *Annual Review of Clinical Psychology,* 7, 39-61.

FICK, D. M., AGOSTINI, J. V. & INOUYE, S. K. 2002. Delirium superimposed on dementia: a systematic review. *J Am Geriatr Soc,* 50, 1723-32.

FIELD, R., CAMPION, S., WARREN, C., MURRAY, C. & CUNNINGHAM, C. 2010. Systemic challenge with the TLR3 agonist poly I:C induces amplified IFNalpha/beta and IL-1beta responses in the diseased brain and exacerbates chronic neurodegeneration. *Brain Behav Immun,* 24, 996-1007.

FIELD, R. H., GOSSEN, A. & CUNNINGHAM, C. 2012. Prior Pathology in the Basal Forebrain Cholinergic System Predisposes to Inflammation-Induced Working Memory Deficits: Reconciling

Inflammatory and Cholinergic Hypotheses of Delirium. *The Journal of Neuroscience,* 32, 6288-6294.

FIORE, M., PROBERT, L., KOLLIAS, G., AKASSOGLOU, K., ALLEVA, E. & ALOE, L. 1996. Neurobehavioral alterations in developing transgenic mice expressing TNF-alpha in the brain. *Brain Behav Immun,* 10, 126-38.

FLIER, J. S., UNDERHILL, L. H., SAPER, C. B. & BREDER, C. D. 1994. The neurologic basis of fever. *New England Journal of Medicine,* 330, 1880-1886.

FOLSTEIN, M. F., BASSETT, S. S., ROMANOSKI, A. J. & NESTADT, G. 1991. The epidemiology of delirium in the community: the Eastern Baltimore Mental Health Survey. *Int Psychogeriatr,* 3, 169-76.

FONG, T. G., JONES, R. N., MARCANTONIO, E. R., TOMMET, D., GROSS, A. L., HABTEMARIAM, D., SCHMITT, E., YAP, L. & INOUYE, S. K. 2012. Adverse Outcomes After Hospitalization and Delirium in Persons With Alzheimer Disease. *Annals of internal medicine,* 156, 848-856.

FONG, T. G., JONES, R. N., SHI, P., MARCANTONIO, E. R., YAP, L., RUDOLPH, J. L., YANG, F. M., KIELY, D. K. & INOUYE, S. K. 2009. Delirium accelerates cognitive decline in Alzheimer disease. *Neurology,* 72, 1570-5.

FONG, Y., TRACEY, K. J., MOLDAWER, L. L., HESSE, D. G., MANOGUE, K. B., KENNEY, J. S., LEE, A. T., KUO, G. C., ALLISON, A. C., LOWRY, S. F. & ET AL. 1989. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1 beta and interleukin 6 appearance during lethal bacteremia. *J Exp Med,* 170, 1627-33.

FORSBERG, L., LEEB, L., THORÉN, S., MORGENSTERN, R. & JAKOBSSON, P. J. 2000. Human glutathione dependent prostaglandin E synthase: gene structure and regulation. *FEBS letters,* 471, 78-82.

FOTENOS, A. F., MINTUN, M. A., SNYDER, A. Z., MORRIS, J. C. & BUCKNER, R. L. 2008. Brain volume decline in aging: evidence for a relation between socioeconomic status, preclinical Alzheimer disease, and reserve. *Arch Neurol,* 65, 113.

FRANCESCA, A. 2001. Immune function of microglia. *Glia,* 36, 165-179.

FRANCESCHI, C., BONAFÈ, M., VALENSIN, S., OLIVIERI, F., DE LUCA, M., OTTAVIANI, E. & DE BENEDICTIS, G. 2000. Inflamm-aging: An Evolutionary Perspective on Immunosenescence. *Annals of the New York Academy of Sciences,* 908, 244-254.

FRANK, M. G., BARRIENTOS, R. M., HEIN, A. M., BIEDENKAPP, J. C., WATKINS, L. R. & MAIER, S. F. 2010. IL-1RA blocks E. coli-induced suppression of Arc and long-term memory in aged F344xBN F1 rats. *Brain Behav Immun,* 24, 254-62.

FRIEDMAN, E. M., HAYNEY, M. S., LOVE, G. D., URRY, H. L., ROSENKRANZ, M. A., DAVIDSON, R. J., SINGER, B. H. & RYFF, C. D. 2005. Social relationships, sleep quality, and interleukin-6 in aging women. *Proceedings of the National Academy of Sciences of the United States of America,* 102, 18757.

GALEA, I., PALIN, K., NEWMAN, T. A., VAN ROOIJEN, N., PERRY, V. H. & BOCHE, D. 2005. Mannose receptor expression specifically reveals perivascular macrophages in normal, injured, and diseased mouse brain. *Glia,* 49, 375-84.

GAO, W., SCHMIDTKO, A., WOBST, I., LU, R., ANGIONI, C. & GEISSLINGER, G. 2009. Prostaglandin D2 produced by hematopoietic prostaglandin D synthase contributes to LPS-induced fever. *J Physiol Pharmacol,* 60, 145-50.

GARCIA-BUENO, B., SERRATS, J. & SAWCHENKO, P. E. 2009. Cerebrovascular cyclooxygenase-1 expression, regulation, and role in hypothalamic-pituitary-adrenal axis activation by inflammatory stimuli. *J Neurosci,* 29, 12970-81.

GAUTHIER, S., REISBERG, B., ZAUDIG, M., PETERSEN, R. C., RITCHIE, K., BROICH, K., BELLEVILLE, S., BRODATY, H., BENNETT, D. & CHERTKOW, H. 2006. Mild cognitive impairment. *The Lancet,* 367, 1262-1270.

GAYKEMA, R., CHEN, C. C. & GOEHLER, L. E. 2007. Organization of immune-responsive medullary projections to the bed nucleus of the stria terminalis, central amygdala, and paraventricular nucleus of the hypothalamus: evidence for parallel viscerosensory pathways in the rat brain. *Brain Research,* 1130, 130-145.

GEORGE, J., BLEASDALE, S. & SINGLETON, S. J. 1997. Causes and prognosis of delirium in elderly patients admitted to a district general hospital. *Age Ageing,* 26, 423-7.

GERSZTEN, R. E., GARCIA-ZEPEDA, E. A., LIM, Y. C., YOSHIDA, M., DING, H. A., GIMBRONE, M. A., JR., LUSTER, A. D., LUSCINSKAS, F. W. & ROSENZWEIG, A. 1999. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature,* 398, 718-23.

GIBERTINI, M., NEWTON, C., FRIEDMAN, H. & KLEIN, T. W. 1995. Spatial learning impairment in mice infected with Legionella pneumophila or administered exogenous interleukin-1-β. *Brain Behav Immun,* 9, 113-128.

GINHOUX, F., GRETER, M., LEBOEUF, M., NANDI, S., SEE, P., GOKHAN, S., MEHLER, M. F., CONWAY, S. J., NG, L. G. & STANLEY, E. R. 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science's STKE,* 330, 841.

GODBOUT, J. P., CHEN, J., ABRAHAM, J., RICHWINE, A. F., BERG, B. M., KELLEY, K. W. & JOHNSON, R. W. 2005. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. *FASEB J,* 19, 1329-31.

GODOY, M. C., TARELLI, R., FERRARI, C. C., SARCHI, M. I. & PITOSSI, F. J. 2008. Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain,* 131, 1880-94.

GOEHLER, L. E., GAYKEMA, R. P., NGUYEN, K. T., LEE, J. E., TILDERS, F. J., MAIER, S. F. & WATKINS, L. R. 1999. Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? *J Neurosci,* 19, 2799-806.

GOMEZ-ISLA, T., BLESA, R., BOADA, M., CLARIMON, J., DEL SER, T., DOMENECH, G., FERRO, J. M., GOMEZ-ANSON, B., MANUBENS, J. M., MARTINEZ-LAGE, J. M., MUNOZ, D., PENA-CASANOVA, J. & TORRES, F. 2008. A randomized, double-blind, placebo controlled-trial of triflusal in mild cognitive impairment: the TRIMCI study. *Alzheimer Dis Assoc Disord,* 22, 21-9.

GORDON, S. 2002. Pattern Recognition Receptors:: Doubling Up for the Innate Immune Response. *Cell,* 111, 927-930.

GOSHEN, I., KREISEL, T., OUNALLAH-SAAD, H., RENBAUM, P., ZALZSTEIN, Y., BEN-HUR, T., LEVY-LAHAD, E. & YIRMIYA, R. 2007. A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology,* 32, 1106-1115.

GOSSELIN, D. & RIVEST, S. 2007. Role of IL-1 and TNF in the brain: Twenty years of progress on a Dr. Jekyll/Mr. Hyde duality of the innate immune system. *Brain, Behavior, and Immunity,* 21, 281-289.

GOSSELIN, D. & RIVEST, S. 2008. MyD88 signaling in brain endothelial cells is essential for the neuronal activity and glucocorticoid release during systemic inflammation. *Mol Psychiatry,* 13, 480-97.

GOTZ, J. & ITTNER, L. M. 2008. Animal models of Alzheimer's disease and frontotemporal dementia. *Nat Rev Neurosci,* 9, 532-544.

GRANOWITZ, E. V., CLARK, B. D., VANNIER, E., CALLAHAN, M. V. & DINARELLO, C. A. 1992. Effect of interleukin-1 (IL-1) blockade on cytokine synthesis: I. IL-1 receptor antagonist inhibits IL-1-induced cytokine synthesis and blocks the binding of IL-1 to its type II receptor on human monocytes. *Blood,* 79, 2356-63.

GRANOWITZ, E. V., PORAT, R., MIER, J. W., ORENCOLE, S. F., CALLAHAN, M. V., CANNON, J. G., LYNCH, E. A., YE, K., POUTSIAKA, D. D., VANNIER, E. & ET AL. 1993. Hematologic and immunomodulatory effects of an interleukin-1 receptor antagonist coinfusion during low-dose endotoxemia in healthy humans. *Blood,* 82, 2985-90.

GREENHILL, C. J., ROSE-JOHN, S., LISSILAA, R., FERLIN, W., ERNST, M., HERTZOG, P. J., MANSELL, A. & JENKINS, B. J. 2011. IL-6 Trans-Signaling Modulates TLR4-Dependent Inflammatory Responses via STAT3. *The Journal of Immunology,* 186, 1199-1208.

GRIFFIN, D. E., WESSELINGH, S. L. & MCARTHUR, J. C. 1994. Elevated central nervous system prostaglandins in human immunodeficiency virus-associated dementia. *Ann Neurol,* 35, 592-7.

GRIFFIN, R., NALLY, R., NOLAN, Y., MCCARTNEY, Y., LINDEN, J. & LYNCH, M. A. 2006. The age-related attenuation in long-term potentiation is associated with microglial activation. *J Neurochem,* 99, 1263-72.

GRIFFIN, W., SHENG, J., ROBERTS, G. & MRAK, R. 1995. Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. *J Neuropathol Exp Neurol,* 54, 276.

GRIGOLEIT, J. S., KULLMANN, J. S., WOLF, O. T., HAMMES, F., WEGNER, A., JABLONOWSKI, S., ENGLER, H., GIZEWSKI, E., OBERBECK, R. & SCHEDLOWSKI, M. 2011. Dose-Dependent Effects of Endotoxin on Neurobehavioral Functions in Humans. *PloS one,* 6, e28330.

GRIGOLEIT, J. S., OBERBECK, J. R., LICHTE, P., KOBBE, P., WOLF, O. T., MONTAG, T., REY, A., GIZEWSKI, E. R., ENGLER, H. & SCHEDLOWSKI, M. 2010. Lipopolysaccharide-induced experimental immune activation does not impair memory functions in humans. *Neurobiology of learning and memory,* 94, 561-567.

GUENTHER, K., DEACON, R. M., PERRY, V. H. & RAWLINS, J. N. 2001. Early behavioural changes in scrapie-affected mice and the influence of dapsone. *Eur J Neurosci,* 14, 401-9.

GUTIERREZ, E. G., BANKS, W. A. & KASTIN, A. J. 1993. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. *J Neuroimmunol,* 47, 169-76.

GUTIERREZ, E. G., BANKS, W. A. & KASTIN, A. J. 1994. Blood-borne interleukin-1 receptor antagonist crosses the blood-brain barrier. *J Neuroimmunol,* 55, 153-60.

HAEFFNER-CAVAILLON, N., ROUSSELLIER, N., PONZIO, O., CARRENO, M., LAUDE, M., CARPENTIER, A. & KAZATCHKINE, M. 1989. Induction of interleukin-1 production in patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg,* 98, 1100-1106.

HALLE, A., HORNUNG, V., PETZOLD, G. C., STEWART, C. R., MONKS, B. G., REINHECKEL, T., FITZGERALD, K. A., LATZ, E., MOORE, K. J. & GOLENBOCK, D. T. 2008. The NALP3 inflammasome is involved in the innate immune response to amyloid-[beta]. *Nat Immunol,* 9, 857-865.

HAN, J. H., SHINTANI, A., EDEN, S., MORANDI, A., SOLBERG, L. M., SCHNELLE, J., DITTUS, R. S., STORROW, A. B. & ELY, E. W. 2010. Delirium in the Emergency Department: An Independent Predictor of Death Within 6 Months. *Annals of Emergency Medicine,* 56, 244-252.e1.

HARDEN, L. M., DU PLESSIS, I., POOLE, S. & LABURN, H. P. 2006. Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behavior. *Physiol Behav,* 89, 146-55.

HARDEN, L. M., DU PLESSIS, I., ROTH, J., LORAM, L. C., POOLE, S. & LABURN, H. P. 2011. Differences in the relative involvement of peripherally released interleukin (IL)-6, brain IL-1β and prostanoids in mediating lipopolysaccharide-induced fever and sickness behavior. *Psychoneuroendocrinology,* 36, 608-622.

HARDY, J. & SELKOE, D. J. 2002. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science,* 297, 353-356.

HART, B. L. 1988. Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral Reviews,* 12, 123-137.

HART, R. P., BEST, A. M., SESSLER, C. N. & LEVENSON, J. L. 1997. Abbreviated cognitive test for delirium. *Journal of Psychosomatic Research,* 43, 417-423.

HART, R. P., LEVENSON, J. L., SESSLER, C. N., BEST, A. M., SCHWARTZ, S. M. & RUTHERFORD, L. E. 1996. Validation of a Cognitive Test for Delirium in Medical ICU Patients. *Psychosomatics,* 37, 533-546.

HATA, A. N. & BREYER, R. M. 2004. Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune modulation. *Pharmacology & Therapeutics,* 103, 147-166.

HAYAISHI, O. 2000. Molecular mechanisms of sleep–wake regulation: a role of prostaglandin D2. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* 355, 275-280.

HEIN, A. M. & O'BANION, M. K. 2009. Neuroinflammation and memory: the role of prostaglandins. *Mol Neurobiol,* 40, 15-32.

HEIN, A. M., STASKO, M. R., MATOUSEK, S. B., SCOTT-MCKEAN, J. J., MAIER, S. F., OLSCHOWKA, J. A., COSTA, A. C. & O'BANION, M. K. 2010. Sustained hippocampal IL-1beta overexpression impairs contextual and spatial memory in transgenic mice. *Brain Behav Immun,* 24, 243-53.

HEIN, A. M., STUTZMAN, D. L., BLAND, S. T., BARRIENTOS, R. M., WATKINS, L. R., RUDY, J. W. & MAIER, S. F. 2007. Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1 beta injections into the dorsal hippocampus. *Neuroscience,* 150, 754-63.

HEINRICH, P. C., BEHRMANN, I., HAAN, S., HERMANNS, H. M., MÜLLER-NEWEN, G. & SCHAPER, F. 2003. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochemical Journal,* 374, 1.

HENEKA, M. T., O'BANION, M. K., TERWEL, D. & KUMMER, M. P. 2010. Neuroinflammatory processes in Alzheimer's disease. *Journal of neural transmission,* 117, 919-947.

HOFFMANN, A., CONRADT, H. S., GROSS, G., NIMTZ, M., LOTTSPEICH, F. & WURSTER, U. 1993. Purification and chemical characterization of beta-trace protein from human cerebrospinal fluid: its identification as prostaglandin D synthase. *J Neurochem,* 61, 451-6.

HOGAN, D., GAGE, L., BRUTO, V., BURNE, D., CHAN, P. & WIENS, C. 2006. National Guidelines for Seniors' Mental Health—The assessment and Treatment of Delirium. *Toronto (ON): Canadian Coalition for Seniors' Mental Health.*

HOLMES, C., CUNNINGHAM, C., ZOTOVA, E., CULLIFORD, D. & PERRY, V. H. 2011. Proinflammatory cytokines, sickness behavior, and Alzheimer disease. *Neurology,* 77, 212-218.

HOLMES, C., CUNNINGHAM, C., ZOTOVA, E., WOOLFORD, J., DEAN, C., KERR, S., CULLIFORD, D. & PERRY, V. H. 2009. Systemic inflammation and disease progression in Alzheimer disease. *Neurology,* 73, 768-74.

HOLMES, C., EL-OKL, M., WILLIAMS, A. L., CUNNINGHAM, C., WILCOCKSON, D. & PERRY, V. H. 2003. Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease. *J Neurol Neurosurg Psychiatry,* 74, 788-9.

HOOZEMANS, J. J., ROZEMULLER, A. J., JANSSEN, I., DE GROOT, C. J., VEERHUIS, R. & EIKELENBOOM, P. 2001. Cyclooxygenase expression in microglia and neurons in Alzheimer's disease and control brain. *Acta Neuropathol,* 101, 2-8.

HOPKINS, S. J. 2007. Central nervous system recognition of peripheral inflammation: a neural, hormonal collaboration. *Acta Biomed,* 78 Suppl 1, 231-47.

HOSHINO, K., TAKEUCHI, O., KAWAI, T., SANJO, H., OGAWA, T., TAKEDA, Y., TAKEDA, K. & AKIRA, S. 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *The Journal of Immunology,* 162, 3749-3752.

HOSOI, T., OKUMA, Y., MATSUDA, T. & NOMURA, Y. 2005. Novel pathway for LPS-induced afferent vagus nerve activation: possible role of nodose ganglion. *Auton Neurosci,* 120, 104-7.

HOSOI, T., OKUMA, Y. & NOMURA, Y. 2000. Electrical stimulation of afferent vagus nerve induces IL-1β expression in the brain and activates HPA axis. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 279, R141-R147.

HSHIEH, T. T., FONG, T. G., MARCANTONIO, E. R. & INOUYE, S. K. 2008. Cholinergic deficiency hypothesis in delirium: a synthesis of current evidence. *J Gerontol A Biol Sci Med Sci,* 63, 764-72.

HUANG, Z. L., SATO, Y., MOCHIZUKI, T., OKADA, T., QU, W. M., YAMATODANI, A., URADE, Y. & HAYAISHI, O. 2003. Prostaglandin E2 activates the histaminergic system via the EP4 receptor to induce wakefulness in rats. *The Journal of Neuroscience,* 23, 5975-5983.

IADECOLA, C., NIWA, K., NOGAWA, S., ZHAO, X., NAGAYAMA, M., ARAKI, E., MORHAM, S. & ROSS, M. E. 2001. Reduced susceptibility to ischemic brain injury and N-methyl-d-aspartate-mediated neurotoxicity in cyclooxygenase-2-deficient mice. *Proceedings of the National Academy of Sciences,* 98, 1294-1299.

IKEDA-MATSUO, Y., IKEGAYA, Y., MATSUKI, N., UEMATSU, S., AKIRA, S. & SASAKI, Y. 2005. Microglia-specific expression of microsomal prostaglandin E2 synthase-1 contributes to lipopolysaccharide-induced prostaglandin E2 production. *J Neurochem,* 94, 1546-1558.

IKEJIMA, T., OKUSAWA, S., GHEZZI, P., VAN DER MEER, J. W. & DINARELLO, C. A. 1990. Interleukin-1 induces tumor necrosis factor (TNF) in human peripheral blood mononuclear cells in vitro and a circulating TNF-like activity in rabbits. *J Infect Dis,* 162, 215-23.

IN 'T VELD, B. A., RUITENBERG, A., HOFMAN, A., LAUNER, L. J., VAN DUIJN, C. M., STIJNEN, T., BRETELER, M. M. B. & STRICKER, B. H. C. 2001. Nonsteroidal Antiinflammatory Drugs and the Risk of Alzheimer's Disease. *New England Journal of Medicine,* 345, 1515-1521.

INOUYE, S. K. 2006. Delirium in older persons. *N Engl J Med,* 354, 1157-65.

INOUYE, S. K. & CHARPENTIER, P. A. 1996. Precipitating factors for delirium in hospitalized elderly persons. Predictive model and interrelationship with baseline vulnerability. *JAMA,* 275, 852-7.

INOUYE, S. K., RUSHING, J. T., FOREMAN, M. D., PALMER, R. M. & POMPEI, P. 1998. Does delirium contribute to poor hospital outcomes? *Journal of General Internal Medicine,* 13, 234-242.

INOUYE, S. K., VAN DYCK, C. H., ALESSI, C. A., BALKIN, S., SIEGAL, A. P. & HORWITZ, R. I. 1990. Clarifying confusion: the confusion assessment method. A new method for detection of delirium. *Ann Intern Med,* 113, 941-8.

INUI, A. 2001. Cytokines and sickness behavior: implications from knockout animal models. *Trends Immunol,* 22, 469-73.

ISHIHARA, K. & HIRANO, T. 2002. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine & growth factor reviews,* 13, 357-368.

IWASHYNA, T. J., ELY, E. W., SMITH, D. M. & LANGA, K. M. 2010. Long-term Cognitive Impairment and Functional Disability Among Survivors of Severe Sepsis. *JAMA: The Journal of the American Medical Association,* 304, 1787-1794.

JACKSON, J. C., GORDON, S. M., HART, R. P., HOPKINS, R. O. & ELY, E. W. 2004. The association between delirium and cognitive decline: a review of the empirical literature. *Neuropsychol Rev,* 14, 87-98.

JACOBSON, S. & JERRIER, H. 2000. EEG in delirium. *Seminars in clinical neuropsychiatry,* 5, 86-92.

JAKOBSSON, P. J., THOREN, S., MORGENSTERN, R. & SAMUELSSON, B. 1999. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc Natl Acad Sci U S A,* 96, 7220-5.

JANEWAY, C. A., TRAVERS, P., WALPORT, M. & CAPRA, J. D. 2001. *Immunobiology: the immune system in health and disease*, Current Biology.

JATURAPATPORN, D., ISAAC, M. G., MCCLEERY, J. & TABET, N. 2012. Aspirin, steroidal and non-steroidal anti-inflammatory drugs for the treatment of Alzheimer's disease. *Cochrane Database Syst Rev,* 2, CD006378.

JHA, S., SRIVASTAVA, S. Y., BRICKEY, W. J., IOCCA, H., TOEWS, A., MORRISON, J. P., CHEN, V. S., GRIS, D., MATSUSHIMA, G. K. & TING, J. P.-Y. 2010. The Inflammasome Sensor, NLRP3, Regulates CNS Inflammation and Demyelination via Caspase-1 and Interleukin-18. *The Journal of Neuroscience,* 30, 15811-15820.

JONES, R. N., FONG, T. G., METZGER, E., TULEBAEV, S., YANG, F. M., ALSOP, D. C., MARCANTONIO, E. R., CUPPLES, L. A., GOTTLIEB, G. & INOUYE, S. K. 2010. Aging, brain disease, and reserve: implications for delirium. *Am J Geriatr Psychiatry,* 18, 117-27.

JONES, R. N., YANG, F. M., ZHANG, Y., KIELY, D. K., MARCANTONIO, E. R. & INOUYE, S. K. 2006. Does educational attainment contribute to risk for delirium? A potential role for cognitive reserve. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 61, 1307-1311.

JONES, S. A., RICHARDS, P. J., SCHELLER, J. & ROSE-JOHN, S. 2005. IL-6 transsignaling: the in vivo consequences. *J Interferon Cytokine Res,* 25, 241-53.

JURGENS, H. A., AMANCHERLA, K. & JOHNSON, R. W. 2012. Influenza Infection Induces Neuroinflammation, Alters Hippocampal Neuron Morphology, and Impairs Cognition in Adult Mice. *The Journal of Neuroscience,* 32, 3958-3968.

KALISVAART, K. J., VREESWIJK, R., DE JONGHE, J. F. M., VAN DER PLOEG, T., VAN GOOL, W. A. & EIKELENBOOM, P. 2006. Risk Factors and Prediction of Postoperative Delirium in Elderly Hip-Surgery Patients: Implementation and Validation of a Medical Risk Factor Model. *Journal of the American Geriatrics Society,* 54, 817-822.

KAT, M. G., VREESWIJK, R., DE JONGHE, J. F., VAN DER PLOEG, T., VAN GOOL, W. A., EIKELENBOOM, P. & KALISVAART, K. J. 2008. Long-term cognitive outcome of delirium in elderly hip surgery patients. A prospective matched controlled study over two and a half years. *Dement Geriatr Cogn Disord,* 26, 1-8.

KATSUKI, H., NAKAI, S., HIRAI, Y., AKAJI, K., KISO, Y. & SATOH, M. 1990. Interleukin-1 [beta] inhibits long-term potentiation in the CA3 region of mouse hippocampal slices. *European journal of pharmacology,* 181, 323-326.

KAUFMANN, W. E., WORLEY, P. F., PEGG, J., BREMER, M. & ISAKSON, P. 1996. COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proceedings of the National Academy of Sciences,* 93, 2317-2321.

KAWAI, T., TAKEUCHI, O., FUJITA, T., INOUE, J.-I., MÜHLRADT, P. F., SATO, S., HOSHINO, K. & AKIRA, S. 2001. Lipopolysaccharide Stimulates the MyD88-Independent Pathway and Results in Activation of IFN-Regulatory Factor 3 and the Expression of a Subset of Lipopolysaccharide-Inducible Genes. *The Journal of Immunology,* 167, 5887-5894.

KAWANO, T., ANRATHER, J., ZHOU, P., PARK, L., WANG, G., FRYS, K. A., KUNZ, A., CHO, S., ORIO, M. & IADECOLA, C. 2006. Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. *Nat Med,* 12, 225-9.

KHAN, B. A., ZAWAHIRI, M., CAMPBELL, N. L. & BOUSTANI, M. A. 2011. Biomarkers for Delirium—A Review. *Journal of the American Geriatrics Society,* 59, S256-S261.

KIECOLT-GLASER, J. K., MCGUIRE, L., ROBLES, T. F. & GLASER, R. 2002. Psychoneuroimmunology and Psychosomatic Medicine: Back to the Future. *Psychosomatic Medicine,* 64, 15-28.

KIELY, D. K., JONES, R. N., BERGMANN, M. A., MURPHY, K. M., ORAV, E. J. & MARCANTONIO, E. R. 2006. Association Between Delirium Resolution and Functional Recovery Among Newly Admitted Postacute Facility Patients. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 61, 204-208.

KITAOKA, S., FURUYASHIKI, T., NISHI, A., SHUTO, T., KOYASU, S., MATSUOKA, T., MIYASAKA, M., GREENGARD, P. & NARUMIYA, S. 2007. Prostaglandin E2 acts on EP1 receptor and amplifies both dopamine D1 and D2 receptor signaling in the striatum. *J Neurosci,* 27, 12900-7.

KITAZAWA, M., ODDO, S., YAMASAKI, T. R., GREEN, K. N. & LAFERLA, F. M. 2005. Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease. *J Neurosci,* 25, 8843-53.

KLUGER, M. J. & ROTHENBURG, B. A. 1979. Fever and reduced iron: their interaction as a host defense response to bacterial infection. *Science,* 203, 374-376.

KO, C.-Y., CHANG, L.-H., LEE, Y.-C., STERNECK, E., CHENG, C.-P., CHEN, S.-H., HUANG, A. M., TSENG, J. T. & WANG, J.-M. 2012. CCAAT/enhancer binding protein delta (CEBPD) elevating PTX3 expression inhibits macrophage-mediated phagocytosis of dying neuron cells. *Neurobiology of Aging,* 33, 422.e11-422.e25.

KOJ, A. 1996. Initiation of acute phase response and synthesis of cytokines. *Biochim Biophys Acta,* 1317, 84-94.

KONSMAN, J., VEENEMAN, J., COMBE, C., POOLE, S., LUHESHI, G. & DANTZER, R. 2008. Central nervous action of interleukin-1 mediates activation of limbic structures and behavioural depression in response to peripheral administration of bacterial lipopolysaccharide. *European Journal of Neuroscience,* 28, 2499-2510.

KONSMAN, J. P., LUHESHI, G. N., BLUTHE, R. M. & DANTZER, R. 2000. The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. *Eur J Neurosci,* 12, 4434-46.

KONSMAN, J. P., PARNET, P. & DANTZER, R. 2002. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci,* 25, 154-9.

KOTILINEK, L. A., WESTERMAN, M. A., WANG, Q., PANIZZON, K., LIM, G. P., SIMONYI, A., LESNE, S., FALINSKA, A., YOUNKIN, L. H., YOUNKIN, S. G., ROWAN, M., CLEARY, J., WALLIS, R. A., SUN, G. Y., COLE, G., FRAUTSCHY, S., ANWYL, R. & ASHE, K. H. 2008. Cyclooxygenase-2 inhibition improves amyloid-beta-mediated suppression of memory and synaptic plasticity. *Brain,* 131, 651-64.

KYRKANIDES, S., TALLENTS, R., MILLER, J.-N., OLSCHOWKA, M., JOHNSON, R., YANG, M., OLSCHOWKA, J., BROUXHON, S. & O'BANION, M. K. 2011. Osteoarthritis accelerates and exacerbates Alzheimer's disease pathology in mice. *J Neuroinflammation,* 8, 112.

LACROIX, S. & RIVEST, S. 1998. Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. *J Neurochem,* 70, 452-66.

LAFLAMME, N. & RIVEST, S. 1999. Effects of systemic immunogenic insults and circulating proinflammatory cytokines on the transcription of the inhibitory factor kappaB alpha within specific cellular populations of the rat brain. *J Neurochem,* 73, 309-21.

LAFOSSE, J. M., REED, B. R., MUNGAS, D., STERLING, S. B., WAHBEH, H. & JAGUST, W. J. 1997. Fluency and memory differences between ischemic vascular dementia and Alzheimer's disease. *Neuropsychology,* 11, 514.

LAWLOR, P. G., FAINSINGER, R. L. & BRUERA, E. D. 2000. Delirium at the end of life. *JAMA: The Journal of the American Medical Association,* 284, 2427-2429.

LAWRENCE, C. B., ALLAN, S. M. & ROTHWELL, N. J. 1998. Interleukin-1β and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rat. *European Journal of Neuroscience,* 10, 1188-1195.

LAWS, S. M., PERNECZKY, R., WAGENPFEIL, S., MÜLLER, U., FÖRSTL, H., MARTINS, R. N., KURZ, A. & RIEMENSCHNEIDER, M. 2005. TNF polymorphisms in Alzheimer disease and functional implications on CSF beta-amyloid levels. *Human Mutation,* 26, 29-35.

LAZARUS, M., YOSHIDA, K., COPPARI, R., BASS, C. E., MOCHIZUKI, T., LOWELL, B. B. & SAPER, C. B. 2007. EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. *Nat Neurosci,* 10, 1131-3.

LEE, J., CHAN, S. L. & MATTSON, M. P. 2002. Adverse effect of a presenilin-1 mutation in microglia results in enhanced nitric oxide and inflammatory cytokine responses to immune challenge in the brain. *Neuromolecular Med,* 2, 29-45.

LEE, S., JANG, E., KIM, J.-H., KIM, J.-H., LEE, W.-H. & SUK, K. 2012. Lipocalin-type prostaglandin D2 synthase regulates glial cell migration and morphology through marcks: prostaglandin D2-independent effects. *Journal of Biological Chemistry*.

LEMSTRA, A. W., KALISVAART, K. J., VREESWIJK, R., VAN GOOL, W. A. & EIKELENBOOM, P. 2008. Pre-operative inflammatory markers and the risk of postoperative delirium in elderly patients. *Int J Geriatr Psychiatry,* 23, 943-8.

LENCZOWSKI, M. J. P., BLUTHÉ, R.-M., ROTH, J., REES, G. S., RUSHFORTH, D. A., VAN DAM, A.-M., TILDERS, F. J. H., DANTZER, R., ROTHWELL, N. J. & LUHESHI, G. N. 1999. Central administration of rat IL-6 induces HPA activation and fever but not sickness behavior in rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 276, R652-R658.

LEOUTSAKOS, J. M., MUTHEN, B. O., BREITNER, J. C. & LYKETSOS, C. G. 2011. Effects of non-steroidal anti-inflammatory drug treatments on cognitive decline vary by phase of pre-clinical Alzheimer disease: findings from the randomized controlled Alzheimer's Disease Anti-inflammatory Prevention Trial. *Int J Geriatr Psychiatry.*

LESLIE, D. L. & INOUYE, S. K. 2011. The importance of delirium: economic and societal costs. *J Am Geriatr Soc,* 59 Suppl 2, S241-3.

LESLIE, D. L., MARCANTONIO, E. R., ZHANG, Y., LEO-SUMMERS, L. & INOUYE, S. K. 2008. One-year health care costs associated with delirium in the elderly population. *Arch Intern Med,* 168, 27-32.

LEWIS, M. C. & BARNETT, S. R. 2004. Postoperative delirium: the tryptophan dyregulation model. *Med Hypotheses,* 63, 402-6.

LIANG, X., WANG, Q., HAND, T., WU, L., BREYER, R. M., MONTINE, T. J. & ANDREASSON, K. 2005. Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. *J Neurosci,* 25, 10180-7.

LICASTRO, F., CANDORE, G., LIO, D., PORCELLINI, E., COLONNA-ROMANO, G., FRANCESCHI, C. & CARUSO, C. 2005. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. *Immun Ageing,* 2, 8.

LIPOWSKI, Z. J. 1983. Transient cognitive disorders (delirium, acute confusional states) in the elderly. *Am J Psychiatry,* 140, 1426-36.

LIPOWSKI, Z. J. 1987. Delirium (acute confusional states). *JAMA: The Journal of the American Medical Association,* 258, 1789-1792.

LIU, D., WU, L., BREYER, R., MATTSON, M. P. & ANDREASSON, K. 2005. Neuroprotection by the PGE2 EP2 receptor in permanent focal cerebral ischemia. *Ann Neurol,* 57, 758-61.

LODDICK, S. A., WONG, M. L., BONGIORNO, P. B., GOLD, P. W., LICINIO, J. & ROTHWELL, N. J. 1997. Endogenous interleukin-1 receptor antagonist is neuroprotective. *Biochemical and Biophysical Research Communications,* 234, 211-215.

LOOI, J. C. L. & SACHDEV, P. S. 1999. Differentiation of vascular dementia from AD on neuropsychological tests. *Neurology,* 53, 670.

LU, Y.-C., YEH, W.-C. & OHASHI, P. S. 2008. LPS/TLR4 signal transduction pathway. *Cytokine,* 42, 145-151.

LUENGO-FERNANDEZ, R., LEAL, J. & GRAY, A. 2010. Dementia 2010: The economic burden of dementia and associated research funding in the United Kingdom. *Cambridge: Alzheimer's Research Trust.*

LUTERMAN, J. D., HAROUTUNIAN, V., YEMUL, S., HO, L., PUROHIT, D., AISEN, P. S., MOHS, R. & PASINETTI, G. M. 2000. Cytokine gene expression as a function of the clinical progression of Alzheimer disease dementia. *Arch Neurol,* 57, 1153-60.

LYKETSOS, C. G., BREITNER, J. C., GREEN, R. C., MARTIN, B. K., MEINERT, C., PIANTADOSI, S. & SABBAGH, M. 2007. Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. *Neurology,* 68, 1800-8.

LYNCH, M. A. 1998. Analysis of the mechanisms underlying the age-related impairment in long-term potentiation in the rat. *Reviews in the Neurosciences,* 9, 169.

LYNCH, M. A. 2004. Long-term potentiation and memory. *Physiol Rev,* 84, 87-136.

LYNCH, M. A. 2010. Age-related neuroinflammatory changes negatively impact on neuronal function. *Front Aging Neurosci,* 1, 6.

MACDONALD, A., ADAMIS, D., TRELOAR, A. & MARTIN, F. 2007. C-reactive protein levels predict the incidence of delirium and recovery from it. *Age and Ageing,* 36, 222-225.

MACLULLICH, A. M., BEAGLEHOLE, A., HALL, R. J. & MEAGHER, D. J. 2009. Delirium and long-term cognitive impairment. *Int Rev Psychiatry,* 21, 30-42.

MACLULLICH, A. M., EDELSHAIN, B. T., HALL, R. J., DE VRIES, A., HOWIE, S. E., PEARSON, A., MIDDLETON, S. D., GILLIES, F., ARMSTRONG, I. R., WHITE, T. O., CUNNINGHAM, C., DE ROOIJ, S. E. & VAN

MUNSTER, B. C. 2011. Cerebrospinal fluid interleukin-8 levels are higher in people with hip fracture with perioperative delirium than in controls. *J Am Geriatr Soc,* 59, 1151-3.

MACLULLICH, A. M., FERGUSON, K. J., MILLER, T., DE ROOIJ, S. E. & CUNNINGHAM, C. 2008. Unravelling the pathophysiology of delirium: a focus on the role of aberrant stress responses. *J Psychosom Res,* 65, 229-38.

MACLULLICH, A. M. & HALL, R. J. 2011. Who understands delirium? *Age Ageing,* 40, 412-4.

MAGRI, F., CRAVELLO, L., BARILI, L., SARRA, S., CINCHETTI, W., SALMOIRAGHI, F., MICALE, G. & FERRARI, E. 2006. Stress and dementia: the role of the hypothalamicpituitary-adrenal axis. *Aging clinical and experimental research,* 18, 167.

MANEPALLI, J., GROSSBERG, G. T. & MUELLER, C. 1990. Prevalence of Delirium and Urinary Tract Infection in a Psychogeriatric Unit. *Journal of Geriatric Psychiatry and Neurology,* 3, 198-202.

MARINKOVIC, S., JAHREIS, G. P., WONG, G. G. & BAUMANN, H. 1989. IL-6 modulates the synthesis of a specific set of acute phase plasma proteins in vivo. *J Immunol,* 142, 808-12.

MARSLAND, A. L., GIANAROS, P. J., ABRAMOWITCH, S. M., MANUCK, S. B. & HARIRI, A. R. 2008. Interleukin-6 covaries inversely with hippocampal grey matter volume in middle-aged adults. *Biol Psychiatry,* 64, 484-90.

MARTIN, B. K., SZEKELY, C., BRANDT, J., PIANTADOSI, S., BREITNER, J. C., CRAFT, S., EVANS, D., GREEN, R. & MULLAN, M. 2008. Cognitive function over time in the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT): results of a randomized, controlled trial of naproxen and celecoxib. *Arch Neurol,* 65, 896-905.

MARTIN, S., GRIMWOOD, P. & MORRIS, R. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci,* 23, 649-711.

MARTINON, F., PETRILLI, V., MAYOR, A., TARDIVEL, A. & TSCHOPP, J. 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature,* 440, 237-241.

MATHURIN, K., GALLANT, M. A., GERMAIN, P., ALLARD-CHAMARD, H., BRISSON, J., IORIO-MORIN, C., DE BRUM FERNANDES, A., CARON, M. G., LAPORTE, S. A. & PARENT, J.-L. 2011. An Interaction between L-prostaglandin D Synthase and Arrestin Increases PGD2 Production. *Journal of Biological Chemistry,* 286, 2696-2706.

MATOUSEK, S. B., HEIN, A. M., SHAFTEL, S. S., OLSCHOWKA, J. A., KYRKANIDES, S. & O'BANION, M. K. 2010. Cyclooxygenase-1 mediates prostaglandin E(2) elevation and contextual memory impairment in a model of sustained hippocampal interleukin-1beta expression. *J Neurochem,* 114, 247-58.

MATSUMOTO, Y., YAMAGUCHI, T., WATANABE, S. & YAMAMOTO, T. 2004. Involvement of arachidonic acid cascade in working memory impairment induced by interleukin-1 beta. *Neuropharmacology,* 46, 1195-1200.

MATSUMURA, K., KAIHATSU, S., IMAI, H., TERAO, A., SHIRAKI, T. & KOBAYASHI, S. 2000. Cyclooxygenase in the vagal afferents: is it involved in the brain prostaglandin response evoked by lipopolysaccharide? *Autonomic Neuroscience,* 85, 88-92.

MATSUOKA, T., HIRATA, M., TANAKA, H., TAKAHASHI, Y., MURATA, T., KABASHIMA, K., SUGIMOTO, Y., KOBAYASHI, T., USHIKUBI, F. & AZE, Y. 2000. Prostaglandin D2 as a mediator of allergic asthma. *Science's STKE,* 287, 2013.

MATSUOKA, Y., FURUYASHIKI, T., BITO, H., USHIKUBI, F., TANAKA, Y., KOBAYASHI, T., MURO, S., SATOH, N., KAYAHARA, T., HIGASHI, M., MIZOGUCHI, A., SHICHI, H., FUKUDA, Y., NAKAO, K. & NARUMIYA, S. 2003. Impaired adrenocorticotropic hormone response to bacterial endotoxin in mice deficient in prostaglandin E receptor EP1 and EP3 subtypes. *Proc Natl Acad Sci U S A,* 100, 4132-7.

MATSUOKA, Y., FURUYASHIKI, T., YAMADA, K., NAGAI, T., BITO, H., TANAKA, Y., KITAOKA, S., USHIKUBI, F., NABESHIMA, T. & NARUMIYA, S. 2005. Prostaglandin E receptor EP1 controls impulsive behavior under stress. *Proc Natl Acad Sci U S A,* 102, 16066-71.

MAUBACH, K., DAVIS, R., CLARK, D., FENTON, G., LOCKEY, P., CLARK, K., OXFORD, A., HAGAN, R., ROUTLEDGE, C. & COLEMAN, R. 2009. BGC20-1531, a novel, potent and selective prostanoid EP4 receptor antagonist: a putative new treatment for migraine headache. *Br J Pharmacol,* 156, 316-327.

MCAVAY, G. J., VAN NESS, P. H., BOGARDUS, S. T., ZHANG, Y., LESLIE, D. L., LEO-SUMMERS, L. S. & INOUYE, S. K. 2006. Older Adults Discharged from the Hospital with Delirium: 1-Year Outcomes. *Journal of the American Geriatrics Society,* 54, 1245-1250.

MCCOLL, B. W., ROTHWELL, N. J. & ALLAN, S. M. 2007. Systemic Inflammatory Stimulus Potentiates the Acute Phase and CXC Chemokine Responses to Experimental Stroke and Exacerbates Brain Damage via Interleukin-1- and Neutrophil-Dependent Mechanisms. *The Journal of Neuroscience,* 27, 4403-4412.

MCCOY, M. K. & TANSEY, M. G. 2008. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. *J Neuroinflammation,* 5, 45.

MCCULLERS, J. A., FACCHINI, S., CHESNEY, P. J. & WEBSTER, R. G. 1999. Influenza B Virus Encephalitis. *Clinical Infectious Diseases,* 28, 898-900.

MCCULLOUGH, L., WU, L., HAUGHEY, N., LIANG, X., HAND, T., WANG, Q., BREYER, R. M. & ANDREASSON, K. 2004. Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *J Neurosci,* 24, 257-68.

MCGEER, P. L., SCHULZER, M. & MCGEER, E. G. 1996. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology,* 47, 425-32.

MCINTOSH, T. K., BUSH, H. L., YESTON, N. S., GRASBERGER, R., PALTER, M., AUN, F. & EGDAHL, R. H. 1985. Beta-endorphin, cortisol and postoperative delirium: a preliminary report. *Psychoneuroendocrinology,* 10, 303-313.

MCKHANN, G., DRACHMAN, D., FOLSTEIN, M., KATZMAN, R., PRICE, D. & STADLAN, E. M. 1984. Clinical diagnosis of Alzheimer's disease. *Neurology,* 34, 939-939.

MEAGHER, D. 2009. Motor subtypes of delirium: Past, present and future. *International Review of Psychiatry,* 21, 59-73.

MEAGHER, D., ADAMIS, D., TRZEPACZ, P. & LEONARD, M. 2012. Features of subsyndromal and persistent delirium. *The British Journal of Psychiatry,* 200, 37-44.

MEAGHER, D. J., MORAN, M., RAJU, B., GIBBONS, D., DONNELLY, S., SAUNDERS, J. & TRZEPACZ, P. T. 2007. Phenomenology of delirium: Assessment of 100 adult cases using standardised measures. *The British Journal of Psychiatry,* 190, 135-141.

MICHAUD, L., BÜLA, C., BERNEY, A., CAMUS, V., VOELLINGER, R., STIEFEL, F. & BURNAND, B. 2007. Delirium: guidelines for general hospitals. *Journal of Psychosomatic Research,* 62, 371-383.

MILLER, A. H., MALETIC, V. & RAISON, C. L. 2009. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. *Biol Psychiatry,* 65, 732-741.

MILLER, S. B. Prostaglandins in health and disease: an overview. 2006. Elsevier, 37-49.

MITCHELL, J. A., LARKIN, S. & WILLIAMS, T. J. 1995. Cyclooxygenase-2: regulation and relevance in inflammation. *Biochem Pharmacol,* 50, 1535-42.

MOHRI, I., KADOYAMA, K., KANEKIYO, T., SATO, Y., KAGITANI-SHIMONO, K., SAITO, Y., SUZUKI, K., KUDO, T., TAKEDA, M., URADE, Y., MURAYAMA, S. & TANIIKE, M. 2007. Hematopoietic prostaglandin D synthase and DP1 receptor are selectively upregulated in microglia and astrocytes within senile plaques from human patients and in a mouse model of Alzheimer disease. *J Neuropathol Exp Neurol,* 66, 469-80.

MOHRI, I., TANIIKE, M., TANIGUCHI, H., KANEKIYO, T., ARITAKE, K., INUI, T., FUKUMOTO, N., EGUCHI, N., KUSHI, A. & SASAI, H. 2006. Prostaglandin D2-mediated microglia/astrocyte interaction enhances astrogliosis and demyelination in twitcher. *The Journal of Neuroscience,* 26, 4383-4393.

MONTINE, T. J., SIDELL, K. R., CREWS, B. C., MARKESBERY, W. R., MARNETT, L. J., ROBERTS, L. J., 2ND & MORROW, J. D. 1999. Elevated CSF prostaglandin E2 levels in patients with probable AD. *Neurology,* 53, 1495-8.

MORENO, B., JUKES, J.-P., VERGARA-IRIGARAY, N., ERREA, O., VILLOSLADA, P., PERRY, V. H. & NEWMAN, T. A. 2011. Systemic inflammation induces axon injury during brain inflammation. *Ann Neurol,* 70, 932-942.

MORITA, I., SCHINDLER, M., REGIER, M. K., OTTO, J. C., HORI, T., DEWITT, D. L. & SMITH, W. L. 1995. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and- 2. *Journal of Biological Chemistry,* 270, 10902-10908.

MORITA, T., HIRAI, K., SAKAGUCHI, Y., TSUNETO, S. & SHIMA, Y. 2004. Family-Perceived Distress From Delirium-Related Symptoms of Terminally Ill Cancer Patients. *Psychosomatics,* 45, 107-113.

MORMÈDE, C., CASTANON, N., MÉDINA, C. & DANTZER, R. 2003. Conditioned place aversion with interleukin-1[beta] in mice is not associated with activation of the cytokine network. *Brain, Behavior, and Immunity,* 17, 110-120.

MORRIS, J. & PRICE, J. 2001. Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage alzheimer's disease. *Journal of Molecular Neuroscience,* 17, 101-118.

MURAKAMI, M., NAKASHIMA, K., KAMEI, D., MASUDA, S., ISHIKAWA, Y., ISHII, T., OHMIYA, Y., WATANABE, K. & KUDO, I. 2003. Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and-2. *Journal of Biological Chemistry,* 278, 37937-37947.

MURAKI, T., FUJIMORI, K., ISHIZAKA, M., OHE, Y., URADE, Y., OKAJIMA, F. & ISHIKAWA, K. 2004. Effects of interleukin-1beta and prostaglandin E2 on prostaglandin D synthase production in cultivated rat leptomeningeal cells. *J Cereb Blood Flow Metab,* 24, 409-18.

MURRAY, C., SANDERSON, D. J., BARKUS, C., DEACON, R. M., RAWLINS, J. N., BANNERMAN, D. M. & CUNNINGHAM, C. 2012. Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium. *Neurobiol Aging,* 33, 603-616 e3.

MURRAY, C. L., SKELLY, D. T. & CUNNINGHAM, C. 2011. Exacerbation of CNS inflammation and neurodegeneration by systemic LPS treatment is independent of circulating IL-1beta and IL-6. *J Neuroinflammation,* 8, 50.

NAGASAKA, T., HIRAIDE, M., SUGIMOTO, T., SHINDO, K., SHIOZAWA, Z. & YOKOTA, S. 2004. Localization of lipocaline-type prostaglandin D synthase in rat brain: immunoelectron microscopic study. *Histochemistry and Cell Biology,* 121, 483-491.

NAGATA, A., SUZUKI, Y., IGARASHI, M., EGUCHI, N., TOH, H., URADE, Y. & HAYAISHI, O. 1991. Human brain prostaglandin D synthase has been evolutionarily differentiated from lipophilic-ligand carrier proteins. *Proc Natl Acad Sci U S A,* 88, 4020-4.

NAKAMURA, K., MATSUMURA, K., KANEKO, T., KOBAYASHI, S., KATOH, H. & NEGISHI, M. 2002. The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. *The Journal of Neuroscience,* 22, 4600-4610.

NAKAYAMA, M., UCHIMURA, K., ZHU, R. L., NAGAYAMA, T., ROSE, M. E., STETLER, R. A., ISAKSON, P. C., CHEN, J. & GRAHAM, S. H. 1998. Cyclooxygenase-2 inhibition prevents delayed death of CA1 hippocampal neurons following global ischemia. *Proceedings of the National Academy of Sciences,* 95, 10954-10959.

NATHAN, C. 2002. Points of control in inflammation. *NATURE-LONDON-,* 846-852.

NELSON, P. T., BRAAK, H. & MARKESBERY, W. R. 2009. Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship. *J Neuropathol Exp Neurol,* 68, 1.

NESTLER, E. J. & HYMAN, S. E. 2010. Animal models of neuropsychiatric disorders. *Nat Neurosci,* 13, 1161-1169.

NGUYEN, M. D., D'AIGLE, T., GOWING, G., JULIEN, J. P. & RIVEST, S. 2004. Exacerbation of motor neuron disease by chronic stimulation of innate immunity in a mouse model of amyotrophic lateral sclerosis. *J Neurosci,* 24, 1340-9.

NILSBERTH, C., ELANDER, L., HAMZIC, N., NORELL, M., LONN, J., ENGSTROM, L. & BLOMQVIST, A. 2009. The role of interleukin-6 in lipopolysaccharide-induced fever by mechanisms independent of prostaglandin E2. *Endocrinology,* 150, 1850-60.

NIWA, K., HAENSEL, C., ROSS, M. E. & IADECOLA, C. 2001. Cyclooxygenase-1 Participates in Selected Vasodilator Responses of the Cerebral Circulation. *Circulation Research,* 88, 600-608.

NOMURA, F., AKASHI, S., SAKAO, Y., SATO, S., KAWAI, T., MATSUMOTO, M., NAKANISHI, K., KIMOTO, M., MIYAKE, K. & TAKEDA, K. 2000. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. *The Journal of Immunology,* 164, 3476.

O'MAHONY, R., MURTHY, L., AKUNNE, A. & YOUNG, J. 2011. Synopsis of the National Institute for Health and Clinical Excellence Guideline for Prevention of Delirium. *Annals of internal medicine,* 154, 746.

OGINO, K., HATANAKA, K., KAWAMURA, M., KATORI, M. & HARADA, Y. 1997. Evaluation of pharmacological profile of meloxicam as an anti-inflammatory agent, with particular reference to its relative selectivity for cyclooxygenase-2 over cyclooxygenase-1. *Pharmacology,* 55, 44-53.

OITZL, M., VAN OERS, H., SCHOBITZ, B. & DE KLOET, E. R. 1993. Interleukin-1β, but not interleukin-6, impairs spatial navigation learning. *Brain Research,* 613, 160-163.

OKA, T., OKA, K., SCAMMELL, T. E., LEE, C., KELLY, J. F., NANTEL, F., ELMQUIST, J. K. & SAPER, C. B. 2000. Relationship of EP1-4 prostaglandin receptors with rat hypothalamic cell groups involved in lipopolysaccharide fever responses. *The Journal of Comparative Neurology,* 428, 20-32.

ORTIZ, L. A., DUTREIL, M., FATTMAN, C., PANDEY, A. C., TORRES, G., GO, K. & PHINNEY, D. G. 2007. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proceedings of the National Academy of Sciences,* 104, 11002-11007.

ORZACK, S. H. & SOBER, E. 1994. Optimality models and the test of adaptationism. *American Naturalist,* 361-380.

OSBORN, L., HESSION, C., TIZARD, R., VASSALLO, C., LUHOWSKYJ, S., CHI-ROSSO, G. & LOBB, R. 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell,* 59, 1203-11.

OSERA, C., PASCALE, A., AMADIO, M., VENTURINI, L., GOVONI, S. & RICEVUTI, G. 2011. Pentraxins and Alzheimer's disease: At the interface between biomarkers and pharmacological targets. *Ageing Res Rev.*

OVERSHOTT, R., KARIM, S. & BURNS, A. 2008. Cholinesterase inhibitors for delirium. *Cochrane Database Syst Rev,* 1.

PAAKKARI, I. & LINDSBERG, P. 1995. Nitric oxide in the central nervous system. *Annals of medicine,* 27, 369-77.

PACE, J. L., RUSSELL, S., TORRES, B., JOHNSON, H. & GRAY, P. 1983. Recombinant mouse gamma interferon induces the priming step in macrophage activation for tumor cell killing. *The Journal of Immunology,* 130, 2011-2013.

PALIN, K., CUNNINGHAM, C., FORSE, P., PERRY, V. H. & PLATT, N. 2008. Systemic inflammation switches the inflammatory cytokine profile in CNS Wallerian degeneration. *Neurobiol Dis,* 30, 19-29.

PARK, B. S., SONG, D. H., KIM, H. M., CHOI, B. S., LEE, H. & LEE, J. O. 2009. The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. *Nature,* 458, 1191-1195.

PARK, J. Y., PILLINGER, M. H. & ABRAMSON, S. B. 2006. Prostaglandin E2 synthesis and secretion: The role of PGE2 synthases. *Clinical Immunology,* 119, 229-240.

PARRISH, W. R., ROSAS-BALLINA, M., GALLOWITSCH-PUERTA, M., OCHANI, M., OCHANI, K., YANG, L. H., HUDSON, L., LIN, X., PATEL, N., JOHNSON, S. M., CHAVAN, S., GOLDSTEIN, R. S., CZURA, C. J., MILLER, E. J., AL-ABED, Y., TRACEY, K. J. & PAVLOV, V. A. 2008. Modulation of TNF release by choline requires alpha7 subunit nicotinic acetylcholine receptor-mediated signaling. *Mol Med,* 14, 567-74.

PEARSON, A., DE VRIES, A., MIDDLETON, S. D., GILLIES, F., WHITE, T. O., ARMSTRONG, I. R., ANDREW, R., SECKL, J. R. & MACLULLICH, A. M. 2010. Cerebrospinal fluid cortisol levels are higher in patients with delirium versus controls. *BMC Res Notes,* 3, 33.

PECCHI, E., DALLAPORTA, M., THIRION, S., SALVAT, C., BERENBAUM, F., JEAN, A. & TROADEC, J. D. 2006. Involvement of central microsomal prostaglandin E synthase-1 in IL-1{beta}-induced anorexia. *Physiol. Genomics,* 25, 485-492.

PERRY, R. T., COLLINS, J. S., WIENER, H., ACTON, R. & GO, R. C. P. 2001. The role of TNF and its receptors in Alzheimer's disease. *Neurobiology of Aging,* 22, 873-883.

PERRY, V. H., CUNNINGHAM, C. & HOLMES, C. 2007. Systemic infections and inflammation affect chronic neurodegeneration. *Nat Rev Immunol,* 7, 161-7.

PETERSON, J. F., PUN, B. T., DITTUS, R. S., THOMASON, J. W. W., JACKSON, J. C., SHINTANI, A. K. & ELY, E. 2006. Delirium and its motoric subtypes: a study of 614 critically ill patients. *Journal of the American Geriatrics Society,* 54, 479-484.

PICKERING, M. & O'CONNOR, J. J. 2007. Pro-inflammatory cytokines and their effects in the dentate gyrus. *Prog Brain Res,* 163, 339-54.

PISANI, M. A., MCNICOLL, L. & INOUYE, S. K. 2003. Cognitive impairment in the intensive care unit. *Clinics in chest medicine,* 24, 727.

PLASCHKE, K., FICHTENKAMM, P., SCHRAMM, C., HAUTH, S., MARTIN, E., VERCH, M., KARCK, M. & KOPITZ, J. 2010. Early postoperative delirium after open-heart cardiac surgery is associated with decreased bispectral EEG and increased cortisol and interleukin-6. *Intensive care medicine,* 36, 2081-2089.

PUGH, C. R., KUMAGAWA, K., FLESHNER, M., WATKINS, L. R., MAIER, S. F. & RUDY, J. W. 1998. Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning. *Brain Behav Immun,* 12, 212-29.

RADILOVA, H., LIBRA, A., HOLASOVA, S., SAFAROVA, M., VISKOVA, A., KUNC, F. & BUNCEK, M. 2009a. COX-1 is coupled with mPGES-1 and ABCC4 in human cervix cancer cells. *Molecular and cellular biochemistry,* 330, 131-140.

RADILOVA, H., LIBRA, A., HOLASOVA, S., SAFAROVA, M., VISKOVA, A., KUNC, F. & BUNCEK, M. 2009b. COX-1 is coupled with mPGES-1 and ABCC4 in human cervix cancer cells. *Mol Cell Biochem,* 330, 131-40.

RADTKE, F. M., GAUDREAU, J. D. & SPIES, C. 2010. Diagnosing delirium. *JAMA,* 304, 2125; author reply 2126-7.

RAHKONEN, T., ELONIEMI-SULKAVA, U., PAANILA, S., HALONEN, P., SIVENIUS, J. & SULKAVA, R. 2001. Systematic intervention for supporting community care of elderly people after a delirium episode. *International psychogeriatrics,* 13, 37-49.

RAHKONEN, T., LUUKKAINEN-MARKKULA, R., PAANILA, S., SIVENIUS, J. & SULKAVA, R. 2000. Delirium episode as a sign of undetected dementia among community dwelling elderly subjects: a 2 year follow up study. *Journal of Neurology, Neurosurgery & Psychiatry,* 69, 519-521.

RAJAKARIAR, R., HILLIARD, M., LAWRENCE, T., TRIVEDI, S., COLVILLE-NASH, P., BELLINGAN, G., FITZGERALD, D., YAQOOB, M. M. & GILROY, D. W. 2007. Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyΔ12–14 PGJ2. *Proceedings of the National Academy of Sciences,* 104, 20979-20984.

RANSOHOFF, R. M. & PERRY, V. H. 2009. Microglial physiology: unique stimuli, specialized responses. *Annu Rev Immunol,* 27, 119-45.

REICHENBERG, A., YIRMIYA, R., SCHULD, A., KRAUS, T., HAACK, M., MORAG, A. & POLLMACHER, T. 2001. Cytokine-associated emotional and cognitive disturbances in humans. *Archives of General Psychiatry,* 58, 445.

REINES, S. A., BLOCK, G. A., MORRIS, J. C., LIU, G., NESSLY, M. L., LINES, C. R., NORMAN, B. A. & BARANAK, C. C. 2004. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. *Neurology,* 62, 66-71.

RICARDO, J. A. & TONGJU KOH, E. 1978. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Research,* 153, 1-26.

RIDER, P., CARMI, Y., GUTTMAN, O., BRAIMAN, A., COHEN, I., VORONOV, E., WHITE, M. R., DINARELLO, C. A. & APTE, R. N. 2011. IL-1α and IL-1β Recruit Different Myeloid Cells and Promote Different Stages of Sterile Inflammation. *The Journal of Immunology,* 187, 4835-4843.

RIVEST, S. 2001. How circulating cytokines trigger the neural circuits that control the hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology,* 26, 761-88.

ROBERTS, R. O., GEDA, Y. E., KNOPMAN, D. S., CHA, R. H., PANKRATZ, V. S., BOEVE, B. F., IVNIK, R. J., TANGALOS, E. G., PETERSEN, R. C. & ROCCA, W. A. 2008. The Mayo Clinic Study of Aging: design and sampling, participation, baseline measures and sample characteristics. *Neuroepidemiology,* 30, 58-69.

ROBERTSSON, B., BLENNOW, K., BRANE, G., EDMAN, A., KARLSSON, I., WALLIN, A. & GOTTFRIES, C. G. 2001a. Hyperactivity in the hypothalamic-pituitary-adrenal axis in demented patients with delirium. *Int Clin Psychopharmacol,* 16, 39-47.

ROBERTSSON, B., BLENNOW, K., BRÅNE, G., EDMAN, Å., KARLSSON, I., WALLIN, A. & GOTTFRIES, C. G. 2001b. Hyperactivity in the hypothalamic-pituitary-adrenal axis in demented patients with delirium. *International clinical psychopharmacology,* 16, 39.

ROCK, K. L., LATZ, E., ONTIVEROS, F. & KONO, H. 2010. The Sterile Inflammatory Response. *Annu Rev Immunol,* 28, 321-342.

ROCKWOOD, K., COSWAY, S., CARVER, D., JARRETT, P., STADNYK, K. & FISK, J. 1999. The risk of dementia and death after delirium. *Age Ageing,* 28, 551-556.

ROGERS, M. P., LIANG, M. H., DALTROY, L. H., EATON, H., PETEET, J., WRIGHT, E. & ALBERT, M. 1989. Delirium after elective orthopedic surgery: risk factors and natural history. *Int J Psychiatry Med,* 19, 109-21.

ROLFSON, D. B., MCELHANEY, J. E., ROCKWOOD, K., FINNEGAN, B. A., ENTWISTLE, L. M., WONG, J. F. & SUAREZ-ALMAZOR, M. E. 1999. Incidence and risk factors for delirium and other adverse outcomes in older adults after coronary artery bypass graft surgery. *Can J Cardiol,* 15, 771-6.

ROLLINS, B. J., YOSHIMURA, T., LEONARD, E. J. & POBER, J. S. 1990. Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. *Am J Pathol,* 136, 1229-33.

ROMAN, G. C. 2004. Brain hypoperfusion: a critical factor in vascular dementia. *Neurol Res,* 26, 454-8.

ROMANOVSKY, A. A., IVANOV, A. I. & KARMAN, E. K. 1999. Blood-borne, albumin-bound prostaglandin E2 may be involved in fever. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 276, R1840-R1844.

RONALD DE KOLOET, E. 1997. Why dexamethasone poorly penetrates in brain. *Stress: The International Journal on the Biology of Stress,* 2, 13-19.

ROSENBERG, G. A. 2002. Matrix metalloproteinases in neuroinflammation. *Glia,* 39, 279-291.

ROTHWELL, N., ALLAN, S. & TOULMOND, S. 1997. The role of interleukin 1 in acute neurodegeneration and stroke: pathophysiological and therapeutic implications. *Journal of Clinical Investigation,* 100, 2648.

RUDOLPH, J. L., JONES, R. N., LEVKOFF, S. E., ROCKETT, C., INOUYE, S. K., SELLKE, F. W., KHURI, S. F., LIPSITZ, L. A., RAMLAWI, B. & LEVITSKY, S. 2009. Derivation and validation of a preoperative prediction rule for delirium after cardiac surgery. *Circulation,* 119, 229-236.

SANG, N., ZHANG, J., MARCHESELLI, V., BAZAN, N. G. & CHEN, C. 2005. Postsynaptically synthesized prostaglandin E2 (PGE2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. *J Neurosci,* 25, 9858-70.

SANSONETTI, P. J., PHALIPON, A., ARONDEL, J., THIRUMALAI, K., BANERJEE, S., AKIRA, S., TAKEDA, K. & ZYCHLINSKY, A. 2000. Caspase-1 Activation of IL-1β and IL-18 Are Essential for Shigella flexneri–Induced Inflammation. *Immunity,* 12, 581-590.

SAPER, C. B. 2010. The dance of the perivascular and endothelial cells: mechanisms of brain response to immune signaling. *Neuron,* 65, 4-6.

SATZ, P., COLE, M. A., HARDY, D. J. & RASSOVSKY, Y. 2011. Brain and cognitive reserve: mediator(s) and construct validity, a critique. *J Clin Exp Neuropsychol,* 33, 121-30.

SCHNEIDER, A., ZHANG, Y., ZHANG, M., LU, W. J., RAO, R., FAN, X., REDHA, R., DAVIS, L., BREYER, R. M., HARRIS, R., GUAN, Y. & BREYER, M. D. 2004. Membrane-associated PGE synthase-1 (mPGES-1) is coexpressed with both COX-1 and COX-2 in the kidney. *Kidney Int,* 65, 1205-13.

SCHRAM, M. T., EUSER, S. M., DE CRAEN, A. J., WITTEMAN, J. C., FROLICH, M., HOFMAN, A., JOLLES, J., BRETELER, M. M. & WESTENDORP, R. G. 2007. Systemic markers of inflammation and cognitive decline in old age. *J Am Geriatr Soc,* 55, 708-16.

SEMMLER, A., HERMANN, S., MORMANN, F., WEBERPALS, M., PAXIAN, S. A., OKULLA, T., SCHÄFERS, M., KUMMER, M. P., KLOCKGETHER, T. & HENEKA, M. T. 2008. Sepsis causes neuroinflammation and concomitant decrease of cerebral metabolism. *J Neuroinflammation,* 5, 38.

SERRATS, J., SCHILTZ, J. C., GARCIA-BUENO, B., VAN ROOIJEN, N., REYES, T. M. & SAWCHENKO, P. E. 2010. Dual roles for perivascular macrophages in immune-to-brain signaling. *Neuron,* 65, 94-106.

SHENG, J. G., BORA, S. H., XU, G., BORCHELT, D. R., PRICE, D. L. & KOLIATSOS, V. E. 2003. Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid beta peptide in APPswe transgenic mice. *Neurobiol Dis,* 14, 133-45.

SHI, J., JOHANSSON, J., WOODLING, N. S., WANG, Q., MONTINE, T. J. & ANDREASSON, K. 2010. The prostaglandin E2 E-prostanoid 4 receptor exerts anti-inflammatory effects in brain innate immunity. *J Immunol,* 184, 7207-18.

SHIE, F. S., BREYER, R. M. & MONTINE, T. J. 2005a. Microglia lacking E Prostanoid Receptor subtype 2 have enhanced Abeta phagocytosis yet lack Abeta-activated neurotoxicity. *Am J Pathol,* 166, 1163-72.

SHIE, F. S., MONTINE, K. S., BREYER, R. M. & MONTINE, T. J. 2005b. Microglial EP2 is critical to neurotoxicity from activated cerebral innate immunity. *Glia,* 52, 70-7.

SHIMIZU, I., ADACHI, N., LIU, K., LEI, B., NAGARO, T. & ARAI, T. 1999. Sepsis facilitates brain serotonin activity and impairs learning ability in rats. *Brain Research,* 830, 94-100.

SHITASHIGE, M., MORITA, I. & MUROTA, S. 1998. Different substrate utilization between prostaglandin endoperoxide H synthase-1 and-2 in NIH3T3 fibroblasts. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism,* 1389, 57-66.

SIMI, A., TSAKIRI, N., WANG, P. & ROTHWELL, N. 2007. Interleukin-1 and inflammatory neurodegeneration. *Biochemical Society Transactions,* 35, 1122-1126.

SINGH, A. & JIANG, Y. 2004. How does peripheral lipopolysaccharide induce gene expression in the brain of rats? *Toxicology,* 201, 197-207.

SKINNER, R. A., GIBSON, R. M., ROTHWELL, N. J., PINTEAUX, E. & PENNY, J. I. 2009. Transport of interleukin-1 across cerebromicrovascular endothelial cells. *Br J Pharmacol,* 156, 1115-23.

SKROBIK, Y. 2010. Delirium treatment: an unmet challenge. *The Lancet,* 376, 1805-7.
SLY, L. M., KRZESICKI, R. F., BRASHLER, J. R., BUHL, A. E., MCKINLEY, D. D., CARTER, D. B. & CHIN, J. E. 2001. Endogenous brain cytokine mRNA and inflammatory responses to lipopolysaccharide are elevated in the Tg2576 transgenic mouse model of Alzheimer's disease. *Brain Res Bull,* 56, 581-8.
SMITH, C., EMSLEY, H., GAVIN, C., GEORGIOU, R., VAIL, A., BARBERAN, E., DEL ZOPPO, G., HALLENBECK, J., ROTHWELL, N. & HOPKINS, S. 2004. Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. *BMC neurology,* 4, 2.
SMITH, C. J., ZHANG, Y., KOBOLDT, C. M., MUHAMMAD, J., ZWEIFEL, B. S., SHAFFER, A., TALLEY, J. J., MASFERRER, J. L., SEIBERT, K. & ISAKSON, P. C. 1998. Pharmacological analysis of cyclooxygenase-1 in inflammation. *Proceedings of the National Academy of Sciences,* 95, 13313-13318.
SMITH, P. J., ATTIX, D. K., WELDON, B. C., GREENE, N. H. & MONK, T. G. 2009. Executive function and depression as independent risk factors for postoperative delirium. *Anesthesiology,* 110, 781.
SMITH, W. L., MEADE, E. A. & DEWITT, D. L. 1994a. Interactions of PGH synthase isozymes-1 and -2 with NSAIDs. *Ann N Y Acad Sci,* 744, 50-7.
SMITH, W. L., MEADE, E. A. & DEWITT, D. L. 1994b. Pharmacology of prostaglandin endoperoxide synthase isozymes-1 and -2. *Ann N Y Acad Sci,* 714, 136-42.
SOININEN, H., WEST, C., ROBBINS, J. & NICULESCU, L. 2007. Long-term efficacy and safety of celecoxib in Alzheimer's disease. *Dement Geriatr Cogn Disord,* 23, 8-21.
SOIZA, R. L., SHARMA, V., FERGUSON, K., SHENKIN, S. D., SEYMOUR, D. G. & MACLULLICH, A. M. J. 2008. Neuroimaging studies of delirium: A systematic review. *Journal of Psychosomatic Research,* 65, 239-248.
SOMMER, B. R., WISE, L. C. & KRAEMER, H. C. 2002. Is dopamine administration possibly a risk factor for delirium? *Critical care medicine,* 30, 1508.
SPARKMAN, N. L., BUCHANAN, J. B., HEYEN, J. R., CHEN, J., BEVERLY, J. L. & JOHNSON, R. W. 2006. Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers. *J Neurosci,* 26, 10709-16.
SPENCER, A. G., WOODS, J. W., ARAKAWA, T., SINGER, I. I. & SMITH, W. L. 1998. Subcellular localization of prostaglandin endoperoxide H synthases-1 and-2 by immunoelectron microscopy. *Journal of Biological Chemistry,* 273, 9886.
STARKSTEIN, S. E., SABE, L., VÁZQUEZ, S., TESÓN, A., PETRACCA, G., CHEMERINSKI, E., DI LORENZO, G. & LEIGUARDA, R. 1996. Neuropsychological, Psychiatric, and Cerebral Blood Flow Findings in Vascular Dementia and Alzheimer's Disease. *Stroke,* 27, 408-414.
STEEG, P., MOORE, R., JOHNSON, H. & OPPENHEIM, J. 1982. Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. *J Exp Med,* 156, 1780.
STEINMAN, L. 2004. Elaborate interactions between the immune and nervous systems. *Nat Immunol,* 5, 575-581.
STERN, Y. 2009. Cognitive reserve. *Neuropsychologia,* 47, 2015-28.
STREIT, W. J., MRAK, R. E. & GRIFFIN, W. S. T. 2004. Microglia and neuroinflammation: a pathological perspective. *J Neuroinflammation,* 1, 14.
SUGIMOTO, Y. & NARUMIYA, S. 2007. Prostaglandin E receptors. *J Biol Chem,* 282, 11613-7.
SWANEPOEL, T., HARVEY, B. H., HARDEN, L. M., LABURN, H. P. & MITCHELL, D. 2011. Dissociation between learning and memory impairment and other sickness behaviours in rats given fibroblast-stimulating lipopeptide-1 (FSL-1). *Brain Behav Immun.*
SWARDFAGER, W., LANCTÔT, K., ROTHENBURG, L., WONG, A., CAPPELL, J. & HERRMANN, N. 2010. A Meta-Analysis of Cytokines in Alzheimer's Disease. *Biol Psychiatry,* 68, 930-941.
SWIERGIEL, A. H. & DUNN, A. J. 1999. The roles of IL-1, IL-6, and TNFalpha in the feeding responses to endotoxin and influenza virus infection in mice. *Brain Behav Immun,* 13, 252-65.
SWIERGIEL, A. H. & DUNN, A. J. 2007. Effects of interleukin-1beta and lipopolysaccharide on behavior of mice in the elevated plus-maze and open field tests. *Pharmacol Biochem Behav,* 86, 651-9.
SZE, C. I., TRONCOSO, J. C., KAWAS, C., MOUTON, P., PRICE, D. L. & MARTIN, L. J. 1997. Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J Neuropathol Exp Neurol,* 56, 933-44.

TAEPAVARAPRUK, P. & SONG, C. 2010. Reductions of acetylcholine release and nerve growth factor expression are correlated with memory impairment induced by interleukin-1β administrations: effects of omega-3 fatty acid EPA treatment. *J Neurochem,* 112, 1054-1064.

TAGA, T., HIBI, M., HIRATA, Y., YAMASAKI, K., YASUKAWA, K., MATSUDA, T., HIRANO, T. & KISHIMOTO, T. 1989. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell,* 58, 573-81.

TAN, Z. S., BEISER, A. S., VASAN, R. S., ROUBENOFF, R., DINARELLO, C. A., HARRIS, T. B., BENJAMIN, E. J., AU, R., KIEL, D. P., WOLF, P. A. & SESHADRI, S. 2007. Inflammatory markers and the risk of Alzheimer disease. *Neurology,* 68, 1902-1908.

TANIOKA, T., NAKATANI, Y., SEMMYO, N., MURAKAMI, M. & KUDO, I. 2000. Molecular Identification of Cytosolic Prostaglandin E2 Synthase That Is Functionally Coupled with Cyclooxygenase-1 in Immediate Prostaglandin E2Biosynthesis. *Journal of Biological Chemistry,* 275, 32775-32782.

TARTAGLIA, L. A. & GOEDDEL, D. V. 1992. Two TNF receptors. *Immunology today,* 13, 151.

TAZI, A., DANTZER, R., CRESTANI, F. & LE MOAL, M. 1988. Interleukin-1 induces conditioned taste aversion in rats: a possible explanation for its pituitary-adrenal stimulating activity. *Brain Research,* 473, 369-371.

TEATHER, L. A., PACKARD, M. G. & BAZAN, N. G. 2002. Post-training cyclooxygenase-2 (COX-2) inhibition impairs memory consolidation. *Learn Mem,* 9, 41-7.

TEELING, J. L., CUNNINGHAM, C., NEWMAN, T. A. & PERRY, V. H. 2010. The effect of non-steroidal anti-inflammatory agents on behavioural changes and cytokine production following systemic inflammation: Implications for a role of COX-1. *Brain Behav Immun,* 24, 409-19.

TEELING, J. L., FELTON, L. M., DEACON, R. M., CUNNINGHAM, C., RAWLINS, J. N. & PERRY, V. H. 2007. Sub-pyrogenic systemic inflammation impacts on brain and behavior, independent of cytokines. *Brain Behav Immun,* 21, 836-50.

TERRANDO, N., MONACO, C., MA, D., FOXWELL, B. M., FELDMANN, M. & MAZE, M. 2010a. Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline. *Proc Natl Acad Sci U S A,* 107, 20518-22.

TERRANDO, N., REI FIDALGO, A., VIZCAYCHIPI, M., CIBELLI, M., MA, D., MONACO, C., FELDMANN, M. & MAZE, M. 2010b. The impact of IL-1 modulation on the development of lipopolysaccharide-induced cognitive dysfunction. *Crit Care,* 14, R88.

TERRY, R. D., MASLIAH, E., SALMON, D. P., BUTTERS, N., DETERESA, R., HILL, R., HANSEN, L. A. & KATZMAN, R. 1991. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol,* 30, 572-80.

THORNBERRY, N. A., BULL, H. G., CALAYCAY, J. R., CHAPMAN, K. T., HOWARD, A. D., KOSTURA, M. J., MILLER, D. K., MOLINEAUX, S. M., WEIDNER, J. R., AUNINS, J. & ET AL. 1992. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature,* 356, 768-74.

THORNBERRY, N. A. & LAZEBNIK, Y. 1998. Caspases: enemies within. *Science,* 281, 1312-1316.

THORNTON, P., MCCOLL, B. W., GREENHALGH, A., DENES, A., ALLAN, S. M. & ROTHWELL, N. J. 2010. Platelet interleukin-1α drives cerebrovascular inflammation. *Blood,* 115, 3632-3639.

TILLEY, S. L., COFFMAN, T. M. & KOLLER, B. H. 2001. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest,* 108, 15-23.

TRACEY, K. J. 2002. The inflammatory reflex. *Nature,* 420, 853-9.

TRACEY, K. J. 2009. Reflex control of immunity. *Nat Rev Immunol,* 9, 418-28.

TRZEPACZ, P. Is there a final common neural pathway in delirium? Focus on acetylcholine and dopamine. 2000. 132.

TRZEPACZ, P. T. 1999. Update on the neuropathogenesis of delirium. *Dement Geriatr Cogn Disord,* 10, 330-334.

TRZEPACZ, P. T., LEAVITT, M. & CIONGOLI, K. 1992. An animal model for delirium. *Psychosomatics: Journal of Consultation Liaison Psychiatry.*

TUNE, L. E., HOLLAND, A., FOLSTEIN, M. F., DAMLOUJI, N. F., GARDNER, T. J. & COYLE, J. T. 1981. Association of postoperative delirium with raised serum levels of anticholinergic drugs. *The Lancet,* 318, 651-653.

TURRIN, N. P. & RIVEST, S. 2004. Unraveling the molecular details involved in the intimate link between the immune and neuroendocrine systems. *Exp Biol Med (Maywood),* 229, 996-1006.

UCHIKADO, H., AKIYAMA, H., KONDO, H., IKEDA, K., TSUCHIYA, K., KATO, M., ODA, T., TOGO, T., ISEKI, E. & KOSAKA, K. 2004. Activation of vascular endothelial cells and perivascular cells by systemic

inflammation-an immunohistochemical study of postmortem human brain tissues. *Acta Neuropathol,* 107, 341-51.
UENO, N., TAKEGOSHI, Y., KAMEI, D., KUDO, I. & MURAKAMI, M. 2005. Coupling between cyclooxygenases and terminal prostanoid synthases. *Biochem Biophys Res Commun,* 338, 70-6.
URADE, Y. & HAYAISHI, O. 2000. Prostaglandin D synthase: structure and function. *Vitam Horm,* 58, 89-120.
USHIKUBI, F., SEGI, E., SUGIMOTO, Y., MURATA, T., MATSUOKA, T., KOBAYASHI, T., HIZAKI, H., TUBOI, K., KATSUYAMA, M., ICHIKAWA, A., TANAKA, T., YOSHIDA, N. & NARUMIYA, S. 1998. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature,* 395, 281-4.
VALENZUELA, M. J. & SACHDEV, P. 2006. Brain reserve and dementia: a systematic review. *Psychological Medicine,* 36, 441-454.
VALLES, A., GRIJPINK-ONGERING, L., DE BREE, F. M., TUINSTRA, T. & RONKEN, E. 2006. Differential regulation of the CXCR2 chemokine network in rat brain trauma: implications for neuroimmune interactions and neuronal survival. *Neurobiol Dis,* 22, 312-22.
VAN DEN BOOGAARD, M., RAMAKERS, B. P., VAN ALFEN, N., VAN DER WERF, S. P., FICK, W. F., HOEDEMAEKERS, C. W., VERBEEK, M. M., SCHOONHOVEN, L., VAN DER HOEVEN, J. G. & PICKKERS, P. 2010. Research Endotoxemia-induced inflammation and the effect on the human brain.
VAN DER MAST, R. 1998. Pathophysiology of delirium. *Journal of Geriatric Psychiatry and Neurology,* 11, 138.
VAN EIJK, M. M. J., ROES, K. C. B., HONING, M. L. H., KUIPER, M. A., KARAKUS, A., VAN DER JAGT, M., SPRONK, P. E., VAN GOOL, W. A., VAN DER MAST, R. C., KESECIOGLU, J. & SLOOTER, A. J. C. 2010. Effect of rivastigmine as an adjunct to usual care with haloperidol on duration of delirium and mortality in critically ill patients: a multicentre, double-blind, placebo-controlled randomised trial. *The Lancet,* 376, 1829-1837.
VAN EXEL, E., DE CRAEN, A. J. M., REMARQUE, E. J., GUSSEKLOO, J., HOUX, P., BOOTSMA–VAN DER WIEL, A., FRÖLICH, M., MACFARLANE, P. W., BLAUW, G. J. & WESTENDORP, R. G. J. 2003. Interaction of atherosclerosis and inflammation in elderly subjects with poor cognitive function. *Neurology,* 61, 1695-1701.
VAN GOOL, W. A., VAN DE BEEK, D. & EIKELENBOOM, P. 2010. Systemic infection and delirium: when cytokines and acetylcholine collide. *Lancet,* 375, 773-5.
VAN MUNSTER, B. C., BISSCHOP, P. H., ZWINDERMAN, A. H., KOREVAAR, J. C., ENDERT, E., WIERSINGA, W. J., VAN OOSTEN, H. E., GOSLINGS, J. C. & DE ROOIJ, S. E. 2010. Cortisol, interleukins and S100B in delirium in the elderly. *Brain Cogn,* 74, 18-23.
VAN MUNSTER, B. C., KOREVAAR, J. C., ZWINDERMAN, A. H., LEVI, M., WIERSINGA, W. J. & DE ROOIJ, S. E. 2008. Time-course of cytokines during delirium in elderly patients with hip fractures. *J Am Geriatr Soc,* 56, 1704-9.
VANE, J., BAKHLE, Y. & BOTTING, R. 1998. CYCLOOXYGENASES 1 AND 2. *Annual Review of Pharmacology and Toxicology,* 38, 97-120.
VANHOUTTE, P. M. 2009. COX-1 and Vascular Disease. *Clin Pharmacol Ther,* 86, 212-215.
VARFOLOMEEV, E. E. & ASHKENAZI, A. 2004. Tumor necrosis factor: an apoptosis JuNKie? *Cell,* 116, 491-497.
VILLARAN, R. F., ESPINOSA-OLIVA, A. M., SARMIENTO, M., DE PABLOS, R. M., ARGUELLES, S., DELGADO-CORTES, M. J., SOBRINO, V., VAN ROOIJEN, N., VENERO, J. L., HERRERA, A. J., CANO, J. & MACHADO, A. 2010. Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson`s disease. *J Neurochem,* 114, 1687-700.
VLAD, S. C., MILLER, D. R., KOWALL, N. W. & FELSON, D. T. 2008. Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology,* 70, 1672-1677.
WANG, J., ANDO, T. & DUNN, A. J. 1997. Effect of homologous interleukin-1, interleukin-6 and tumor necrosis factor-alpha on the core body temperature of mice. *Neuroimmunomodulation,* 4, 230-6.
WANG, J. & DUNN, A. J. 1998. Mouse interleukin-6 stimulates the HPA axis and increases brain tryptophan and serotonin metabolism. *Neurochemistry International,* 33, 143-154.
WARNER, T. D., GIULIANO, F., VOJNOVIC, I., BUKASA, A., MITCHELL, J. A. & VANE, J. R. 1999. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with

human gastrointestinal toxicity: A full in vitro analysis. *Proceedings of the National Academy of Sciences,* 96, 7563-7568.

WATANABE-FUKUNAGA, R., BRANNAN, C. I., COPELAND, N. G., JENKINS, N. A. & NAGATA, S. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature,* 356, 314-7.

WATSON, K. & FAN, G. H. 2005. Macrophage inflammatory protein 2 inhibits beta-amyloid peptide (1-42)-mediated hippocampal neuronal apoptosis through activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways. *Mol Pharmacol,* 67, 757-65.

WEAVER, J. D., HUANG, M. H., ALBERT, M., HARRIS, T., ROWE, J. W. & SEEMAN, T. E. 2002. Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging. *Neurology,* 59, 371-8.

WEIDENFELD, J., CRUMEYROLLE-ARIAS, M. & HAOUR, F. 1995. Effect of Bacterial Endotoxin and Interleukin-1 on Prostaglandin Biosynthesis by the Hippocampus of Mouse Brain: Role of Interleukin-1 Receptors and Glucocorticoids. *Neuroendocrinology,* 62, 39-46.

WEINTRAUB, D., DIETZ, N., DUDA, J. E., WOLK, D. A., DOSHI, J., XIE, S. X., DAVATZIKOS, C., CLARK, C. M. & SIDEROWF, A. 2012. Alzheimer's disease pattern of brain atrophy predicts cognitive decline in Parkinson's disease. *Brain,* 135, 170-180.

WHO 1992. The ICD-10 classification of mental and behavioural disorders. Diagnostic criteria for research.: World Health Organisation (Geneva).

WILSON, C. J., COHEN, H. J. & PIEPER, C. F. 2003. Cross-linked fibrin degradation products (D-dimer), plasma cytokines, and cognitive decline in community-dwelling elderly persons. *J Am Geriatr Soc,* 51, 1374-81.

WINBLAD, B., ANDREASEN, N., MINTHON, L., FLOESSER, A., IMBERT, G., DUMORTIER, T., MAGUIRE, R. P., BLENNOW, K., LUNDMARK, J., STAUFENBIEL, M., ORGOGOZO, J.-M. & GRAF, A. 2012. Safety, tolerability, and antibody response of active A? immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study. *The Lancet Neurology,* 11, 597-604.

WITLOX, J., EURELINGS, L. S., DE JONGHE, J. F., KALISVAART, K. J., EIKELENBOOM, P. & VAN GOOL, W. A. 2010. Delirium in elderly patients and the risk of postdischarge mortality, institutionalization, and dementia: a meta-analysis. *JAMA,* 304, 443-51.

WONG, C. L., HOLROYD-LEDUC, J., SIMEL, D. L. & STRAUS, S. E. 2010. Does this patient have delirium?: value of bedside instruments. *JAMA,* 304, 779-86.

WOOD, A. J. J., FITZGERALD, G. A. & PATRONO, C. 2001. The coxibs, selective inhibitors of cyclooxygenase-2. *New England Journal of Medicine,* 345, 433-442.

WOOD, J. A., WOOD, P. L., RYAN, R., GRAFF-RADFORD, N. R., PILAPIL, C., ROBITAILLE, Y. & QUIRION, R. 1993. Cytokine indices in Alzheimer's temporal cortex: no changes in mature IL-1 [beta] or IL-1RA but increases in the associated acute phase proteins IL-6,[alpha] 2-macroglobulin and C-reactive protein. *Brain Research,* 629, 245-252.

WOODRUFF, R. T., SCHORPP, K. M., LAWRENCZYK, A. J., CHAKRABORTY, T. & KUSNECOV, A. W. 2011. Effects of acute and repeated administration of Staphylococcal enterotoxin A on Morris water maze learning, corticosterone and hippocampal IL-1β and TNFα. *Brain Behav Immun,* 25, 938-946.

WRIGHT, C. B., SACCO, R. L., RUNDEK, T. R., DELMAN, J. B., RABBANI, L. E. & ELKIND, M. S. 2006. Interleukin-6 is associated with cognitive function: the Northern Manhattan Study. *J Stroke Cerebrovasc Dis,* 15, 34-8.

WYSS-CORAY, T. 2006. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? *Nat Med,* 12, 1005-15.

XIE, Q. W., KASHIWABARA, Y. & NATHAN, C. 1994. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. *Journal of Biological Chemistry,* 269, 4705-4708.

XING, Z., GAULDIE, J., COX, G., BAUMANN, H., JORDANA, M., LEI, X. F. & ACHONG, M. K. 1998. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest,* 101, 311-20.

YAFFE, K., KANAYA, A., LINDQUIST, K., SIMONSICK, E. M., HARRIS, T., SHORR, R. I., TYLAVSKY, F. A. & NEWMAN, A. B. 2004. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA,* 292, 2237-42.

YAFFE, K., LINDQUIST, K., PENNINX, B. W., SIMONSICK, E. M., PAHOR, M., KRITCHEVSKY, S., LAUNER, L., KULLER, L., RUBIN, S. & HARRIS, T. 2003. Inflammatory markers and cognition in well-functioning African-American and white elders. *Neurology,* 61, 76-80.

YAMAGATA, K., MATSUMURA, K., INOUE, W., SHIRAKI, T., SUZUKI, K., YASUDA, S., SUGIURA, H., CAO, C., WATANABE, Y. & KOBAYASHI, S. 2001. Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J Neurosci,* 21, 2669-77.

YANG, F. M., MARCANTONIO, E. R., INOUYE, S. K., KIELY, D. K., RUDOLPH, J. L., FEARING, M. A. & JONES, R. N. 2009. Phenomenological subtypes of delirium in older persons: patterns, prevalence, and prognosis. *Psychosomatics,* 50, 248.

YASOJIMA, K., SCHWAB, C., MCGEER, E. G. & MCGEER, P. L. 1999. Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs. *Brain Res,* 830, 226-36.

YASUKAWA, H., OHISHI, M., MORI, H., MURAKAMI, M., CHINEN, T., AKI, D., HANADA, T., TAKEDA, K., AKIRA, S. & HOSHIJIMA, M. 2003. IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. *Nature immunology,* 4, 551-556.

YERMAKOVA, A. V., ROLLINS, J., CALLAHAN, L. M., ROGERS, J. & O'BANION, M. K. 1999. Cyclooxygenase-1 in human Alzheimer and control brain: quantitative analysis of expression by microglia and CA3 hippocampal neurons. *J Neuropathol Exp Neurol,* 58, 1135-46.

YIRMIYA, R. & GOSHEN, I. 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav Immun,* 25, 1181-2213.

YOUNG, J. & INOUYE, S. K. 2007. Delirium in older people. *BMJ,* 334, 842-6.

YOUNG, R. S. & ARSEVEN, A. 2010. Diagnosing delirrium. *JAMA,* 304, 2125-6; author reply 2126-7.

ZHANG, J., FUJII, S., WU, Z., HASHIOKA, S., TANAIKA, Y., SHIRATSUCHI, A., NAKANISHI, Y. & NAKANISHI, H. 2006. Involvement of COX-1 and up-regulated prostaglandin E synthases in phosphatidylserine liposome-induced prostaglandin E2 production by microglia. *Journal of neuroimmunology,* 172, 112-120.

ZHANG, J. & RIVEST, S. 2003. Is survival possible without arachidonate metabolites in the brain during systemic infection? *News Physiol Sci,* 18, 1137-42.