Low-Grade Inflammation and Cognitive Functioning - Emotion Recognition, Reinforcement Learning, and Attention

By

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A thesis submitted to

The University of Birmingham

For the degree of DOCTOR OF PHILOSOPHY

School of Psychology

College of Life and Environmental Sciences

University of Birmingham

April 2019

ABSTRACT

The research in this thesis investigated low-grade inflammation as a factor in cognitive function, focussing on three cognitive domains: social cognition, motivated behaviour, and attention and psychomotor processes. Vaccination-induced acute low-grade inflammation reduced emotion recognition and perceived loneliness predicted the magnitude of the inflammatory response to this induction. The effects of acute low-grade inflammation on emotion recognition were replicated in age- and BMI-related chronic low-grade inflammation. Next, acute inflammation affected selective aspects of motivated learning (e.g., rate of learning, flexibility). These results were again partly replicated in chronic inflammation. Finally, older age and high BMI were both associated with psychomotor slowing, and inflammation appeared to be a mediator. Behavioural responses to the Attention Network Task appeared unaffected by both acute and chronic low-grade inflammation. However, EEG analysis demonstrated that acute low-grade inflammation affected the underlying neurophysiological process that underpins attentional alerting functions, as evident by greater cue-induced suppression of alpha power. This result suggests greater deployment of mental effort to maintain adequate performance. While prior research has mostly focussed on inflammation as a possible determinant of psychopathology, the present results indicate that low-grade inflammation in the absence of illness likewise impact cognitive function, suggesting also relevance for every-day cognitive functioning.

ACKNOWLEDGEMENTS

I am grateful for the opportunity I was given to develop myself as researcher and as person. I have had the luxury of being inspirited and supported by so many people, who I would like to cordially thank.

First and foremost, I would like to thank Jane Raymond, Jos Bosch, and Suzanne Higgs for their intellectual enthusiasm, mentorship, for providing the backbone for all of my doctoral work, and for shaping me as a researcher. Jane, you inspired me from start to finish with your talent to effortlessly produce understandable examples of complex matters, meeting after meeting. Thank you for sharing your wealth of knowledge and analytical eye, which helped me realise the power of critical reasoning. In extension of that I would like to thank Jos. Your limitless patience, writing skills, and ability to convey a message have been an invaluable set of skills that greatly contributed to my development and the quality of this dissertation. Thank you for your profound belief in my abilities and your honest, passionate, and dedicated attitude. We have been a perfect team. It has been an absolute honour to learn from you. Suzanne, thank you for always making time for a meeting, a call or fast email reply. You deserve the first prize for fast reply (ok, shared with Jos). Your faith and energy have been hugely valuable for me. I would also like to thank Ali Mazaheri for collaborating. Ali, your infinite passion, and your open and welcoming attitude are priceless. Thank you for sharing your EEG skills and for showing me how much more there is still to learn. Working with you was a privilege. Sasha Hulsken, Sarah Aldred, Jet Veldhuijzen van Zanten, and Mark Drayson thank you for collaborating. The ethical application process would have been much more difficult without you. And, of course, a huge thank you to both the University of Birmingham and University of Amsterdam assessment committee for taking the time to read this dissertation: Dr. Vanessa van Ast, Prof. Dr. Neil Harrison, Prof. Dr. Willem Kop, Prof. Dr. Mats Lekander, Prof. Dr. Sanne Schagen, Dr. Bernhard Staresina, and Dr. Bruno Verschuere. I am looking forward to hear your thoughts!

I thank the University of Birmingham and the University of Amsterdam for giving me the opportunity to conduct research and learn from inspiring and highly competent people. I would like to acknowledge the helpful and erudite people I met on this journey. First, I would like to thank my fellow Visual Experience Lab mates of the University of Birmingham, Sara Assecondi, Alberto Aviles, Andrew Clouter, Daniel Dodgson, Lily FitzGibbon, Kelly Garner, Chris Nolan, Scott Jones, Daniel Lindh, and Aleksandra Pastuszak. The lab meetings with you, Jane Raymond, Kim Shapiro, and Howard Bowman, were immensely supportive and refreshing. It was a privilege to be part of such a knowledgeable lab. My time in the office could not be better, thanks for the good (tea)time in the office, Lily, Kelly, and Scott. I am grateful to have been part of the Eating Behaviour Lab of Suzanne Higgs too and to meet Maartje Spetter, Panagiota Kaisari, Angela Meadows, Emily Collins, Jinyu Liu, and all the other lab members. Thank you Suzanne and lab members for the good times. Thanks also to Richard Elsworthy for helping with the lab work.

I would also like to thank all the other people I met in Birmingham. You made my time there unforgettable: Marit Petzka, Steffen Buergers, Tara van Viegen, Charlotte Poulisse, Claudio Tennie, Kelly Garner, Chris Nolan, Giulio Degano, Cati Ferreira, Sebastian Michelmann, Rodika Sokoliuk, Juan Linde, Jonathan Catling, thank you for such an enjoyable time. Claudio, thank you for being such a warm and lovely person. I still look back with a big smile to our trip in East-Germany and visiting each other in Tübingen and Amsterdam (and soon Sweden). Marit, I am glad to have met you. I value the many science and non-science conversations we have had. Thanks also to Steffen, Tara, Judo, and Marit for staying at your house when I came over for yet another visit after I moved to Amsterdam.

Sasha Hulsken, Linda Geven, Wouter Cox, Carlijn Wibbelink, Olivier de Vries, Duygu Yakin, and Laura Steenbergen, thank you for such a warm welcome in Amsterdam halfway through my PhD. Linda, Wouter, Olivier, and Sasha, it was an honour to share an office together, I will miss the smell of peanut butter and microwave meals of Olivier, the blue cheese of Linda (your new office mate will hopefully accept you the way you are), and the peaceful slicing of cucumber how only Wouter can do. Thank you for the unforgettable time.

Furthermore, I would like to thank all colleagues at the department of Clinical Psychology at the University of Amsterdam. It was an absolute pleasure to work there.

Linda, Wouter, and Carlijn thank you for being so supportive and having a brilliant sense of humour. I look forward to visiting each other wherever life will take us. A special thank you to Sasha. I am grateful for knowing such a warm and honest person like you. Thank you for being my friend, for sharing important and slightly less important things in and outside of the office, dancing together, having dinner, travelling, and relaxing in the park. There is still much more to come! Gaby, it feels like I have known you for so much longer. Thanks for all the good laughs and conversations while running, cycling, and building muscles. Experiencing the different 'disciplines of life' together with you created unforgettable moments. I look forward to collecting more of such memories. Nick, you are a friend I can share everything with, nothing is too crazy for you. I feel honoured to know you and be able to call you my friend. Cédric, I feel so fortunate to have you as a friend. I look forward to many more (mountain) adventures. Milan, our friendship means everything to me. Thank you for your unconditional friendship. I would also like to thank the people of Beacon Roads Cycling Club and Birmingham Running, Athletics and Triathlon Club (BRAT) for the many hours running and cycling around in the UK. I also met amazing people at Amsterdam Triathlon and Cycling (ATAC) and WV de Amstel. It is great to run and cycle with like-minded people. The training camps on Tenerife, Mallorca, as well as the trips to Sweden, Belgium, and other places fuelled me with the energy I needed. I would also like to thank my running shoes and road bikes for always waiting for me. No matter what time or how wet, warm or windy it was, you always gave me the relaxation through exertion, fuelled me with vitamin D, and gave me fresh ideas and insights.

Finally, I am grateful for having such a close loving family. Thank you, mam en pap, for raising me to become the person I am today, for giving me endless support, and for creating the best home I could wish for. Thanks to my sister Anne, for blazing a path into science ahead of me and for your advice and support throughout my (PhD) life. You are the smartest person I know. Thank you my brother Benno, to let me be a 'wheelsucker' and for making me a stronger

person. You are my favourite brother.

With love,

Leonie

LIST OF PUBLICATIONS

Published articles

Balter, L. J. T., Hulsken, S., Aldred, S., Drayson, M. T., Higgs, S., Veldhuijzen van Zanten, J. J. C. S., Raymond, J. E., & Bosch, J. A. (2018). Low-grade inflammation decreases emotion recognition – Evidence from the vaccination model of inflammation. *Brain, Behavior, and Immunity*. doi: 10.1016/j.bbi.2018.05.006.

Articles under review

Balter, L. J. T., Raymond, J. A., Aldred, S., Drayson, M. T., Veldhuijzen van Zanten, J. J. C. S., Higgs, S., & Bosch, J. A. (under review). Loneliness in Healthy Young Adults Predicts Inflammatory Responsiveness a Mild Immune Challenge in Vivo.

Balter, L. J. T., Higgs, S., Aldred, S., Bosch, J. A., & Raymond, J. E., (under review). Inflammation Mediates Body Weight and Ageing Effects on Psychomotor Slowing.

Balter, L. J. T., Bosch, J. A., Aldred, S., Drayson, M. T., Higgs, S., Veldhuijzen van Zanten, J. J. C. S., Raymond, J., E., & Mazaheri, A. (under review). Selective Effects of Acute Low-Grade Inflammation on Human Visual Attention

Articles in preparation

Balter, L. J. T., Bosch, J. A., Aldred, S., Drayson, M. T., Veldhuijzen van Zanten, J. J. C. S., Higgs, S., & Raymond, J. E. (in preparation). Where is My Motivation – Experimentally Induced Low Grade Inflammation Selectively Impairs Motivated Learning.

Balter, L. J. T., Bosch, J. A., Aldred, S., Raymond, J. E., & Higgs, S. (in preparation). Inflammation, Motivated Learning and Emotion Recognition – Associations with Age- and Body Weight-Related Inflammation.

Balter, L. J. T., Bosch, J. A., & Raymond, J. E. (in preparation). Social Traits Predict Behavioural Inflexibility in Socially-Rewarded Tasks.

Balter, L.J.T., & Higgs, S. (in preparation). Physiological and Cognitive Responses Associated with Successful Weight Loss Maintenance: A Cross-Sectional Study.

Balter, L. J. T., & Raymond, J. A. (in preparation). Aging and Spatial Working Memory Capacity Affect Generalization of Learned Information.

AUTHOR CONTRIBUTION STATEMENT

The candidate was the principal researcher with respect to designing the experiments, applying for ethical approval via the University of Birmingham Research Ethics Committee and Research Ethics Committee of the National Health Service (NHS), executing the experiments, undertaking data analyses and writing the papers. The authorship indicates the collaborative work for each chapter. The work has been co-authored by the candidate's supervisors, Prof Jane Raymond, Dr Jos Bosch, and Prof Suzanne Higgs, and co-workers, Drs Sasha Hulsken, Dr Ali Mazaheri, Dr Sarah Aldred, Dr Jet Veldhuijzen van Zanten, and Prof Mark Drayson. Volunteers and Bachelor and Master degree students of the School of Psychology and School of Sport, Exercise and Rehabilitation Sciences aided in recruiting and testing of participants and the nursing staff of the School of Nursing injected the vaccination. This thesis does not contain any material, which has been written or published by another person except where referenced above. Chapter 2 to 7 contains material that has been prepared for publications in peer-reviewed journals.

LIST OF CONTENTS

Chapter 1	General introduction	**3**
Chapter 2	Low-grade inflammation decreases emotion recognition - Evidence from the vaccination model of inflammation	**21**
Chapter 3	Loneliness in healthy young adults predicts inflammatory responsiveness to a mild immune challenge in vivo	**39**
Chapter 4	Where is my motivation - Experimentally induced low-grade inflammation reduces motivated learning	**51**
Chapter 5	Inflammation, motivated learning, and emotion recognition - Associations with age- and body weight-related inflammation	**79**
Chapter 6	Inflammation mediates body weight and ageing effects on psychomotor slowing	**111**
Chapter 7	Selective effects of acute low-grade inflammation on human visual attention	**143**
Chapter 8	Summary and general discussion	**175**
List of references		**187**

CHAPTER 1

General Introduction

Cognitive function is a broad concept that subsumes all mental capacities that underlie or support our ability to think, feel or act. It determines the ability to perceive, process, understand, store and retrieve information, and to decide and produce appropriate responses (Neisser, 1976). Cognition can be studied as distinct, but interdependent, functions such as attention, learning, motivation, memory, motor behaviour, and emotional processing. Disturbances in cognitive functions are common in conditions associated with elevated inflammatory activity. These include inflammatory diseases (e.g., rheumatoid arthritis, inflammatory bowel diseases), organ dysfunctions (e.g., chronic kidney disease, cardiovascular diseases), but also non-disease states like old age and obesity (Appenzeller, Bertolo, & Costallat, 2004; Chan et al., 2013; Drew & Weiner, 2014; Evans et al., 2005; Petruo, Zeißig, Schmelz, Hampe, & Beste, 2017).

Related to its effects on cognitive function, elevated inflammatory activity generally negatively impacts overall functioning and wellbeing. Patients with inflammatory diseases have a 3 to 5 times higher incidence of depression as compared to healthy controls (Marrie et al., 2018; Neuendorf, Harding, Stello, Hanes, & Wahbeh, 2016). Even more prevalent is presence of debilitating fatigue and its components, like tiredness and lack of motivation, whereby depending on disease type and severity 30% to 70% of patients with an inflammatory disease report severe levels of fatigue and related motivational symptoms (Graff et al., 2011). There has been mounting evidence, both from experimental work and epidemiological data, that elevated inflammatory activity may, at least partially, account for the fatigue symptoms and psychological comorbidity seen in these patients. Importantly, psychological comorbidity is associated with unfavourable clinical outcomes, poor medical compliance, and interferes with role behaviours essential to the patient's life, e.g., being an employee, maintaining a social life, and being a parent (DiMatteo, Lepper, & Croghan, 2000; Naess, Lunde, Brogger, & Waje-Andreassen, 2010).

What is inflammation?

Inflammation is a physiological defence mechanism to tissue damage, infection or foreign bodies (e.g., toxins). Integral is the release of cytokines; signalling molecules that orchestrate responses to such insults (Janeway, 2012; Medzhitov, 2008). Activated immune cells release these cytokines, which in turn transmit signals to the central nervous system via endocrine and neural routes (CNS) (Miller, Haroon, Raison, & Felger, 2013). This, in turn, results in a host of physiological, cognitive, and behavioural changes. Examples are upregulation of body temperature, nausea, malaise, mood changes, and fatigue. Under ideal circumstances, the inflammatory response is a short-lived and adaptive process, intended to promote healing and prevent spread of infection (Medzhitov, 2008). However, in a chronic state of inflammation damaging effects tend to dominate. The adaptive versus maladaptive nature of the inflammatory response may also hold true for the behavioural symptoms. As an example, high plasma levels of inflammatory cytokines, i.e., as when having the flu, induce social withdrawal, fatigue and lack of motivation that may help limit energy expenditure, such that the immune system more effectively utilises these limited resources for recuperation (Dantzer & Kelley, 2007; Karshikoff, Sundelin, & Lasselin, 2017). However, in a chronic inflammatory state, these biobehavioural effects may increase vulnerability to the development and exacerbation of clinical symptoms that characterise depression and other psychiatric conditions (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008).

The negative effects of protracted inflammation are not relevant only to those with infectious or inflammatory diseases, but may also hold true for non-pathological states associated with elevated inflammatory activity such as overweight and ageing (Capuron et al., 2014; Shelton & Miller, 2010). In those with a high body mass index (BMI) elevated inflammatory activity is mainly due to adipose cells and local immune cells that produce copious amounts of inflammatory cytokines (Cancello & Clément, 2006). In ageing, factors such as oxidative stress, immunosenescence (i.e., the gradual deterioration of the immune system), endocrinosenescence (e.g., declining levels of sex hormones), epigenetic modifications, and age-related diseases (e.g., atherosclerosis) all contribute to age-related elevated inflammatory activity, a phenomenon denoted as "inflammageing" (Franceschi et al., 2007; Horstman, Dillon,

Urban, & Sheffield-Moore, 2012; Vitale, Salvioli, & Franceschi, 2013; Xia et al., 2016). Environmental exposures (i.e., air pollution), lifestyle (i.e., stress, smoking behaviour), and stress exposure are additional factors that may also contribute to modulation of inflammation (Schmidt et al., 2015). Likely no single determinant explains all aspects of age- and BMI-related inflammation; it seems likely that multiple processes contribute and that all are intertwined with inflammatory activity.

Why may inflammation affect behaviour and cognition?

Animal and human research support the idea that inflammation causes alterations in cognition and the frontostriatal circuit has been thought to be the primary central nervous system target of inflammatory mediators. Complex cognitive functions such as motivation, reward processing, and responding to social threat (i.e., anxiety and arousal) are primarily subserved by this neural network (Miller, Haroon, Raison, & Felger, 2013). This finding has engendered speculation as to whether reward-motivated and social behaviours are specifically sensitive to the deleterious effects of inflammation.

Given that heightened inflammation signals a vulnerable situation, from an evolutionary point of view, social withdrawal and reduced motivation might imply a survival advantage (D'Acquisto, 2017; Jaremka, Fagundes, Glaser, et al., 2013). Reduced interest in others during times of sickness, may be self-protective by avoiding interaction with potentially harmful others, but may also help to prevent the spread of pathogens through social networks (Inagaki, Muscatell, Irwin, Cole, & Eisenberger, 2012). However, being cut off from social groups makes individuals more prone to injury and infection. An upregulated inflammatory response may thus be evolutionary adaptive when alone (Cole, 2013; Inagaki et al., 2012). However, in today's Western societies, the risk to get wounded by predators or enemy attacks is much less likely as compared to about 12,000 years ago, and the harmful effects of an elevated inflammatory state when feeling lonely may not be proportional to the risk of bacterial infection. Altogether, it has been suggested that inflammation does not unidirectionally induce social withdrawal but that

psychological components may also exert immune changes (e.g., Jaremka et al., 2013). Indeed, in the late 1950s, the first evidence in support of bidirectional communication among the brain and the immune system emerged. Rasmussen (1957) formed the conceptual leap by demonstrating that stressor exposure in animals could affect the course of viral infection.

The subjective feeling of loneliness, which may follow upon social withdrawal, has been interpreted as an adaptive response to social disconnection that provides the motivation to reintegrate with social groups (Cacioppo, Cacioppo, & Boomsma, 2014). Whereas increased approach-related behaviour towards close others may increase the chance for support or care (Cacioppo et al., 2014), non-social (i.e., monetary) rewards, on the other hand, cannot directly provide help and care and do not seem useful for survival and energy-preserving purposes. Indeed, inflammation is generally accompanied by reduced motivation to obtain non-social rewards (Boyle et al., 2019; Draper et al., 2017; Inagaki et al., 2015). However, increased engagement in effortful reward-motivated behaviours in some contexts has been reported too, for example when there is a high chance to receive a high reward (Boyle et al., 2019; Lasselin, Treadway, et al., 2016), suggesting that inflammation alters cognitive processes in more complex ways than merely reducing energy expenditure.

While social behaviour and motivation seem to be prime targets of inflammatory mediators, other cognitive functions, such as attention, memory, and motor responses, have been shown to be affected by inflammation too, although its relationship is less well defined (e.g., Lasselin et al., 2016). It is possible that there is specificity in the inflammation-brain link. For example, enzymes involved in the inflammatory process are more abundant in limbic structures than in primary sensory cortices. Limbic structures may thus incur increased risk of damage from inflammation (Raz & Rodrigue, 2006). Moreover, studies that administered large sets of cognitive tests have documented domain-specificity in the inflammation-cognition link. For example, Lin et al. (2018) showed that inflammation was linked to reduced processing speed but not to short-term memory performance and Tegeler et al. (2016) showed that higher inflammation was correlated with poorer executive function and processing speed, but not with memory. These data suggest that some functions may be more preserved than others. However,

differences could also be attributed to study methodology, e.g., variability in the tests used to assess the cognitive domain in question, severity of inflammation, or presence of other factors than inflammation that may themselves contribute to reduced cognitive function (e.g., depression, poor cardiovascular health) (Dhar & Barton, 2016). For example, whereas attention deficits in middle-aged adults have been tentatively linked to chronic inflammation (Marsland et al., 2006), experimental models of acute inflammation provided little evidence of degraded selective attention (reviewed in Bollen, Trick, Llewellyn, & Dickens, 2017). Interestingly, an experimental study of inflammation showed that interferon (IFN)-α induced inflammation did not impair visuospatial attention behaviourally, however IFN-α treated patients recruited an additional portion of dorsal anterior cingulate cortex (ACC) region that was not observed in controls, which was interpreted as greater deployment of mental effort to maintain performance (Capuron et al., 2005a). Similarly, Brydon et al. (2008) showed that additional brain regions were recruited during a task that required inhibitory control (Stroop task), while behavioural performance was not affected. Making an effort to maintain attention under inflammation seems beneficial from an evolutionary perspective, as there should be a system that incidentally scans the environment for opportunities and dangers. Together these data suggest that absence of behavioural effects does not imply the absence of underlying neurophysiological effects of inflammation. Understanding inflammation-induced neurophysiological changes may open up possibilities for early markers for those at risk to develop cognitive function. This requires sensitive methods that can detect subtle aspects of cognitive dysfunction. It has been shown that electrophysiological methods can identify early signs of cognitive decline in pathological states, such as mild cognitive impairment in Parkinson's disease (Bonanni et al., 2015; Klassen et al., 2011), making this method highly suitable for probing neural effects of low-grade inflammation. Nevertheless, it remains unclear why behaviour is sometimes unaffected by inflammation while underlying neurophysiological processes indicate significant alterations. One reason may be that overt behavioural effects of inflammation require persistent (i.e., chronic) or severe inflammation before compensatory mechanisms that maintain performance fail to cope with the

negative effects of inflammation. Intertwined with this question is whether there may be differential cognitive consequences of acute and chronic low-grade inflammation.

As reviewed above, ageing and overweight or obesity are independently associated with chronic inflammation. Levels of inflammatory mediators typically increase with age and body fat, even in the absence of acute infection or physiologic stress (Dhar & Barton, 2016). While it is clear that age and obesity independently exert adverse effects on aspects of cognition, studies appear inconsistent regarding possible synergistic effects of ageing and obesity on inflammation and cognition. Animal research has shown that a single immune challenge superimposed upon an existing neurodegenerative disease exaggerated the CNS response to acute inflammation, leading to acute increased neuronal cell death, accelerated disease progression and acute cognitive dysfunction (Nguyen, Killcross, & Jenkins, 2014; Singh & Newman, 2011). However, it remains to be determined whether one inflammatory state, i.e., high BMI, superimposed on another inflammatory state, i.e., ageing, drastically increases inflammation and perhaps accelerates cognitive decline. Human research has shown both synergistic, i.e., obesity related cognitive deficits increase with age (Stanek et al., 2013), and additive effects, i.e., relationships between cognition and BMI do not vary with age (Gunstad et al., 2007). Some even report protective effects of obesity against cognitive decline in older age (Kuo et al., 2006), referred to as the "obesity paradox". These conflicting results emphasise the need for research aimed to clarify possible overlapping and distinctive effects of acute versus chronic low-grade inflammation on cognition.

Methods to investigate the consequences of inflammation

One of the effects of peripherally produced inflammatory cytokines is activation of central microglia, which are the brain's primary immune defence cells, support cells and which are differentiated macrophages (Dantzer et al., 2008). Activated microglia release inflammatory mediators locally in the CNS that can alter production, metabolism, and transport of neurotransmitter systems, and hereby affect neurocircuits underlying cognitive processes such

as mood and motivation. For example, cytokines can induce synthesis of enzymes such as the indoleamine 2,3-dioxygenase (IDO) and GTP-cyclohydrolase 1 (GTP-CH1), which results in significant alterations in the biosynthesis of key monoamines (e.g., serotonin, dopamine) (Dantzer et al., 2008). Cytokines additionally contribute to oxidative stress through generation of radicals, glutamate dysregulation, changes in neuropeptide systems, and decreases in growth factors, further exacerbating circuit dysfunction and behavioural pathology (Dantzer & Kelley, 2007; Felger & Lotrich, 2013; Haroon, Miller, & Sanacora, 2017).

Further evidence that inflammation affects brain processes stems from several lines of evidence that will be briefly expanded on in the next sections: 1) observational research in patients with inflammatory conditions or depression, 2) clinical interventions such as IFN-α treatment, 3) experimental animal research; and, 4) experimental research in healthy humans (Dantzer et al., 2008; Raison, Capuron, & Miller, 2006).

Observational research in patients. Correlational analyses examining cognitive performance in populations with elevated inflammatory activity, such as elderly, those with overweight, or other causes of protracted inflammatory states, generally reveal negative correlations between inflammation and cognitive performance such as processing speed and executive functions (Lin et al., 2018; Marsland et al., 2006; but see also Singh-Manoux et al., 2014). Common complaints of mild cognitive deficits in conditions associated with chronic inflammation (e.g., ageing, obesity, kidney disease, rheumatoid arthritis, virus infection, and neurodegenerative diseases) or acute inflammation (e.g., injury or commonplace infections) include impaired concentration (Vollmer-Conna et al., 2004), cognitive sluggishness (Smith, 2012), and fatigue (Villoria et al., 2017). Moreover, patients with inflammatory diseases show higher incidence of depression and worse disease prognosis as compared to matched controls without the disease and independent of the increased prevalence of physical comorbidities such as chronic pain (Bernstein et al., 2018; Marrie et al., 2018; Neuendorf et al., 2016; Wang et al., 2008). Interestingly, subsets of patients with major depressive disorder show increased expression of inflammatory cytokines too, without presence of medical diseases, and a growing body of literature suggests that those who show inflammatory activity, may benefit from anti-inflammatory medication as an adjuvant to

standard antidepressant treatment (Köhler-Forsberg et al., 2019; Köhler, Krogh, Mors, & Eriksen Benros, 2016; Raison et al., 2013). The putative link between inflammation and mental functioning is further supported by evidence in patients facing similar types of disability, but accompanied with (e.g., multiple sclerosis) or without (e.g., car accident victims) inflammation, with the former reporting higher levels of depression than the latter (Ron & Logsdail, 1989).

Clinical interventions and observations. IFN-α is a cytokine that is administered for the treatment of infectious diseases such as hepatitis C and for some forms of cancer. Up to 50% of patients who are administered the cytokine IFN-α develop a variety of neuropsychiatric adverse effects including severe fatigue, depression, and cognitive complaints (for a review see Raison et al., 2006). IFN-α treatment induced increased ACC activity in response to a visuospatial attention task, which is similarly seen in individuals vulnerable to psychiatric conditions (Paulus, Feinstein, Simmons, & Stein, 2004). Increased ACC activity has been suggested to represent increased sensitivity to negative events (Capuron et al., 2005b). Moreover, IFN-α has been shown to alter dopamine metabolism in the basal ganglia (Juengling et al., 2000), decrease activation in the basal ganglia during unexpected delivery of reward which was in turn associated with decreased motivation and increased fatigue (Capuron et al., 2012) and IFN-α induced striatal microstructural reorganisation was predictive of development of fatigue (Dowell et al., 2016). These findings suggest that alterations in dopamine pathways may be involved in inflammation-induced depressive-like behaviours. Further corroborating this hypothesis is the finding that tetrahydrobiopterin (BH4) activity, a cofactor that is necessary for the synthesis of dopamine, is reduced in IFN-α treated patients. Decreased BH4 in cerebrospinal fluid (CSF) was further correlated with increased CSF interleukin-6 (IL-6) (i.e., an inflammatory cytokine), suggesting a link with central inflammation (Felger et al., 2013).

Experimental animal models of inflammation. Experimental animal models have further provided data on the pathophysiology of inflammation-induced behavioural changes and reinforced the link between inflammatory cytokines and cognitive dysfunction. Animal studies have as a major advantage that they allow more detailed mechanistic studies that would be impossible or unethical with human subjects. A rich body of animal studies show that activation

of immune pathways, as a results of administration of immune modulating substances such as cytokines like IL-1β or immune activators like lipopolysaccharide (LPS), can induce behaviours that are homologous to depression, including reduced motivation (e.g., decreased effortful responding for reward), decreased locomotor activity, and reduced social exploration (Nunes et al., 2014; Vichaya, Hunt, & Dantzer, 2014). These studies show, amongst others, that inflammation is linked to lower CSF concentrations of dopamine metabolites, which negatively correlated with depressive behaviour (i.e., time spent huddling) (Felger et al., 2007; Felger & Miller, 2012). Furthermore, in vivo microdialysis on IFN-α treated monkeys showed inflammation-associated reduced striatal dopamine, which correlated with reduced effort-based reward responding (sucrose consumption) and administration of the dopamine precursor levodopa reversed these effects (Felger et al., 2013; Felger, Hernandez, & Miller, 2015). In parallel with IFN-α treated patients (see *Clinical interventions and observations*), rats injected with IFN-α showed reduced BH4 concentrations (Kitagami et al., 2003). One (indirect) pathway through which inflammatory cytokines can reduce BH4 concentrations is via oxidative stress. Inflammation-induced increases in inducible nitric oxide synthases (NOS) activity can usurp BH4, resulting in the generation of reactive oxygen species (ROS). This increase in ROS (oxidative stress) can then contribute to oxidative reduction of BH4, reducing BH4 even more and leaving thus less BH4 available for dopamine synthesis (Cunnington & Channon, 2010). Cytokines can further affect multiple other aspects of dopamine function, directly and indirectly, resulting in decreased synthesis, impaired packaging, increased reuptake, and decreased dopamine receptor availability, all of which may interact to a greater or lesser extent to reduced dopamine function (Felger & Miller, 2012; Felger & Treadway, 2016; Miller et al., 2013). Laboratory animal studies further showed IFN-α decreased brain concentrations of serotonin and dopamine via multiple direct and indirect pathways. For example, inflammation induced activation of p38 mitogen activated protein kinase which has been shown to increase expression and activity of the serotonin transporter (Sanchez et al., 2007). Foremost, these studies point at a key role of dopaminergic systems, however it is unlikely that this is the sole mechanism. Other pathways possibly associated with depressive-like symptoms include alterations in

neuroendocrine function. For example a hyperactive hypothalamus–pituitary–adrenal (HPA) axis is a hallmark of neuropsychiatric disorders including depression (Pariante & Lightman, 2008) and acute cytokine administration has been shown to stimulate the HPA axis. Whether there may be a link between inflammation, depressive-like behaviours, and the HPA-axis activation remains an underdeveloped field of research (Capuron & Miller, 2011; Louati & Berenbaum, 2015). Taken together, experimental animal models of inflammation further shed light onto possible mechanisms underlying inflammation-related behavioural changes.

Experimental human models of inflammation. There are several limitations to animal models that hinder extrapolation to humans. For example, measuring emotional components in animals objectively is an underdeveloped field of research (de Vere & Kuczaj, 2016), which is additionally complicated by trait-differences between species and strains, which can hinder the translation of laboratory animal results to humans (van der Staay, Arndt, & Nordquist, 2009). Therefore, researchers have attempted to develop human equivalents of the animal models of inflammation. Inflammation has been induced experimentally in healthy individuals by means of administration of immune-activating agents including purified lipopolysaccharides (LPS; endotoxin) from *Escherichia coli* or *Salmonella abortus equi*, through administration of a vaccine against *Salmonella Typhi* or an influenza vaccine (Boyle et al., 2019; Harrison et al., 2009; Lasselin, Treadway, et al., 2016). Other methods have used physiological, i.e., non-immune, triggers such as administration of a high-fat load, eccentric exercise, or acute stress (Marsland, Walsh, Lockwood, & John-Henderson, 2017; Paine, Bosch, Ring, Drayson, & Veldhuijzen van Zanten, 2015; Pedersen, 2000; Schmid et al., 2015).

Human experiments using bacterial endotoxin and typhoid vaccine as immune stimuli support the hypothesis that inflammation underlies symptoms including fatigue, changes in social behaviour, altered reward-motivated behaviours, and psychomotor slowing (Boyle et al., 2019; Brydon et al., 2008; Draper et al., 2017; Eisenberger, Moieni, Inagaki, Muscatell, & Irwin, 2017; Karshikoff et al., 2017; Lasselin, Treadway, et al., 2016). However, behavioural changes are not always reported with the typhoid model of inflammation possibly due to the mild nature of typhoid vaccination.

It is important to note that inflammation does not simply reduce responses or behaviour, however, more complex behavioural patterns are observed that possibly include a reorganisation of priorities. For example, a handful of human neuroimaging studies showed reduced reward-related signals in ventral striatum in response to immune challenges such as IFN-α, endotoxin or typhoid vaccination administration (Capuron, 2012; Dowell et al., 2016; Eisenberger et al., 2010; Harrison, Voon, et al., 2015). However, a different picture emerged when looking at social-rewards; increased, instead of decreased, neural activity in reward processing areas (i.e., ventral striatum) was found when endotoxin-exposed individuals viewed images of close others as compared to images of strangers (Inagaki et al., 2015). Socially threatening images or feedback, on the other hand, resulted in increased activity in threat related neural regions (i.e., dorsal ACC, amygdala, dorsomedial prefrontal cortex (DM PFC) (Inagaki et al., 2012; Muscatell et al., 2016). Moreover, endotoxin reduced willingness to exert effort (Boyle et al., 2019; Draper et al., 2017) but also increased willingness to exert effort, dependent on the context, has been reported; i.e., when the reward was highly probable and of high value (Lasselin, Treadway, et al., 2016).

In sum, there is evidence to suggest that inflammation affects motivation-related behaviours and neurophysiological mechanisms associated with it, expressed as inflammation-related fatigue, changes in willingness to invest effort for rewards, altered neural responses to rewards, and changes in neurotransmitter systems and neurocircuits associated with motivation. Nevertheless, remaining questions are whether there may be differential effects of acute and chronic low-grade inflammation, whether there may be aspects of motivation that remain intact or a specifically sensitive to the effects of inflammation (e.g., continuous pursuit of rewards, flexible adaptation to changes), and what neurophysiological mechanism may be associated with these effects. Changes in social cognition are also commonly reported: impaired emotion recognition with inflammation, increased brain responses to social threats in threat-related neural regions (e.g., dACC), increased brain reward responses when viewing close others, and development of cognitive and mood symptoms characteristic of depression in response to potent immune stimuli (e.g., LPS, IFN-α). Experimental research investigating

effects of inflammation rather than sickness behaviour would perhaps benefit from a milder immune stimulus, such as typhoid vaccination, that induces low-grade inflammation but avoids development of confounding severe physical malaise that denotes sickness (e.g., fever, nausea). Lastly, evidence for a role of inflammation in attention-related deficits remains sparse (reviewed in Bollen et al., 2017). However, absence of behavioural effects does not imply the absence of underlying neurophysiological effects of inflammation. Moreover, impaired concentration (Vollmer-Conna et al., 2004), cognitive sluggishness (Smith, 2012), and fatigue (Villoria et al., 2017) are common complaints of conditions associated with inflammation, suggesting that attention processes may be affected by inflammation (Theoharides, Stewart, & Hatziagelaki, 2015; Vollmer-Conna et al., 2004). From an evolutionary perspective, there should be a system that incidentally scans the environment for opportunities and dangers. It is thus possible that efforts are made in order to maintain attention functions with inflammation. This research area may benefit from measures specifically designed to assess attention as well as inclusion of sensitive well-validated measures of attention.

The Salmonella Typhoid model of acute low-grade inflammation

Results obtained from the endotoxin-induced inflammation, from patients who receive IFN-α therapy, or from patients with an inflammatory disorder provide compelling information about the relationship between inflammation and cognition. However, side-effects such as low mood, nausea, and disease-related symptoms (e.g., pain) may confound neuropsychological performance decrements by itself (Kuo et al., 2006). An immune stimulus that substantially minimises such secondary illness effects is provided by vaccination against *Salmonella typhi* (the causal agent of typhoid fever). Typhoid vaccination evokes a low-grade inflammatory state, but typically without physical malaise that characterise sickness (e.g., fever, nausea). This model of low-grade inflammation is further advantageous in terms of its generalisability, as the level of immune activation seen after typhoid vaccination is akin to the low-grade inflammatory levels seen in subsets of depressed individuals, as well as some medical conditions such as diabetes

and atherosclerosis associated with psychological comorbidity and cognitive impairment (Dowlati et al., 2010; Wegner, Araszkiewicz, Piorunska-Stolzmann, Wierusz-Wysocka, & Zozulinska-Ziolkiewicz, 2013). Whether the effects of this acute inflammatory stimulus generalise to chronic inflammation seen in these aforementioned and other conditions remains unclear at this point, and is one of the questions being explored in the current thesis (discussed below).

General aim and approach

The overall objective of the present dissertation was to investigate how low-grade inflammation (e.g., elevations in inflammation in the normal, non-pathological range) may affect cognitive function. Specifically, analyses have focussed on three cognitive domains; social cognition, reward-motivated behaviour, and attention processes, of which the latter is the least well studied (Bollen et al., 2017).

Although several cognitive tasks and outcomes were administered, the present thesis mainly presents the results of three tests that appeared sensitive to the effects of inflammation. First, the reading the mind in the eyes test is considered an advanced test of theory of mind involving mental state attribution and complex emotion recognition from photographs of the eye region of the face (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001; Oakley, Brewer, Bird, & Catmur, 2016). Second, a motivated learning task was developed based on a reinforcement learning task of Frank & Kong (2008). The task used in this dissertation measured the ability to learn reinforcement contingencies, the rate of learning, the stability of performance, and the ability to adapt to changes in reinforcement contingencies (i.e., flexibility) and the relative impact of reinforcement value on learning. Finally, visual attention, i.e., the capacity to prioritise relevant information from the sensory environment was assessed as a critical brain function that underpins higher order cognitive processes. Visual attention is a multi-faceted construct that can be distinguished both theoretically and empirically (Callejas, Lupiáñez, & Tudela, 2004; Fan, McCandliss, Sommer, Raz, & Posner, 2002). The distinct attentional processes

assessed here were alerting (i.e., preparing the brain for upcoming events), orienting (i.e., preparing where to look for task relevant information), and executive control (i.e., prioritising task-relevant information), using the Attention Network Test (ANT).

To study and compare the cognitive and psychological consequences of acute and protracted low-grade inflammation, two sets of studies were performed. In a correlational design, young and old individuals with a low BMI or high BMI (i.e., overweight or obesity) were compared. Old age and high BMI are each independently associated with inflammation and represent a 'normal', i.e., physiological, and stable pro-inflammatory state. Obviously, ageing and high BMI are associated with factors that could confound observed associations between inflammation and cognition, e.g., subclinical illness, which may additionally differ in duration and severity, medication use, and more generic confounding factors related to lifestyle (e.g., alcohol, exercise) and demographics. These correlational investigations were therefore complemented by an experimental study design, using the typhoid vaccination model, in which low-grade inflammation was transiently induced in healthy young individuals. While most research in this area has focussed the potential relevance of inflammation as a risk factor of psychiatric comorbidity and associated issues (e.g., severe fatigue), selection of these low-grade inflammation models could also shed light on the role of inflammation in normal every-day deviations in cognitive performance within and between healthy individuals.

Outline of this thesis

In the first empirical chapter of this thesis (**Chapter 2**), the effects of experimentally induced inflammation on emotion recognition were investigated, using the reading the mind in the eyes test (RMET). This chapter built on existing literature showing that inflammation impairs the ability to adequately interpret the mental state of other persons. However, prior studies induced inflammation using endotoxin, which also causes discomfort and sickness (e.g., pain and fever) presenting possible confounds (Moieni, Irwin, Jevtic, Breen, & Eisenberger,

2015). In Chapter 2, the Salmonella Typhoid model of acute low-grade inflammation was applied in attempt to replicate these observations.

It is known that peripherally produced inflammatory mediators can communicate with the brain and exert changes to cognition, however, immune cells do not autonomously execute prewired defence mechanisms that unidirectionally affect brain processes. Research in the field of behavioural immunology has demonstrated that immune system activity can shape psychological processes and behaviour, but also that psychology can shape immune responsivity too (Dantzer, 2017). Hence, in **Chapter 3** we explored which psychological factors predicted the inflammatory response to vaccination. Based on prior research (Moieni, Irwin, Jevtic, Breen, Cho, et al., 2015) it was tested if perceived loneliness may affect immune system reactivity.

The subsequent chapters expanded observations to include non-social cognition, i.e., reward-motivated behaviour and attention. Using the typhoid model of transient low-grade inflammation, in **Chapter 4** several non-mutually exclusive hypotheses about how inflammation may affect reward-motivated behaviour were tested. Various studies have consistently shown that inflammatory cytokines affect brain reward circuitry (Brydon et al., 2008; Capuron et al., 2007; Eisenberger et al., 2010; Felger & Miller, 2012; Harrison et al., 2015) and more recently it was suggested that inflammation may modulate the willingness to exert effort to obtain rewards (Draper et al., 2017; Lasselin, Treadway, et al., 2016). However, this field is still in its infancy and many important dimensions of motivated behaviour and learning remained untested, e.g., how quickly people learn reward associations, and stability and flexibility of motivated behaviours. In order to understand whether the effects found with experimentally induced inflammation translate to groups with elevated chronic low-grade inflammation (i.e., high BMI and older age), **Chapter 5** served as a potential conceptual replication test of the findings outlined in Chapter 2 (emotion recognition) and Chapter 4 (reward-motivated behaviour). I.e., the analyses presented in Chapter 5 utilised the same outcome measures that were used in Chapter 2 (reading the mind in the eyes test) and Chapter 4 (reinforcement learning task).

In Chapter 6 of this thesis, a role for low-grade inflammation in visual attention processes was assessed using the ANT. Visual attention is a facilitator enhancing performance of other cognitive processes. In **Chapter 6,** young and old individuals with a low or high BMI completed the ANT. Effective attentional processes can be measured by comparing speed and accuracy of different task conditions. For example, orienting attention was computed by taking the difference between trials with spatial cues that validly predict the spatial location of a target and trial with cues offering no prediction. This test additionally provides a robust measure of psychomotor speed (overall mean response time). In **Chapter 7**, typhoid vaccination was used to induce acute low-grade inflammation and participants completed the ANT with concurrent EEG recorded. In addition to comparing speed and accuracy of different task conditions, Chapter 7 additionally assessed changes in brain activity that can be measured electrophysiologically (Theoharides, Stewart, & Hatziagelaki, 2015; Vollmer-Conna et al., 2004). Specifically, modulation of oscillatory EEG activity in the alpha band to the onset of visual attention cues provides neurophysiological information of mental preparatory effort (Fink, Grabner, Neuper, & Neubauer, 2005; Keil, Mussweiler, & Epstude, 2006). The ratio between left and right occipital alpha, referred to as the Alpha Lateralization Index (ALI), was assessed as an index of the efficiency of orienting attention (Haegens, Handel, & Jensen, 2011; Händel, Haarmeier, & Jensen, 2011) and frontal theta activity served as a measure of executive control (Cavanagh & Frank, 2014). In the final chapter of this thesis, **Chapter 8**, the main findings are summarised and discussed and directions for future research are outlined.

CHAPTER 2

Low-Grade Inflammation Decreases Emotion Recognition – Evidence from the Vaccination Model of Inflammation

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Published as: "Balter, L. J. T., Hulsken, S., Aldred, S., Drayson, M. T., Higgs, S., Veldhuijzen van Zanten, J. J. C. S., Raymond, J. E., & Bosch, J. A. (2018). Low-grade inflammation decreases emotion recognition – Evidence from the vaccination model of inflammation. *Brain, Behavior, and Immunity*. doi: 10.1016/j.bbi.2018.05.006."

ABSTRACT

The ability to adequately interpret the mental state of another person is key to complex human social interaction. Recent evidence suggests that this ability, considered a hallmark of 'theory of mind' (ToM), becomes impaired by inflammation. However, extant supportive empirical evidence is based on experiments that induce not only inflammation but also induce discomfort and sickness, factors that could also account for temporary social impairment. Hence, an experimental inflammation manipulation was applied that avoided this confound, isolating effects of inflammation and social interaction. Forty healthy male participants (mean age = 25, *SD* = 5 years) participated in this double-blind placebo-controlled crossover trial. Inflammation was induced using Salmonella Typhi vaccination (0.025 mg; Typhim Vi, Sanofi Pasteur, UK); saline-injection was used as a control. About 6h30m after injection in each condition, participants completed Reading the Mind in the Eyes Test (RMET), a validated test for assessing how well the mental states of others can be inferred through observation of the eyes region of the face. Vaccination induced systemic inflammation, elevating IL-6 by 419% ($p < .001$), without fever, sickness symptoms (e.g., nausea, light-headedness), or mood changes (all *p's* > .21). Importantly, compared to placebo, vaccination significantly reduced RMET accuracy ($p < .05$). RMET stimuli selected on valence (positive, negative, neutral) provided no evidence of a selective impact of treatment. By utilizing an inflammation-induction procedure that avoided concurrent sicknesses or symptoms in a double-blinded design, the present study provides further support for the hypothesis that immune activation impairs ToM. Such impairment may provide a mechanistic link explaining social-cognitive deficits in psychopathologies that exhibit low-grade inflammation, such as major depression.

2.1 INTRODUCTION

Human and animal studies have identified inflammation as a powerful regulator of social behavior (Fink, Grabner, Neuper, & Neubauer, 2005; Keil, Mussweiler, & Epstude, 2006). Initial animal studies identified social withdrawal as a core-feature of sickness behavior, i.e., the depression-like constellation of symptoms that also includes anhedonia, fatigue, and depressed mood (Eisenberger et al., 2017). Subsequent neurocognitive analyses in humans expanded this understanding beyond mere social withdrawal, and showed, for example, that inflammation induces heightened feelings of social disconnection and alters sensitivity to social threats and rewards (Eisenberger et al., 2010; Inagaki et al., 2012; Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015; Muscatell et al., 2016; Wright, Strike, Brydon, & Steptoe, 2005).

More recently, experimental human research revealed that inflammation-induction reduces the ability to infer the affect and mental states of other people on the basis of facial expressions (Eisenberger et al., 2010; Inagaki et al., 2012; Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015; Muscatell et al., 2016; Wright, Strike, Brydon, & Steptoe, 2005), which is considered indicative of impaired Theory of Mind (ToM). The concept of ToM, sometimes called mentalizing (Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015), was developed in the context of research on autism-spectrum disorders and refers to the ability to interpret someone else's desires, beliefs, and intentions, all of which are essential to human social interaction (Premack & Woodruff, 1978). However, impairments of ToM more broadly characterize a number of mental health disorders, most notably depression (Premack & Woodruff, 1978). Impaired ToM is thought to explain why depressed individuals tend to withdraw from social contacts, report less enjoyment in social interactions, and have fewer social contacts than non-depressed individuals (Bora & Berk, 2016), whereby mood and social interactions may operate in a bidirectional manner (Hirschfeld et al., 2000). Meta-analyses have consistently established that depression is associated with a state of low-grade inflammation (although there is marked heterogeneity between studies) (Bora & Berk, 2016). The observation that inflammation impairs ToM might thus provide a mechanistic link connecting inflammation with the interpersonal difficulties that characterize depression.

Two studies have provided direct human experimental evidence for a link between inflammation and ToM (Dowlati et al., 2010; Leighton et al., 2017). Both induced acute inflammation through the administration of bacterial endotoxin (i.e., lipopolysaccharide, LPS) and assessed ToM using the Reading the Mind in the Eyes Test (RMET), a validated test for assessing ToM (Kullmann et al., 2013; Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015). In this test, participants are presented photographs showing only the eye regions of emotional facial expressions, and are asked to select an emotion word that best describes what the person in the photograph might think or feel (Baron-Cohen, Wheelwright, Hill, et al., 2001). Although Moieni et al. (2015) reported that inflammation impaired RMET performance. Kullmann et al. (2013) found no such impairment, but observed an enhanced response to RMET images in brain regions relevant to ToM (i.e., superior temporal gyrus, temporo-parietal junction), which might suggest that more effort was required to produce impairment-free performance. While potentially important, the interpretation of the above data is somewhat complicated by the fact that endotoxin administration, besides inflammation, also provokes fever and flu-like symptoms including nausea, headache and fatigue in a dose-dependent manner (e.g., Eisenberger et al., 2010; Kullmann et al., 2013; Lasselin et al., 2016; Moieni et al., 2015). Considering that physical discomfort alone may produce neuropsychological performance decrements (Keogh, Moore, Duggan, Payne, & Eccleston, 2013; Smith, 2016), the above observations would benefit from further experimental validation using a model of inflammation that minimizes such secondary illness effects. Such alternative is provided by inflammation-induction through vaccination against *Salmonella typhi* (the causal agent of typhoid fever). Vaccination likewise reliably initiates an acute systemic inflammatory response, lasting up to 12 hours, but without generating flu-like symptoms (Brydon et al., 2009; Harrison et al., 2015; Lacourt et al., 2015; Paine, Ring, Bosch, Drayson, & Veldhuijzen van Zanten, 2013 but also Harrison et al., 2009).

The present study tested whether inflammation would lead to a decrement in the ability to accurately identify mental states in others and whether interpretation of positive versus negative facial expressions would be equally affected. The latter consideration stems from

previous research showing that inflammation increased sensitivity to both positive and negative social feedback (Muscatell et al., 2016).

2.2 METHOD

2.2.1 Participants

Forty healthy young male participants from the University of Birmingham were enrolled as a result of recruitment via online advertisement (*M* age = 24.7, SD = 5.2 years). Mean body mass index (BMI) was 23.7 (SD = 3.2 kg/m^2, 16.6-29.2 kg/m^2). Individuals were excluded if they report a history of or suspected vaccine-related allergy, food allergy/intolerance, inflammatory, cardiovascular, neurological, mental health or immune-related disorders, smokers, visual impairments (unless corrected to normal), and those on any medication 7 days prior to the test days.

Participants received research credits or were paid a minimum of £40 to reimburse travel and expenses. Participants could win an additional £12 depending on task performance during one of the cognitive tasks (reward learning task; data not reported here). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local Research Ethics Committee of the National Health Service (NHS).

2.2.2 Procedures

Participants visited the behavioral immunology laboratory on three separate occasions: the first visit was intended as a familiarization session, followed by two separate test days (i.e., vaccination or control) planned at least one week apart. During the familiarization session, which took about 70 minutes, written informed consent was obtained, inclusion and exclusion criteria were verified, an initial set of questionnaires were completed, and participants performed abbreviated versions of the computer tasks for the purpose of familiarization. Figure 1 presents the time line of the subsequent experimental sessions. On each of the two test days,

participants arrived at the laboratory between 8:00 and 9:00 am after an overnight fast. Participants were instructed to refrain from strenuous physical activity and alcohol intake for at least 24 hours, and were asked to avoid high fat and high sugar products for at least 12 hours prior to the test days. Before vaccination or placebo, participants verified absence of acute illness, and mood and sickness symptoms were assessed (as described below), and tympanic body temperature was measured. Subsequently the first blood sample was taken. After vaccination/placebo, participants received a standardized breakfast (granola with skimmed milk, approx. 430 kcal), and after a 5-hour break participants again received a standardized meal consisting of cheese sandwiches (approx. 328 kcal). For a subset of participants, EEG data was also collected from about 5h30m to 6h30m post-injection (N = 20, not reported here); and these participants arrived back at the laboratory 1 hour earlier to allow EEG preparations. During the 5-hour break participants were instructed to refrain from eating, drinking (except for water and the meals provided), or strenuous physical activity. Fifteen minutes post-lunch a second blood sample was taken and tympanic body temperature was measured; then, a set of cognitive tests that included the computerized RMET were started. Other tasks, not reported here, included measures of memory, attention, learning and response inhibition. Mood was also assessed at several intervals during the test day; before injection and 5h30m and 8 hours post-injection. The final blood sample was taken about 8 hours post-injection. Test timings were identical across visits, as were the procedures, except for the type of injection (vaccine or saline-placebo).

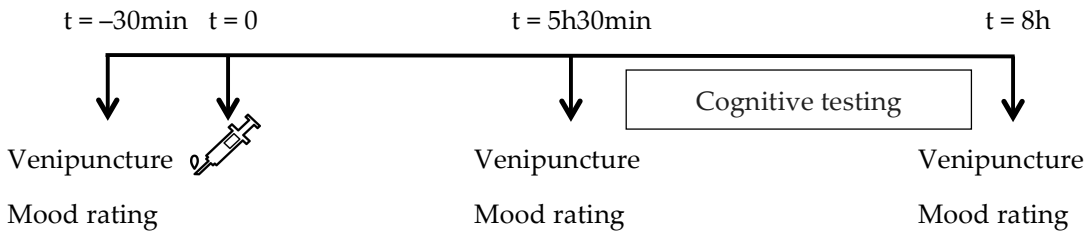

Figure 1. Study timeline. Participants were tested on two occasions, separated by a 7-day wash out, using an identical protocol. Cognitive testing was performed between 5.5h and 8h after application of vaccine or placebo (t=0). Before participation, participants visited the laboratory for a single 70 minutes familiarization session.

2.2.3 Randomization

Participants were randomly assigned to receive vaccine or placebo (control) on the first day of formal testing. Randomization was performed by supporting staff who had no contact with the study participants. Both participants and researchers were blind to condition order, and only the nurse administering the injections was aware of the order whereby a sealed envelope containing information about the condition was handed to the nurse before administering the injection. The nurse followed identical procedures for placebo and vaccine injection ensuring participant's blindness to the condition. The researchers were not present when the injection was administered.

2.3 MATERIALS

2.3.1 Reading the Mind in the Eyes Test (RMET)

The RMET is considered a test of theory of mind, a cognitive process sometimes referred to as "mind-reading" and "social intelligence" (Muscatell et al., 2016). The RMET was developed to measure social sensitivity in typical adults with normal intelligence (Baron-Cohen, Wheelwright, Hill, et al., 2001).

2.3.2 Stimuli

A gray-scale digital image (subtending 9 x° X 3.6 y° of visual angle) of the eye region of a face (including eyes and eyebrows) was presented in the middle of a grey field on a computer monitor. Four words describing mental states accompanied each test stimulus, presented in black Arial font (subtending 2.6 x° X 0.7 x° of visual angle).

2.3.3 Procedure

The test display comprised a test eye image and four words placed in the center of the screen. The participant was instructed to select the word that best described what the person in the test image was thinking or feeling by pressing one of four computer keys (Q, P, A, L) that spatially corresponded to the position of each word. The correct (target) word had the same emotional valence as the accompanying three foil words. For example, the target word, 'panicked', was accompanied by 'arrogant', 'jealous' and 'hateful'. Target words were equally likely to appear in one of the four word locations on the screen. Each test display remained visible until a key response was made; the next test display was immediately presented thereafter. One block comprised of 18 test displays was completed on each day, with a different set of 18 test displays used on the second test day; the order of the display sets used was counterbalanced across participants. The two sets were comparable with regards to the sex of the faces (50% female) and number of items depicting positive, neutral and negative emotional expressions (fully crossed with sex, making three test displays for each sex X expression condition within each block). In line with previous studies, a glossary containing a definition of each word was available to the participant. Accuracy and response time were calculated as the percentage of correct responses and time to complete the task, respectively. Additional accuracy scores were calculated to assess the effect of emotional valence of the mental state discrimination (positive, neutral, negative expressions; see (Baron-Cohen, Wheelwright, Hill, et al., 2001).

2.3.4 Typhoid vaccination

Participants received 0.5 mL *Salmonella typhi* capsular polysaccharide vaccine (0.025 mg in 0.5 mL, Typhim Vi, Sanofi Pasteur, UK) and a saline placebo (0.5 mL) via intra-muscular injection in the deltoid muscle of the non-dominant arm by a certified nurse. Typhoid vaccine was selected as a low-grade inflammatory stimulus, since this vaccine is known to induce increases in circulating pro-inflammatory cytokine levels with no significant effect on body temperature (Paine et al., 2013).

2.3.5 Mood and sickness symptoms

Current mood and presence of sickness symptoms was assessed using a modified version of the Profile of Mood States – Short Form (POMS-SF; (Paine et al., 2013). The POMS-SF consists of 32 items asking 'How are you feeling right now:' followed by the item. Items were rated on a five-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, to 4 = extremely). Scores for POMS subscales (tension-anxiety, anger-hostility, fatigue-inertia, vigour-activity, confusion-bewilderment, depression-dejection) were computed by summing ratings on individual items. Six items were added to assess physical sickness (light-headed, nausea, faint, disgusted) and behavioral sickness (withdrawn, sociable).

2.3.6 Blood sampling

Blood was collected into one 6 ml vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500*g* for 10 min at 4 °C and plasma was aliquoted and stored at -80 °C for later cytokine assessment of plasma interleukin-6 (IL-6). Plasma IL-6 was measured in duplicate using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The limits of detection of this assay was .11 pg/mL, with an intra-assay coefficient of variation (CVs) of 4.2%. All samples from the same participants were assayed in the same run.

2.3.7 Anthropomorphic measures

Participants were asked to remove footwear and coats and empty their pockets before a body composition measurement was taken using a TANITA BC-545N body composition analyser (Tanita Europe, Amsterdam, The Netherlands), a device that uses bioelectrical impedance analysis. A stadiometer was used to measure height.

2.4 STATISTICAL ANALYSIS

All data were analyzed using SPSS v.24.0 (IBM-SPSS Inc., Chicago, IL, USA). Individuals with accuracy scores > 2.5 SD from the mean were excluded from analysis (N = 1). ANOVAs were performed to compare treatment (placebo vs vaccine). For POMS subscales, linear mixed models were used. Model simplicity and likelihood ratio tests were used to select appropriate covariance structures. Treatment and time were fixed and repeated factors; subject was entered as a random factor; and baseline scores were entered as a time-varying covariate. The effects of interest were interactions between time and treatment and main effects of treatment. Tests of simple main effects were performed on the linearly independent pairwise comparisons between the estimated marginal means for all analyses.

2.5 RESULTS

2.5.1 Responses to typhoid vaccination

2.5.1.1 Physiological responses

As shown in Table 1, after typhoid vaccination, participants showed a significantly greater increase in plasma IL-6 at 5h30m (+348%, mean difference 4.1, SE = 0.3) as compared to placebo (-14%, mean difference -0.1, SE = 0.0), $t(32) = -15.14$, $p < .001$. IL-6 remained elevated until at least 8 hours post injection (+227%, mean difference = 2.7, SE = 0.2), $t(33) = -15.41$, $p = <.001$; treatment (placebo, vaccine) x time (0h, 5h30, 8h) interaction, $F(2, 50) = 58.04$, $p < .001$, $\eta_p^2 = .84$). The peak IL-6 response occurred at 5h30m post-injection for most participants (average peak increase was 419%). Core body temperature showed no effect of treatment, $F(2, 54) = 1.32$, $p = .28$, $\eta_p^2 = .05$).

Table 1. Mean (SD) IL-6 in pg/mL for the placebo and vaccine condition. Column labels represent time since vaccination.

2.5.1.2 Affective responses, physical symptoms, and expectancy

No significant time by condition interactions were evident for any of the POMS subscales or total mood score (all *F*'s < 1). POMS data are summarized in Table 2. Results also indicated that participants were blind to their condition at the first visit: on test day 1, 55.6% of the participants reported the condition correctly at the end of the test day, which is at chance level, $\chi^2(1) = .44$, $p = .51$. At the end of test day 2, 83.8% correctly guessed the condition they were in, $\chi^2(1) = 16.89$, $p < .001$. To rule out partial expectancy effects, sensitivity analyses were run including only test day 1 (using between-subject comparisons), which yielded essentially identical results (results presented below).

	0 hours	5h30m	8 hours
Placebo	1.11 (0.58)	0.95 (0.65)	0.97 (0.69)
Vaccine	1.18 (0.62)	5.29 (1.72)	3.86 (1.26)

Table 2. Mean POMS subscales (SD) (mood and physical and behavioural symptoms) separated by condition. No interaction effects between treatment and time were evident (all F's < 1). Column labels represent time since vaccination.

	0 hours		5h30m		8 hours	
	Placebo	Vaccine	Placebo	Vaccine	Placebo	Vaccine
Anger	1.6 (2.2)	1.3 (2.1)	0.7 (1.4)	1.0 (2.4)	1.4 (2.6)	1.4 (2.1)
Confusion	4.0 (2.3)	3.5 (2.3)	3.2 (2.2)	3.4 (2.0)	4.5 (2.2)	4.3 (2.2)
Depression	1.3 (2.0)	1.3 (2.2)	0.6 (1.2)	0.8 (1.9)	1.0 (2.1)	1.4 (2.3)
Fatigue	3.9 (4.1)	3.8 (3.8)	2.8 (4.7)	3.0 (4.5)	5.3 (3.7)	6.0 (3.9)
Tension	1.6 (2.2)	1.2 (1.1)	1.0 (1.9)	1.5 (2.2)	1.7 (2.9)	1.3 (2.0)
Vigour	7.2 (4.2)	7.3 (3.3)	7.0 (3.6)	7.2 (3.4)	4.7 (3.7)	4.3 (3.3)
Behavioral Sickness	2.5 (1.5)	2.2 (1.3)	2.3 (1.3)	2.1 (1.3)	2.6 (1.3)	2.4 (1.3)
Physical Sickness	0.8 (1.6)	0.8 (1.2)	0.8 (1.9)	0.8 (1.4)	1.1 (1.8)	0.8 (1.3)

2.5.2 Reading the Mind in the Eyes Test

As can be seen in Figure 2a, vaccination (vs. placebo) led to a significant decrease in performance on the RMET, $F(1, 38) = 4.78$, $p = .035$, $\eta p2 = .11$. These results remained significant after adjusting for mood, behavioral or physical symptoms as well as for vaccination order. Sensitivity analyses including only test day 1 (using a between-group comparison), yielded virtually identical results, $F(1, 37) = 9.08$, $p = .005$, $\eta p2 = .20$). Similar responses times were observed for vaccine (M = 8.1 sec, SD = 4.4 sec) and placebo (M = 8.2 sec, SD = 4.5 sec), $F(1, 38) = .03$, $p = .867$, $\eta p2 = .00$. Separate analyses of RMET stimuli selected on valence (positive, negative, neutral) provided no evidence of a selective impact of treatment (see Figure 2b), $F(2, 76) = .08$, $p = .921$, $\eta p2 = .00$. Finally, the decreased RMET performance was not correlated with changes in IL-6 in the vaccine condition, $r(36) = .16$, $p = .367$.

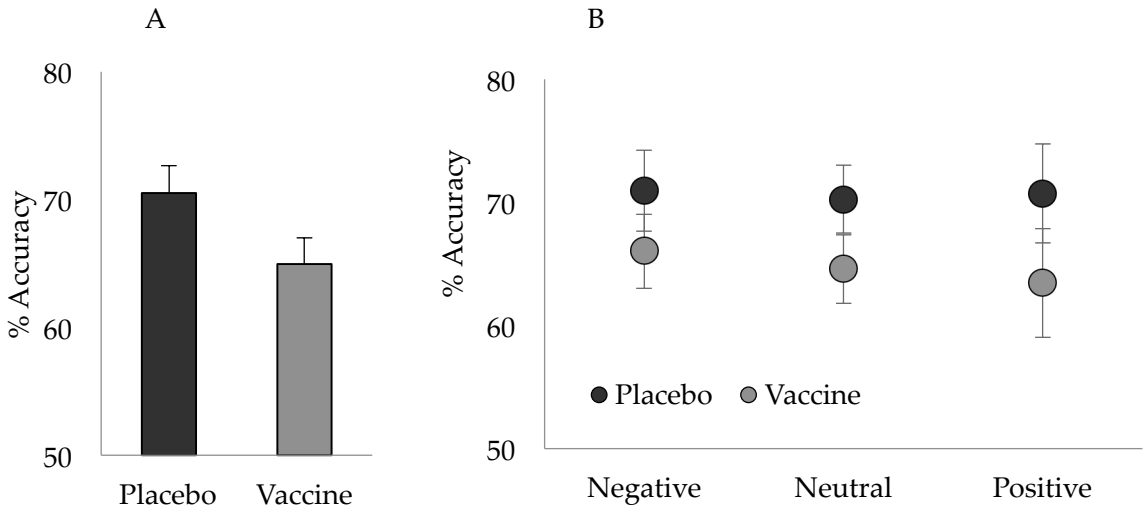

Figure 2. Mean accuracy scores for RMET total score (A), and for each emotional valence (B) for the placebo and vaccine condition. Errors bars indicated SEM. Please note that the y-axes start at 50% accuracy.

2.6 DISCUSSION

The present study tested whether inflammation impairs the ability to infer other's mental states and affect, which is considered a key aspect of Theory of Mind (Frith & Happe, 1999). Inflammation, as measured by IL-6, was effectively induced using a typhoid vaccination without causing sickness symptoms (e.g., fever, light-headedness, nausea, faint, withdrawn) or deterioration of mood. Moreover, analyses indicated that participants were blind to their condition at the first visit, which largely excluded confounding by expectancy effects. In line with the hypothesis, vaccine resulted in poorer performance on the RMET compared to the placebo condition. The current findings provide further experimental support to the idea that inflammation may drive social-cognitive deficits in psychopathologies that exhibit enhanced inflammation, such as major depression (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Eisenberger et al., 2017).

A possible alternative interpretation of the current results is that the lower performance during inflammation reflects reduced motivation. Motivational changes, which are considered a characteristic effect of inflammation, could thus account for the decreased RMET performance (e.g., Draper et al., 2017; Harrison et al., 2015; Lasselin et al., 2016). If this were the case, participants should have performed more poorly and taken less time to carefully inspect the stimuli. However, the results did not seem consistent with this interpretation; the time participants took to complete the task was identical across conditions while performance accuracy was not. Although the current study was not specifically designed to assess social-related motivational changes, the observed pattern of results would not seem consistent with weakened motivation explaining the RMET performance deficits shown here.

The results further suggested that the inflammatory stimulus applied in the current study impaired ToM skills independent of emotional valence. I.e., the ability to infer positive mental states was affected to a similar degree as inferences on negative and neutral mental states. This observation is consisted with that of Muscatell et al. (2016), who showed that endotoxin-induced inflammation enhanced neural responsivity in threat-related (e.g., bilateral amygdala) and reward-related (e.g., ventral striatum) brain regions, as well as in a region involved in inferring mental states of others (dorsomedial prefrontal cortex) to the same degree for negative versus positive social feedback. Muscatell et al. (2016) and Kullmann et al. (2013) further showed endotoxin-induced heightened neural sensitivity in the core region implicated in mentalizing. The fact that Kullmann et al. (2013) failed to find a behavioral effect, may perhaps be taken to suggest that individuals with inflammation may find the RMET task harder and so require more brain resources to achieve the same level of performance.

Clinically, although still somewhat speculatively at this point, the current findings suggest that individuals with inflammation find social interactions more complex, which could possibly contribute to social withdrawal and further amplification of depressive symptoms (Cacioppo, Hughes, Waite, Hawkley, & Thisted, 2006; Heinrich & Gullone, 2006). Likewise, impairments in social emotion recognition may hinder optimal support seeking, whereby patients might be less in tune with their social environment, e.g., less sensitive in picking up

social cues that would otherwise guide symptom reporting and help-seeking behavior. The inflammatory component of depressive disorders may similarly contribute to social impairments and withdrawal that characterize these disorders (Cacioppo, Hughes, Waite, Hawkley, & Thisted, 2006; Heinrich & Gullone, 2006).

A notable feature of the current study is that the typhoid vaccination used here elicited a smaller inflammatory response, raising IL-6 levels 4-fold, compared to the endotoxin manipulations used previously, which raised IL-6 levels between approximately 100-fold with a lower endotoxin dose (0.4 ng/kg body weight) up to roughly 1000-fold with a high dose (2 ng/kg body weight) (Draper et al., 2017; Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009; Grigoleit et al., 2011; Kullmann et al., 2013; Lasselin et al., 2016; Moieni et al., 2015; Muscatell et al., 2016). Although it could be argued that the modest elevation of IL-6 observed here simply reflects diurnal variation (Draper et al., 2017; Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009; Grigoleit et al., 2011; Kullmann et al., 2013; Lasselin, Treadway, et al., 2016; Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015; Muscatell et al., 2016), this account is unlikely as IL-6 changes were specific to the vaccine condition and not found in the placebo condition, even though measurements were obtained at the same time of the day in both conditions. Producing subtle increases in inflammation can be considered an advantage for research aimed at uncovering cognitive consequences of inflammation. For example, in terms of generalizability, the level of immune activation seen here is more akin to the low-grade inflammatory levels seen in depressed individuals, as well as medical conditions such as diabetes and atherosclerosis that have been linked to increased depression risk. Moreover, as argued earlier, the manipulation minimizes potentially confounding side effects such as sickness symptoms or significant mood deterioration (Agorastos et al., 2014; Nilsonne, Lekander, Åkerstedt, Axelsson, & Ingre, 2016). Typhoid vaccination is used to induce inflammation and subsequent neuropsychological effects subsumed under sickness behavior, but typically without physical malaise that more typically denotes sickness (e.g., fever, nausea). This similarly applies to other human data in which low-grade elevated inflammatory activity is present without overt sickness (e.g., such as in depression), but still showing neuropsychological phenomena like fatigue, anhedonia and

motor slowing (e.g., Goldsmith et al., 2016; Treadway, Bossaller, Shelton, & Zald, 2012). Hence, there seems reasonable ground to further discuss whether the term sickness behavior remains appropriate or whether we should consider new terminology (e.g., inflammation-associated cognitive changes). Some studies using typhoid vaccination have reported modest elevations in fatigue after vaccination, although this has not been uniformly observed (Brydon et al., 2008; Harrison et al., 2015; Harrison et al., 2009; but also Paine et al., 2013). Inspection of the data suggests that studies which measured fatigue 2-4 hours post vaccination observed elevated fatigue, whereas those using later time-points did not. However, further studies are needed to establish if timing is indeed a factor. The performance decrement of 5.6% on the RMET we observed in the current study is comparable to the study of Moieni et al. (2015) (about -5%). Interestingly, such differences are also observed in individuals with major depression as compared to controls (Kettle, O'Brien-Simpson, & Allen, 2008; Lee, Harkness, Sabbagh, & Jacobson, 2005; Szanto et al., 2012). However, perhaps the more significant point to be taken from these data goes beyond the exact magnitude of the observed effects, but is the experimental demonstration that mild inflammation affects social cognition. A further strength of the study is the relatively large sample size, which is the largest typhoid vaccination study to date, although it remains possible that some more subtle effects may have been missed: i.e., analyses established that at a power of .80, and assuming an alpha of .05, the current samples size was sufficient to detect small to moderate effect sizes. Limitations are that the current model induces an acute inflammatory state, and generalization to chronic inflammation remains to be determined and only healthy young males were assessed. Even though Moieni et al. (Kettle, O'Brien-Simpson, & Allen, 2008; Lee, Harkness, Sabbagh, & Jacobson, 2005; Szanto et al., 2012) reported no sex differences in acute inflammation or its consequent cognitive effects, further studies are clearly needed to confirm the generalizability of the current effects to females and to younger and older individuals. Indeed, inflammation is a hallmark of aging and replication of results to a relevant group is advised. Moreover, even though Lacourt et al., (2015) showed that vaccination does not affect pain tolerance or pain threshold and no side effects were reported in the current study, pain could have influenced performance and lack of assessing pain is a

limitation of the study. Similarly, even though modified versions of the Profile of Mood Scales-Short Form (POMS-SF) have been used extensively in typhoid vaccination studies (e.g., Brydon et al., 2009; Harrison et al., 2015), the use of a modified POMS-SF to measure mood and sickness can be considered as a limitation of the current study. Finally, similar to the study of Moieni et al. (2015), we found that the magnitude of the vaccine-induced inflammatory response was not related to behavioral performance. They speculated that the RMET might not be sensitive enough to capture incremental changes in inflammation. However, the current study showed that also a modest inflammatory stimulus impairs RMET performance. An alternative explanation might be that IL-6 is not a causal factor, and perhaps more proximal inflammatory biomarkers could be explored (Leighton et al., 2017).

In summary, typhoid vaccination elicited a transient low-grade inflammatory response in healthy young men and decreased performance on the Reading the Mind in the Eyes Test, tested in a double-blinded placebo-controlled crossover design. Hereby the current study provided direct empirical evidence for a link between heightened inflammation and lower ability to infer mental states of others. This finding, together with related recent reports, warrants a more comprehensive program of research linking inflammation and social cognition.

CHAPTER 3

Loneliness in Healthy Young Adults Predicts Inflammatory Responsiveness to a Mild Immune Challenge in Vivo

Leonie JT Balter, Jane E Raymond, Sarah Aldred, Mark T Drayson, Jet JCS Veldhuijzen van Zanten, Suzanne Higgs, & Jos A Bosch

Based on: "Balter, L. J. T., Raymond, J. A., Aldred, S., Drayson, M. T., Veldhuijzen van Zanten, J. J. C. S., Higgs, S., & Bosch, J. A. (under review). Loneliness in Healthy Young Adults Predicts Inflammatory Responsiveness to a Mild Immune Challenge in Vivo."

ABSTRACT

The established link between loneliness and poor health outcomes may stem from aberrant inflammatory regulation. The present study tested whether loneliness predicted the inflammatory response to a standardised in vivo immune challenge. Using a within-subjects double blind placebo-controlled design, 40 healthy men (mean age = 25, *SD* = 5) received a Salmonella Typhi vaccination (0.025 mg; Typhim Vi, Sanofi Pasteur, UK) and placebo (saline) on two separate occasions. Loneliness was assessed using the R-UCLA loneliness scale. Regression analyses showed that those that reported feeling more lonely exhibited an elevated interleukin-6 response (β = .579, 95% confidence interval [.003, .042], p = .025). This association withstood adjustment for potentially confounding variables, including age, sleep quality, socio-emotional factors, and health factors. The present findings are in line with evidence that loneliness may shift immune system responsivity, suggesting a potential biobehavioural pathway linking loneliness to impaired health.INTRODUCTION

Feeling lonely is surprisingly prevalent in today's society, with estimates stating that over 15% of British and nearly 40% of US adults report feeling lonely (Office for National Statistics, 2018; Wilson & Moulton, 2010), Loneliness is increasingly recognised as a significant social problem, whereby the British government recently appointed a Minister of Loneliness. One of the several disruptive effects of loneliness is on physical health. For example, meta-analyses show a 30% increased risk of stroke, myocardial infarction, and mortality in lonelier individuals (Holt-Lunstad, Smith, Baker, Harris, & Stephenson, 2015; Steptoe, Shankar, Demakakos, & Wardle, 2013; Valtorta, Kanaan, Gilbody, Ronzi, & Hanratty, 2016). Immune dysregulation, in the form of enhanced inflammatory responsivity, has been proposed as a mechanism underlying the link between loneliness and health risk (Hawkley, Bosch, England, Marucha, & Cacioppo, 2007). This idea has been supported, amongst others, by evidence that inflammatory gene transcription and epigenetics are altered in lonely individuals, together with studies showing increased immune reactivity to psychological stress in lonelier individuals (Brown, Gallagher, & Creaven, 2018; Steve Cole et al., 2007; Hackett, Hamer, Endrighi, Brydon, & Steptoe, 2012; Jaremka, Fagundes, Peng, et al., 2013). Likewise, the inflammatory response to

an immune challenge (bacterial endotoxin) is elevated in individuals who report feeling socially disconnected (Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015), which is predictive of loneliness (Cacioppo, Hawkley, et al., 2006). However, whether loneliness itself is associated with inflammatory responsivity has yet to be determined. This proposed hypothesis was tested using an existing dataset. The current study addressed the relationship between individual variation in subjective loneliness and immune reactivity in response to a mild immune-mediated inflammatory stimulus. Analyses adjusted for potential confounders such as age, sleep quality, socio-emotional factors (i.e., depression, anxiety, social skills, negative mood), and health factors (i.e., body weight, alcohol intake).

3.1 METHOD

3.1.1 Participants

The study involved a within-subjects double blind placebo-controlled design, presented in detail elsewhere (Balter et al., 2018). Forty healthy young male students from the University of Birmingham were enrolled (*M* age = 24.7, SD = 5.2 years). Individuals were excluded if they self-reported a history of or suspected vaccine- or food-related allergy, inflammatory, cardiovascular, neurological, mental health, visual, or immune-related disorder, being a current smoker, and those on any medication 7 days prior to the test days. Participants received research credits or were paid £40. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local Research Ethics Committee of the National Health Service.

3.1.2 Procedures

Participants visited the behavioural immunology laboratory on three separate occasions (one practice session and two test days): questionnaires were completed once during the first visit, except for negative mood, which was rated on each test day (see Materials). This was followed by two test days scheduled at least one week apart. On each test day, participants

arrived at the laboratory between 8:00 and 9:00 am. A certified nurse administered Salmonella typhi capsular polysaccharide vaccine (0.025 mg in 0.5 mL, Typhim Vi, Sanofi Pasteur, UK) or saline placebo (0.5 mL) via intra-muscular injection in the deltoid muscle of the non-dominant arm; the injection order was counterbalanced across participants. Blood samples were taken before injection, and at 5h30min and 8h post-injection. The time points for the collection of the blood samples was based on the time course and magnitude of a variety of inflammatory indicators published previously by our group (see Paine, Ring, Bosch, Drayson, & Veldhuijzen van Zanten, 2013).

The current analysis is based on the same sample as in Balter et al., (2018) and stem from secondary analysis of a larger study.

3.2 MATERIALS

3.2.1 Questionnaires

Questionnaires were completed in the order as presented below. Higher scores reflect worse functioning.

Alcohol intake. Average alcohol units per week (0 = 0 units, 1 = 1-5 units, 2 = 7-15 units, 3 = >15 units).

Sleep quality. The total score of the 19-item Pittsburgh Sleep Quality Index was used to assess quality of sleep over a 1-month interval. Internal consistency (Cronbach's alpha; α) is 0.80 for the total score (Carpenter & Andrykowski, 1998).

Anxiety. The 21-item Beck Anxiety Inventory was used to assess anxiety (Beck, Epstein, Brown, & Steer, 1988).

Depression. The 21-item Beck Depression Inventory (BDI)-II was used to assess depressive feelings (Beck, Steer, & Brown, 1996). The BDI-II has been found to demonstrate high internal consistency among college students (Cronbach's $\alpha = 0.93$) (Dozois, Dobson, & Ahnberg, 1998).

Loneliness. Loneliness was measured via the 20-item revised UCLA Loneliness Scale (R-UCLA). The Cronbach's α reliability coefficient for the UCLA–R is 0.96 (Russell, Peplau, & Cutrona, 1980).

Social skills. The social skills subscale of the Autism Quotient was used to measure the degree of social skills a person possesses (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). The Cronbach's α for the social skills subscale is 0.75 (Stevenson & Hart, 2017).

Mood. Negative mood on the day of testing was computed by summing five negative subscale scores (anger, confusion, depression, fatigue, and tension) and subtracting the vigour subscale score of the Profile of Mood States Short Form. The Cronbach's α for total negative mood in a healthy sample is 0.88 (Curran, Andrykowski, & Studts, 1995b).

3.2.2 Anthropomorphic measures

A stadiometer was used to measure height and a body composition measurement was taken using a TANITA BC-545N body composition analyser (Tanita Europe, Amsterdam, The Netherlands).

3.2.3 Interleukin-6 analysis

Blood (6 ml) was collected from an antecubital vein in the forearm into a vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500*g* for 10 min at 4 °C and plasma was aliquoted and stored at -80 °C for later cytokine assessment of plasma interleukin-6 (IL-6) using high-sensitivity ELISA (Quantikine HS Human IL-6 ELISA, R&D Systems, UK).

3.3 STATISTICAL ANALYSIS

Data were analysed using SPSS v.24.0 (IBM-SPSS Inc., Chicago, IL, USA). IL-6 data of three participants were excluded because of high baseline values indicative of a possible infection and 5% of IL-6 data was missing. Data were analysed using bivariate correlation

analysis and linear regression analysis with log transformed IL-6 response (difference from baseline to peak IL-6 at 5h30 or 8h post-injection) in the vaccine condition. Model 1 included loneliness, model 2 and 3 additionally included variables previously shown to be associated with inflammation or loneliness: depression, anxiety, negative mood, sleep quality, social skills, alcohol intake (model 2), age, and body mass index (BMI) (model 3).

3.4 RESULTS

Loneliness scores ranged between 22-64 (M = 39, SD = 10) and typhoid vaccination increases in IL-6 ranged from 1.1-8.8 pg/mL (M = 3.8, SD = 1.6) (see also Balter et al., 2018). At baseline, IL-6 was not significantly correlated with loneliness scores. However, as shown in Figure 1, loneliness positively correlated with the IL-6 response to typhoid vaccination ($r(34)$ = .383, p = .026). None of the other socio-emotional variables significantly correlated with the IL-6 response (Table 1). No significant correlations emerged in the placebo condition (p's > .60). Regression analysis showed that individual variation in loneliness was associated with the IL-6 response (model 1), independent of depression, anxiety, negative mood, quality of sleep, social skills, alcohol intake (model 2), age and BMI (model 3) (Table 2).

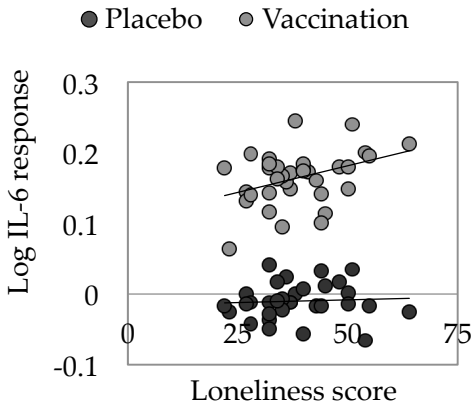

Figure 1. Correlations between log IL-6 response (difference from baseline to peak IL-6 at 5h30 or 8h post-injection) and loneliness separately for the placebo and vaccination condition.

*Table 1. Correlation coefficients between the log IL-6 response (difference from baseline to peak IL-6 at 5h30 or 8h post-injection) to vaccination and socio-emotional variables; * indicates statistical significance.*

	IL-6 response
Loneliness	.383*
Depression	.053
Anxiety	.060
Negative mood	.106
Social skills	.146

*Table 2. Standardised coefficient estimates (β), t- and p-values, and 95% confidence intervals (95% CI) of models predicting the IL-6 response (difference from baseline to peak IL-6 at 5h30 or 8h post-injection) to the immune challenge; * denotes statistical significance.*

	t	β	*p*	95% CI
Model 1 (R^2 = .146)			.026*	
Loneliness	2.343*	.383*	.026*	.001 .029
Model 2 (R^2 = .281)			.302	
Loneliness	2.179*	.517*	.040*	.001 .040
Depression	-0.207	-.055	.838	-.032 .026
Anxiety	0.404	.086	.690	-.014 .021
Sleep quality	-1.558	-.321	.133	-.029 .004
Negative mood	0.421	.105	.678	-.019 .028
Social skills	-0.783	-.182	.442	-.025 .011
Alcohol intake	-1.168	-.238	.255	-.026 .007
Model 3 (R^2 = .347)			.325	
Loneliness	2.407*	.579*	.025*	.003 .042
Depression	-0.772	-.224	.449	-.044 .020
Anxiety	0.278	-.069	.784	-.024 .018
Sleep Quality	-0.802	-.182	.431	-.026 .011
Negative mood	0.755	.194	.458	-.016 .033
Social skills	-0.199	-.056	.844	-.025 .020
Alcohol intake	-1.521	-.374	.143	-.034 .005
Age	-1.031	-.325	.322	-.037 .013
BMI	-0.573	-.121	.572	-.021 .012

3.5 DISCUSSION

The results presented here showed that those that reported feeling more lonely exhibited an enhanced inflammatory response to a mild immune stimulus. This association was robust to adjustment of age, BMI, and socio-emotional variables. A prior study showed that feelings of social disconnection were associated with an elevated immune response to endotoxin, an inflammatory stimulus that raises IL-6 about 100-fold (Moieni, Irwin, Jevtic, Breen, Cho, et al., 2015; Moieni, Irwin, Jevtic, Olmstead, et al., 2015). The current study extends this finding to loneliness, showing that a mild inflammatory stimulus, raising IL-6 levels about 4-fold, similarly evokes an enhanced inflammatory response in more lonely individuals. Although loneliness and social disconnection tend to co-occur, there is a conceptual distinction between the two, whereby feeling lonely is considered a result of social disconnection (Cacioppo, Hawkley, et al., 2006). However, strong genetic overlap between social isolation and loneliness as well as depression has been reported (Matthews et al., 2016). The observation that depression, anxiety, social skills nor negative mood were correlated with the inflammatory response, suggest that the relationship between loneliness (and social disconnection shown by Moieni et al., (2015) and immune responsiveness does not reflect a generalised effect for negative socio-emotional factors.

Since we and others identified loneliness as a predictor of immune dysregulation, screening for loneliness in populations with inflammation-related complaints, and other high-risk populations such as older adults, may be warranted as a target for further study. Admittedly, a causal role of loneliness remains speculative at this point, but the present findings as well as those of others, do provide a rationale to explore if interventions that focus on reducing feelings of loneliness may simultaneously help ameliorate inflammatory dysregulation. Likewise, evidence of a possible causal role of loneliness might be strengthened by studies that manipulate subjective loneliness for example via a false feedback paradigm (see Lamster, Nittel, Rief, Mehl, & Lincoln, 2017). The current findings are limited in terms of generalizability because of the experimental nature of the study and only healthy young males were assessed. Despite this consideration, research could assess whether lonely individuals may also have stronger responses to more naturalistic inflammatory insults such as a cold or flu.

Furthermore, although the present study was aligned with prior research and was hypothesis driven, the current results stem from secondary analysis of existing data, and replication seems therefore warranted.

In summary, the current results showed that, among healthy young adults, those feeling more lonely exhibited a higher inflammatory response to a mild immune challenge, that appeared independent of negative mood and common confounders related to social or health behaviours.

CHAPTER 4

Where is My Motivation? – Experimentally Induced Low-Grade Inflammation Selectively Impairs Motivated Learning

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Based on: "Balter, L. J. T., Bosch, J. A., Aldred, S., Drayson, M. T., Veldhuijzen van Zanten, J. J. C. S., Higgs, S., & Raymond, J. E. (in preparation). Where is My Motivation – Experimentally Induced Low Grade Inflammation Selectively Impairs Motivated Learning."

ABSTRACT

Impairments in motivated behaviour, i.e., fatigue and avolition, are highly prevalent across a broad range of medical and psychiatric disorders and inflammation has been proposed as a biological mechanism. Motivated learning (also "reward" or "reinforcement" learning) is a common method to assess motivation. There is limited data on the effects of inflammation on motivated learning. Using a reinforcement learning task, the current experimental study tested several hypotheses about which aspects of motivated learning may be affected by acute low-grade inflammation: 1) ability to learn (mean response time, mean accuracy, peak learning (i.e., highest achieved accuracy) and rate of learning); 2) stability of learned behaviour (stable performance); 3) the ability to adapt to changing reinforcement contingencies (flexibility); and 4) the relative impact of reinforcement value on learning. Using a double-blinded placebo-controlled within subjects study design, 40 healthy men (mean age = 25, SD = 5) received Salmonella Typhi vaccination (0.025 mg; Typhim Vi, Sanofi Pasteur) or placebo (saline). During the reinforcement learning task abstract symbols had to be associated with monetary rewards/losses and distinguishable aspects of motivated learning were assessed. As expected, vaccination increased IL-6 levels (vaccination +4.9 pg/mL; placebo -0.3 pg/mL; $p < .001$). Participants in the inflammation condition showed a slower rate of learning, slower response times, and reduced cognitive flexibility as compared to the placebo condition (p's $< .01$). Inflammation did not affect other measures of motivated learning: peak learning, stability of performance, and sensitivity to valence or magnitude of the reinforcer. The current results provided evidence for a distinguishable effect of inflammation on motivated learning, whereby acute mild, i.e., low-grade, inflammation may impair learning of cognitively demanding learning behaviours.

4.1 INTRODUCTION

Systemic inflammation involves elevated inflammatory cytokines in the circulation. Inflammatory cytokines act on neural substrates and can hereby induce feelings of sickness, e.g., malaise, fatigue or pain (Dantzer, 2009). Related, a large body of evidence shows that inflammation is associated with psychological and behavioural effects that is strikingly similar to the constellation of symptoms that define depression, including fatigue, anhedonia, cognitive confusion (wooliness) and low mood (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Miller & Raison, 2016). These observations are taken to explain that individuals who exhibit elevated inflammatory activity, such as those with obesity or inflammatory diseases show an elevated prevalence of fatigue and depression (Lim, Hong, Nelesen, & Dimsdale, 2005; Luppino et al., 2010; Marrie et al., 2018; Van Langenberg & Gibson, 2010). Bolstering a link between inflammation and depression-like symptoms is preliminary evidence that anti-inflammatory agents may improve the efficacy of pharmacological antidepressant treatment, particularly for those with evidence of elevated inflammation (Köhler-Forsberg et al., 2019; Köhler et al., 2016; Raison et al., 2013). However, it is also relevant to keep in mind that most fluctuations in inflammatory activity are within a normal range, e.g., due to normal exposures to mild immune-activating stimuli (e.g., exercise, stress, foods, pollution, subclinical infections) and common physiological processes (e.g., overweight, ageing). While this perspective has received little attention in the literature thus far, inflammation may thus also be a relevant determinant of every-day cognitive and psychological functioning (Ackerman, Hill, & Murray, 2018).

Inflammation has been identified as a regulator of reward-motivated behaviours, which reflects a cognitive pathway by which inflammation may increase vulnerability to depression (Eisenberger et al., 2017; Felger & Treadway, 2016). These effects are thought to involve dysregulating effects of inflammatory cytokines on dopamine (DA) functions in mesolimbic brain regions; structures that play important roles in reward-motivated behaviours (Capuron, 2012; Felger & Treadway, 2016). While early understanding of these mechanisms mostly relied on animal research, more recently human models of transient inflammation have contributed further insights (Boyle et al., 2019; Draper et al., 2017; Eisenberger et al., 2010; Harrison, Voon, et

al., 2015; Lasselin, Treadway, et al., 2016). Although a handful of human neuroimaging studies showed reduced signals in reward-related brain areas (e.g., ventral striatum) in response to immune challenges (Capuron, 2012; Dowell et al., 2016; Eisenberger et al., 2010; Harrison, Voon, et al., 2015), inflammation does not simply reduce reward-motivated behaviour. I.e., sensitivity to reward and loss appears unaffected by inflammation (except for one study showing a relative increase in sensitivity to loss versus reward) (Boyle et al., 2019; Draper et al., 2017; Eisenberger et al., 2010; Harrison, Voon, et al., 2015; Lasselin, Treadway, et al., 2016; Nunes et al., 2014; Vichaya et al., 2014). However, data suggest that inflammation is linked to altered incentive motivation, i.e., the extent to which effort investment is conditional on the reward type. For example, injection of bacterial endotoxin, a potent inducer of systemic inflammation, reduced the willingness to perform high effort tasks (Draper et al., 2017). Lasselin et al. (2016) rather observed an increased willingness to exert effort but, importantly, only when this effort was accompanied with a high probability to win a large reward. Whereas these two studies used different paradigms to assess motivated behaviours, the apparent contrasting results may be resolved by assuming that inflammation results in a recalibration of reward-effort trade-offs, i.e., inflammation may induce a shift in the balance between perceived reward value and willingness to exert effort to obtain the reward.

Thus inflammation may induce changes in the willingness to exert effort for a reward. However, the aforementioned studies have possibly neglected important aspects or dimensions of motivated learning such as stability and flexibility of behaviour. Incentive salience models propose that individuals engage in high effort actions if those actions are sufficiently incentivised (Goto & Grace, 2005; Ikemoto, Yang, & Tan, 2015; Westbrook et al., 2016). Incentive cues promote robust maintenance of behaviour. Conversely, to optimise goal-directed behaviour in the ever-changing environment, there is a need for flexibility too. Even though impaired cognitive flexibility has been observed in a range of inflammation-associated states including depression, obesity, and ageing (Cella, Dymond, & Cooper, 2010; Lasselin, Magne, et al., 2016; Wilson, Nusbaum, Whitney, & Hinson, 2018), the role of inflammation in cognitive flexibility and stability of performance has not yet been tested.

In the present study, Salmonella Typhoid vaccination was used to transiently induce low-grade inflammation in healthy young males. The aim of the current study was to extend earlier research by testing several novel hypotheses about how inflammation may affect motivated learning. I.e., a reinforcement learning task was used to assess whether low-grade inflammation affects motivated learning: 1) by blunting reinforcement learning, i.e., mean response time (RT), mean accuracy, peak (maximal performance) or rate (number of trials needed) of learning reward/loss contingencies; 2) by impairing the ability to maintain optimal performance levels, i.e., stability of performance; 3) via poorer adaptation to changes in reward contingencies, i.e., flexibility; 4) by affecting sensitivity to the outcome. The latter is further divided into sensitivity to reward versus loss (valence learning) and large versus small rewards or losses (magnitude learning).

4.2 METHOD

4.2.1 Participants

Forty healthy young male students were recruited from the University of Birmingham, using (online) advertisement (*M* age = 24.7, SD = 5.2 years) in exchange for course credits or £40-£52. Individuals were excluded if they reported being a smoker, having a history of or suspected vaccine-related allergy, food allergy or intolerance, inflammatory, cardiovascular, neurological, mental health or immune-related disorders, visual impairments (unless corrected to normal), and those on any medication 7 days prior to the test days. Individuals having knowledge of Japanese Hiragana were also excluded because these symbols were used in the reinforcement learning task. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local Research Ethics Committee of the National Health Service (NHS).

4.2.2 General Procedures

Participants visited the behavioural immunology laboratory on three separate occasions: the first visit was intended as a familiarization session, followed by two separate test days (i.e., receiving typhoid vaccination or saline placebo, randomly assigned) planned at least one week apart. The study was carried out in a double-blind placebo-controlled crossover fashion. During the familiarisation session, written informed consent was obtained, inclusion and exclusion criteria were verified, height and weight were measured, an initial set of questionnaires were completed, and participants performed shortened versions of the computer tasks for the purpose of familiarisation. The current analysis is based on the same participants as were tested in Balter et al. (2018). On each of the two test days, participants arrived at the laboratory between 8:00 and 9:00 am after an overnight fast. Participants were instructed to refrain from strenuous physical activity and alcohol intake for at least 24 hours, and were asked to avoid high fat and high sugar products for at least 12 hours prior to the test days. Before participants received the injection, mood and sickness symptoms were assessed (as described below), tympanic body temperature (using a Medtronic Genius 2) was measured and the first blood sample was taken. These measurements were repeated at 5h30m and 8 hours post-injection. After injection, participants received a standardised breakfast (granola with skimmed milk, approx. 430 kcal), and after a 5-hour break participants received a standardised meal consisting of cheese sandwiches (approx. 328 kcal). During the 5-hour break participants were instructed to refrain from eating and drinking (except for water), or strenuous physical activity. For a subset of participants, EEG was also recorded (N = 20, see Chapter 7in this thesis) and these participants arrived back at the laboratory 1 hour earlier to allow EEG preparations. Then, a set of cognitive tests was completed including the computerised reinforcement learning task. The reinforcement learning task was completed about 6h30 after injection. Other tasks, reported elsewhere, included measures of attention, and emotion recognition. Since inflammation potentially affects reinforcement learning indirectly via influencing working memory capacity or attention, these results are reported in the Supplementary Materials and in Chapter 8, respectively.

4.2.3 Randomisation

Participants were randomly assigned to receive vaccine or placebo (control) on the first test day. Randomisation was performed by supporting staff who had no contact with the study participants. Both participants and researchers were blind to injection condition order, and only the nurse administering the injections was aware of the order whereby a sealed envelope containing information about the condition was handed to the nurse before administering the injection. The nurse followed identical procedures for placebo and vaccine injection ensuring participant's blindness to the condition. The researchers were not present when the injection was administered.

4.3 MATERIALS

4.3.1 Reinforcement Learning Task

In this task, participants associate abstract symbols with monetary reward or loss that differ in magnitude (large (+5 or -5) and small (+1 or -1)) or a neutral outcome (zero (0)). The primary goal of the task is to learn, by trial-and-error, the relationship between a symbol and an outcome (shown in Figure 1). The reinforcement learning task comprises three learning phases; acquisition, maintenance, and adaptation to contingency change. The task was designed to assess: 1) the ability to learn (mean RT, mean accuracy, peak and rate of learning); 2) stability of performance; 3) the ability to adapt to changes in reward contingencies (flexibility); 4) the impact of reinforcement value on learning, i.e., sensitivity to the outcome: reward versus loss outcomes (valence) and large versus small outcomes (magnitude). For a detailed description see Section 4.3.3. below.

4.3.2 Procedure

The reinforcement learning task comprised three phases. In Phase 1 (Acquisition), participants completed three blocks of 40 trials each; Phase 2 (Maintenance) consisted of three blocks identical to phase 1; and Phase 3 (Adaptation to Change) began with 20 trials using the

same symbol-outcome assignments as were used in phase 1 and 2 (these extra trials were discarded), and then without interruption, another 40 trials were presented using the same symbols but new symbol-outcome pairings. Another two blocks of 40 trials each using the new pairings were then completed. Each block was separated by a self-paced break. At the start of the task, participants were warned of a potential change in feedback contingency and reminded that the task was to maximise winning and minimise losing. Rule changes were confined to the large values and zero (i.e., +5, -5, 0), restricting potential rule changes to two possibilities that were counterbalanced across participants. The participant was informed that the more points collected the more money would be paid out with a maximum of £6 per session. To minimise carry-over effects from session 1 to session 2, different sets of symbols were used for test day 1 and test day 2 that were counterbalanced across participants. To minimise order effects, the order in which the injection was administered was also counterbalanced across participants. Participants completed a shortened version of the reinforcement learning task, using different symbols, during the familiarisation session.

Each trial began with a 750 ms fixation cross that was immediately followed by a 250 ms pair of symbols. The participant chose the symbol yielding the better outcome by pressing the left and right key on the keyboard to indicate left or right, respectively. Upon response, visual feedback (+5, +1, 0, -1, or -5) for both symbols was presented for 500 ms, and then the visual feedback corresponding to the selected character was presented at its location for a further 500 ms. Immediately following the visual feedback, the next trial began with a 750 ms fixation cross (see Figure 1 for two trial examples). Each symbol was assigned a unique feedback that predicted the outcome with 100% certainty. Symbol-outcome assignments were assigned using a Latin square design, resulting in ten possible outcome pairs (see Table 1). Each combination of outcome pair was equally likely to occur and each symbol was equally likely to appear on the left or right side of the screen. Trial order was fully randomised within a set of 20 trials.

4.3.3 Assessment of motivated learning

The reinforcement learning task was designed to distinguish between several expressions of reinforcement learning.

(1) Learning ability. Mean RT and mean accuracy scores were calculated for each block. The peak learning performance was the highest achieved accuracy score during one of the first 6 blocks of 40 trials each. The rate of learning was determined by calculating the number of blocks needed to reach 80% accuracy of an individual's highest achieved accuracy score across block 1 to 6 (i.e., peak learning performance).

(2) Stable performance. Stable performance was calculated as the number of blocks in which performance was above 80% of the peak learning performance after this was reached for the first time. The rate of learning was taken into account to control for possible differences in rate of acquisition: (rate of learning (in blocks to criterion) + stable performance blocks) / total blocks (6) x 100%). A higher value is indicative of stable performance.

(3) Flexibility. For flexibility, accuracy scores before (block 6) and after (block 7) the unannounced change in symbol-outcome mapping were compared. Only outcome pairs that changed reward contingency (+5, -5, 0)) were included. Negative values indicate inflexibility.

(4) Sensitivity to outcomes. Valence learning (reward vs. loss) was assessed via comparing accuracy scores for reward (+5 vs. 0, +1 vs. 0) and loss (-5 vs. 0, -1 vs. 0) outcome pairs. Magnitude learning (large vs. small) was calculated by comparing accuracy scores for large (+5 vs. 0, -5 vs. 0) and small (+1 vs. 0, -1 vs. 0) outcome pairs. The analyses for sensitivity to the outcomes were performed on the acquisition and maintenance phase.

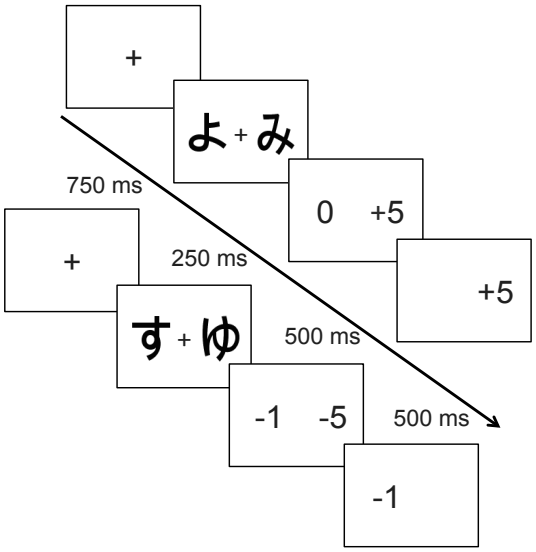

Figure 1. Successive screens of two typical trials.

Table 1. All ten possible outcome pairs.

Outcome pairs	
Large Win / Large Loss	5 / -5
Large Win / Zero	5 / 0
Large Win / Small Win	5 / 1
Large Win / Small Loss	5 / -1
Large Loss / Zero	-5 / 0
Large Loss / Small Win	-5 / 1
Large Loss / Small Loss	-5 / -1
Small Win / Small Loss	1 / -1
Small Win / Zero	1 / 0
Small Loss / Zero	-1 / 0

4.3.3.1 Stimuli

Five different black Japanese hiragana (Arial Unicode MS Hiragana font, each subtending 1.4 x° X 1.8 y° of visual angle) were presented on a gray field. Center-to-center distance along the horizontal meridian between symbols was 4 x° with a black 0.5 x° fixation cross at the midpoint. Two symbols were paired to winning points (+1, +5), two symbols to losing points (-1, -5) and one symbol led to 0 points.

4.3.4 Typhoid vaccination

Participants received 0.5 mL *Salmonella typhi* capsular polysaccharide vaccine (0.025 mg in 0.5 mL, Typhim Vi, Sanofi Pasteur, UK) and a saline placebo (0.5 mL) via intra-muscular injection in the deltoid muscle of the non-dominant arm by a certified nurse. Typhoid vaccine was selected as a low-grade inflammatory stimulus, since this vaccine is known to induce increases in circulating inflammatory cytokine levels without inducing significant secondary illness symptoms (i.e., fever, nausea) (Paine et al., 2013).

4.3.5 Body temperature, mood and sickness symptoms

Mood and sickness symptoms were assessed using a modified version of the Profile of Mood States – Short Form (POMS-SF; Curran, Andrykowski, and Studts, 1995). The POMS-SF consists of 32 items rated on a five-point scale (0 = not at all to 4 = extremely). Scores for POMS subscales (tension-anxiety, anger-hostility, fatigue-inertia, vigour-activity, confusion-bewilderment, depression-dejection) were computed by summing ratings on individual items. Six items were added to assess physical sickness (light-headed, nausea, faint, disgusted) and behavioural sickness (withdrawn, sociable).

4.3.6 Blood sampling

Blood was collected into one 6ml vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500*g* for 10 min at 4°C and plasma was aliquoted and stored at -80°C for later cytokine assessment of plasma interleukin-6 (IL-6). Plasma IL-6 was measured in duplicate using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The limits of detection of this assay was 0.11 pg/mL, with an intra-assay coefficient of variation (CVs) of 4.2%. All samples from the same participants were assayed in the same run.

4.4 STATISTICAL ANALYSIS

Data were examined using Cook's distance and influential data points were removed (*N* = 1). Additionally, non-learners, defined as participants failing to show evidence of learning (accuracy score < 60% in the maintenance phase), were removed from the analysis (*N* = 1). Trials with response times faster than 200 ms were discarded (0.07%). Accuracy scores and response times were calculated for each outcome pair, each block of 40 trials (1-9) and each phase (Acquisition, Maintenance, Adaptation to Change). For the purpose of the study, correct responses were defined as the most optimal choice: i.e., +5 in "+5 vs. 0" or 0 in "0 vs. -1". Outcome measures were calculated as described in section 4.3.3. Data were analysed using

repeated measures (RM) analysis of variance (ANOVA) and independent and paired samples t-tests where appropriate. Post hoc pairwise comparisons were Bonferroni corrected to control for multiple comparisons. For example, a RM ANOVA was performed to assess the effect of injection condition on mean accuracy, including injection condition (placebo, vaccine) and accuracy score of each block (1-9) as within-subjects factors and order of injection included as between-subjects factor. For IL-6 analysis, non-parametric tests were used or log10 transformation was applied because of the skewed distribution of raw IL-6 values. For the IL-6 analysis, three subjects were excluded because of high baseline values indicative of a possible infection and 5% of IL-6 data was missing. Spearman correlations were used to test relationships between IL-6 and performance. Alpha values were set a .05 throughout. In addition to traditional null hypothesis significance testing, Bayes Factors were calculated using Bayesian ANOVAs, t-tests, and correlation analyses using default prior probabilities. Bayes factors provide relative evidence of both the null (H_0) and alternative (H_A) hypothesis, compared to the conclusions about the null hypothesis proffered by traditional null hypothesis significance testing. To allow for clear interpretation, the approximate classification scheme of Wagenmakers et al., (2017) was used which states that an estimated Bayes Factor (BF_{10}; H_0/H_A) value <1 supports evidence in favour of H_0. For example, a BF_{10} of 0.25 indicates that the H_0 is 4 times (1:0.25) more likely than the H_A. Values close to 1 are not informative and a BF_{10} between 1 and 3 provides anecdotal evidence for the H_A, a BF_{10} between 3 and 10 is considered as moderate evidence for H_A, a BF_{10} between 10 and 30 is strong evidence for H_A, and a $BF_{10} > 30$ is very strong or decisive support for H_A. A BF_{10} between 1 and 0.33 provides anecdotal evidence for the H_0 (e.g., 1:3 probability in favour of H_0) and a BF_{10} between 0.33 and 0.10 provides moderate evidence for H_0. A $BF_{10} < 0.10$ provides strong evidence for H_0. All data were analysed using SPSS v.24.0 (IBM-SPSS Inc., Chicago, IL, USA) and JASP (Version 0.9; JASP Team 2019).

4.5 RESULTS

4.5.1 Sample characteristics

Sample characteristics are summarised in Table 2.

Table 2. Descriptive characteristics of the study population.

Characteristic	
N	38
Age (years)	
Mean (*SD*)	25 (5)
Range	19-36
Weight Status	
BMI (kg/m^2) (*SD*)	23.9 (3.3)
Range	16.6-29.7
Native/fluent English	44.7%
Ethnicity	
Caucasian	50%
Chinese	13%
Other Asian	34%
Unknown	3%

4.5.2 Effects of injection order

A RM ANOVA including injection condition (placebo, vaccine), block (1 to 9) and injection order (placebo-vaccine, vaccine-placebo) showed no main effect of injection order on mean accuracy ($F(1, 36) = 0.01$, $p = .985$, $\eta_p^2 = .00$; $BF_{10} = 0.37$). However, importantly, there was a significant interaction between injection condition and injection order with mean accuracy ($F(1, 36) = 9.70$, $p = .004$, $\eta_p^2 = .19$; $BF_{10} = 12.3$), suggesting that the order in which the placebo and vaccine was given significantly affected mean accuracy (see Supplementary Materials Figure S1). No significant effect of injection order was evident for any of the other outcome measures.

Therefore, the mean accuracy analysis was limited to a between-subjects comparison whereby test day 1 was analysed. For the remainder of the analysis the planned within-subjects analysis were performed.

4.5.3 Responses to typhoid vaccination

As reported elsewhere (Balter et al., 2018), a significant Time x Injection Condition (F(2, 50) = 58.04, p < .001, η_p^2 = .84; BF_{10} > 100) indicated that typhoid vaccination increased plasma IL-6 by 4.1 pg/mL (SE = 0.3) at 5h30m as compared to the 0.1 pg/mL (SE = 0.1) decrease in IL-6 seen in the placebo condition (t(34) = -10.23, p < .001; BF_{10} > 100). IL-6 was still significantly elevated at least 8 hours post injection (mean difference from baseline (before injection) = 2.4 pg/mL, SE = 0.4) (t(32) = -15.14, p < .001; BF_{10} > 100). As such, from this point on we will refer to the vaccine condition as the inflammation condition. Core body temperature, and mood and sickness symptoms (POMS-SF) showed no effect of injection condition (see also Balter et al., 2018).

4.5.3.1 Ability to learn: mean RT, mean accuracy, peak, and rate of learning

Mean RT. A RM ANOVA including injection condition (placebo, vaccine), RT for each block (1 to 9) and injection order (placebo-vaccine, vaccine-placebo) was conducted. As expected, RTs became faster across the blocks (F(8, 296) = 14.50, p < .001, η_p^2 = .28; BF_{10} > 100). RT decreased from 1131 ms (SE = 52 ms) in block 1 to 994 ms (SE = 38 ms) in block 3, stabilised during the maintenance phase (M = 964 ms, SE = 37 ms), and then slowed down to 1167 ms (SE = 54 ms) in block 7 and accelerated to 1038 ms (SE = 39 ms) in block 9. As can be seen from Figure 2, across all blocks RT was slower for the inflammation (M = 1077 ms, SE = 39 ms) as compared to the placebo (M = 1005 ms, SE = 46 ms) condition (F(1, 37) = 14.50, p < .001, η_p^2 = .28; BF_{10} > 100). Injection condition did not interact with block (F < 1; BF_{10} = 0.00). Including accuracy as a covariate did not alter these results. No effect of injection order was evident (F < 1; BF_{10} = 0.44).

Mean accuracy. Because there was a significant interaction between injection condition and injection order with mean accuracy (see Section 4.5.2), a between-subjects comparison was performed whereby test day 1 was analysed. As shown in Figure 2, accuracy scores changed

across the blocks in the expected manner ($F(8, 296) = 41.54$, $p < .001$, $\eta_p^2 = .53$; $BF_{10} > 100$). Accuracy increased during acquisition from 73.0% (SE = 2.2%) in block 1 to 86.2% (SE = 2.2%) in block 3, levelled off during the maintenance phase ($M = 92.5\%$, SE = 1.1%), and then dropped to 78.2% (SE = 1.4%) in block 7 and recovered to 92.1% (SE = 1.1%) in block 9 during the adaptation to change phase. No significant effect of injection condition was found ($F < 1$; $BF_{10} = 0.31$), nor an interaction between injection condition and block ($F < 1$; $BF_{10} = 0.05$).

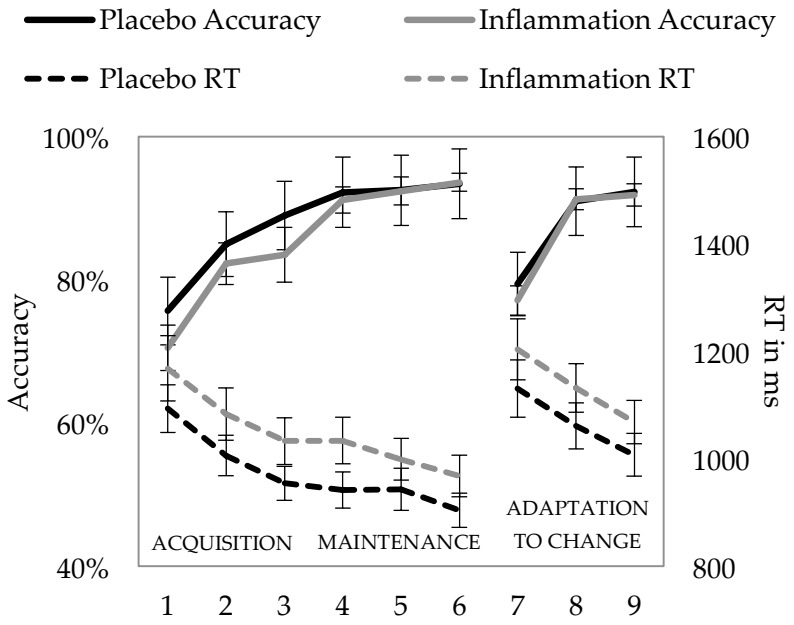

Figure 2. Accuracy learning curves (between-subjects) and RTs (within-subjects) for the acquisition (block 1-3), maintenance (block 4-6), and adaptation to change (block 7-9) phase separated by injection condition; error bars represent standard error of the mean; solid lines represent accuracy scores (primary y-axis); dashed lines represent RTs (secondary y-axis). Note: accuracy scores for the adaptation to change phase also include outcome pairs that did not change (i.e., outcome pairs with +1 and -1).

Peak and rate of learning. The peak learning performance was similar across conditions (inflammation M = 96.4%, SE = 0.7; placebo 96.5%, SE = 0.8) ($t(37) = -.081$, $p = .936$, $d = -0.013$; $BF_{10} = 0.18$), indicating that individuals in the inflammation condition were able to reach a similarly high accuracy score as in the placebo condition. However, as shown in Figure 3a, a slower rate

of learning was found for participants in the inflammation condition (inflammation M = 1.9 blocks, SE = 0.2; placebo M = 1.4 blocks, SE = 0.1) ($t(37) = -2.779$, $p = .009$, $d = -0.451$; $BF_{10} = 4.77$). Since rate of learning showed a marginally significant effect of order ($F(1, 36) = 3.28$, $p = .078$, $\eta_p^2 = .08$; $BF_{10} = 1.45$), the analysis was repeated as a between-subjects analysis, which yielded essentially identical results (results presented in Supplementary Materials 4.7.2).

4.5.3.2 Stable performance and flexibility

As can been seen from Figure 3d, no effect of injection condition was found on the number of blocks in which performance was above 80% of an individual's peak learning performance after this peak was reached, which is taken to indicate that stability of performance was unaffected by inflammation (inflammation $M = 97.9\%$, SE = 1.1%; placebo $M = 95.2\%$, SE = 2.3%) ($t(37) = -.529$, $p = .600$, $d = -0.086$; $BF_{10} = 1.08$). Next, a RM ANOVA with Outcome Type (+5 vs. 0; -5 vs. 0, +5 vs. -5), Block (6 before change, 7 after change), Injection Order, and Injection Condition was performed to assess flexible adaptation to unannounced changes in reward/loss contingencies. As shown in Figure 3b, a significant injection condition x block interaction indicated that the drop in performance after the contingency change was significantly larger in the inflammation ($M = -17.1\%$, SE = 2.3) relative to placebo ($M = -11.0\%$, SE = 1.9) condition ($F(1, 37) = 4.35$ $p = .044$, $\eta_p^2 = .11$; $BF_{10} = 2.92$), suggesting reduced flexibility with inflammation. A block x outcome pair interaction indicated that the drop in accuracy was 7.9% (SE = 2.7) larger on large loss vs. zero outcome pairs as compared to large win vs. large loss outcome pairs ($F(2, 74) = 4.32$ $p = .017$, $\eta_p^2 = .09$; $BF_{10} = 1.99$). Injection condition did not significantly interact with outcome pair ($F(2, 74) = 1.89$, $p = .158$, $\eta_p^2 = .38$; $BF_{10} = 0.32$). Exploratory post-hoc analysis indicated that the larger drop in performance in the inflammation condition was mostly driven by reduced performance on large win vs. zero outcome types (inflammation: $M = -16.4\%$, SE = 0.0; placebo: $M = -7.2\%$, SE = 0.3) ($t(37) = -2.572$, $p = .014$, $d = -0.417$; $BF_{10} = 3.07$). No effect of injection condition was found for the large loss vs. zero ($BF_{10} = 0.61$) and large win vs. large loss ($BF_{10} = 0.18$) outcome types.

4.5.3.3 Sensitivity to outcomes: valence and magnitude

Valence and magnitude learning. To test if learning in the acquisition and maintenance phase was differentially impacted by valence (reward, loss) or magnitude (large, small) of the outcome, a RM ANOVA was conducted with Magnitude (large (5) vs. zero (0), small (1) vs. zero (0)), Valence (reward (+) vs. zero (0), loss (-) vs. zero (0)), Phase (acquisition, maintenance), and Injection condition (inflammation, placebo). Results are presented in Figure 3c. Performance was 19% (SE = 2.1%) better for large as compared to small outcome pairs ($F(1, 37) = 81.27$, $p < .001$, $\eta_P^2 = .69$; $BF_{10} > 100$). Injection condition did not interact with any of the factors ($F's < 1$, $BF's_{10} < 0.23$), suggesting that inflammation did not affect sensitivity to valence or magnitude of the reinforcement. The magnitude of the IL-6 response to the vaccine was not or marginally correlated with the outcome measures.

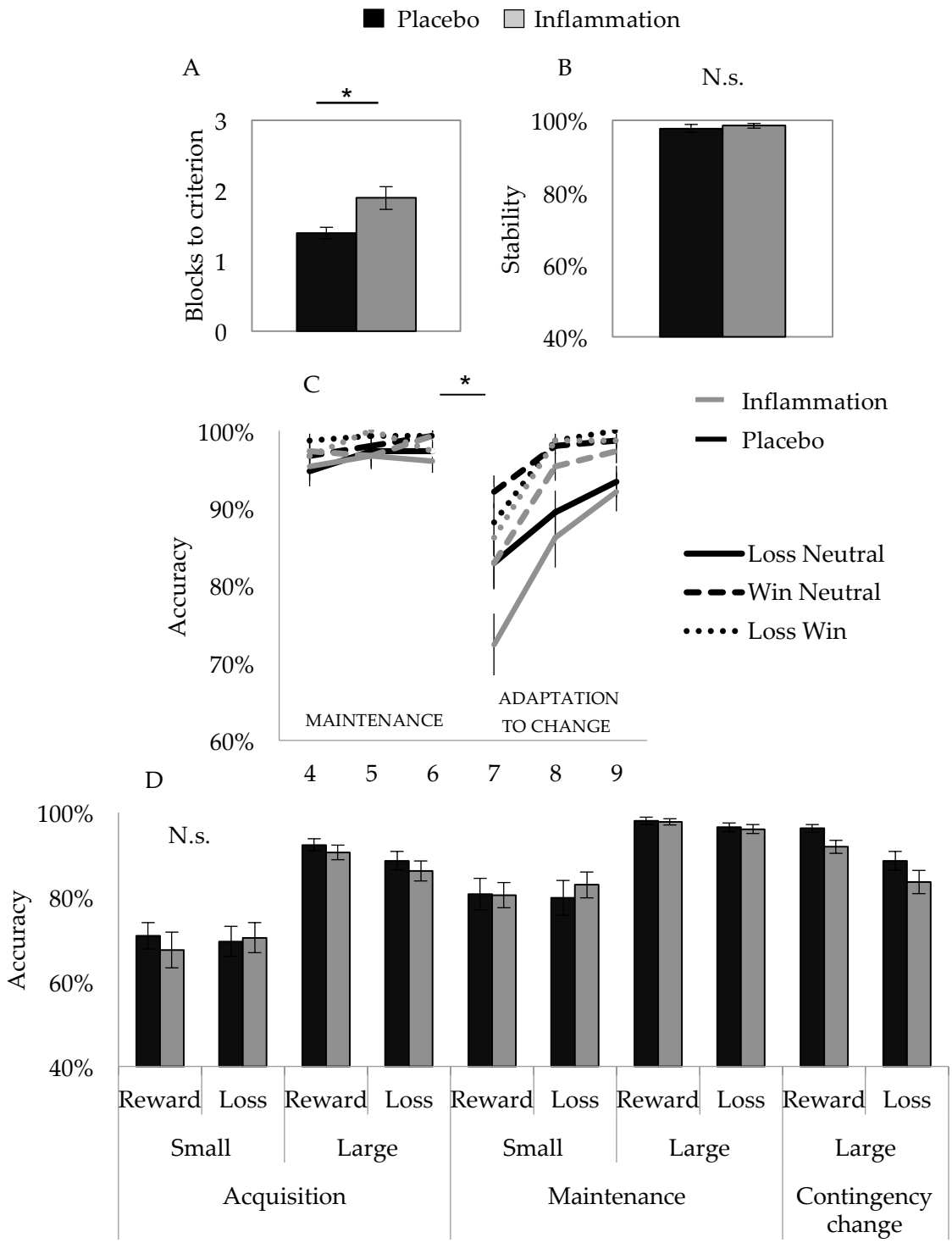
Placebo
Inflammation
A
*
Blocks to criterion
3
2
1
0
B
N.s.
Stability
100%
80%
60%
40%
C
*
Inflammation
Placebo
Accuracy
100%
90%
80%
70%
60%
MAINTENANCE
ADAPTATION TO CHANGE
4
5
6
7
8
9
Loss Neutral
Win Neutral
Loss Win
D
N.s.
Accuracy
100%
80%
60%
40%
Reward
Loss
Small
Large
Acquisition
Maintenance
Contingency change

*Figure 3. Rate of learning, i.e., number of blocks needed to reach 80% of the individual's peak learning score (A); Stability of performance; higher value indicates stability (B); Adaptation to change (i.e., flexibility) shown for the outcome types that change (C); Sensitivity to outcome: valence learning (reward, loss) and magnitude learning (large, small) for the acquisition and maintenance phase (D); Error bars represent standard error of the mean; * indicates significant effect of injection condition $p < .05$; N.s. = No significant effect of injection condition. Grey and black colour represent inflammation and placebo condition, respectively.*

4.6 DISCUSSION

The current study tested the effect of low-grade inflammation on motivation by assessment of distinct expressions of reinforcement learning; i.e., the ability to learn reward contingencies. These included mean RT, mean accuracy, peak and rate of learning), stability of optimal performance, flexibility, and sensitivity to outcome types (valence, magnitude). Chiefly, two effects of inflammation were observed: First, the results indicated that while the capacity to learn reward contingencies remained intact (i.e., mean accuracy and the peak learning performance were not affected by inflammation), the rate (i.e., number of blocks) at which the reward contingencies were acquired was slower in the inflammation condition. The lower acquisition rate may be related to the second observation, which that mean response time (RT) was slower. Slower RT might be the result of post-error slowing, i.e., is secondary to more errors due to slower learning, or may suggest that inflammation caused motor slowing (also see Balter et al., (under review) and Chapter 6 in this thesis). Another possibility, elaborated further below, is that higher RT indicates that during inflammation the task became more effortful (e.g., see Chapter 7 in this thesis). Notwithstanding, once association were learned, however, performance remained equally stable and was of the same quality in both conditions. Second, inflammation led to slower adaptation to unannounced changes in the reward environment suggestive of reduced flexibility. Sensitivity to reward features such as valence (reward versus loss) and magnitude (large versus small outcomes) remained unaffected.

Rapid adaptation to changing reward contingencies is a hallmark of flexible goal-directed behaviour (Dolan & Dayan, 2013). Flexible goal-directed behaviour may maximise

reward, but this could be at the expense of investing more effort because habitual behaviours allow fast, efficient performance of actions with minimal cognitive effort (Redgrave et al., 2010; Schneider & Chein, 2003). In everyday life, habit formation allows to operate more efficiently in stable environments, such as taking a standard route to work or other routine operations, but this propensity becomes problematic when such learned information is no longer valid. From that perspective the shift from flexible goal-directed behaviour towards inflexible habit-based behaviour during inflammation is consistent with the idea of inflammation favouring energy-saving behaviour strategies (Draper et al., 2017; Lasselin, Treadway, et al., 2016). However, whereas these authors suggested that acute inflammation reduced the willingness to exert effort, a potential alternative explanation for reduced motivated behaviour is that the same behaviour simply becomes more effortful. In other words, the willingness to exert effort remains essentially the same, however, but appears less because they pose a higher cognitive demand. Supporting this alternative hypothesis, i.e., that the same cognitive operations become more effortful with inflammation, is the finding that acute inflammation induces greater attention cue-induced alpha suppression, while behavioural performance was unaffected (see Chapter 7 in this thesis). The degree to which alpha power is suppressed is thought to reflect the level of cognitive effort required by an upcoming task (reviewed in Van Diepen, Foxe, & Mazaheri, 2019). This result would thus be consistent with the idea that acute inflammation required individuals to exert greater cognitive effort in order to maintain the same behavioural performance. Moreover, the current study revealed slower mean response times with inflammation. Slower response times are sometimes interpreted as a measure of cognitive effort, because RT characteristically slows with increasingly complex or accuracy-demanding tasks (Egner, 2007; Hogarth, 1987; Patten, Kircher, Östlund, Nilsson, & Svenson, 2006; van Winsum, 2018). However, as discussed earlier, the latter interpretation remains tentative and multiple (and non-mutually exclusive) interpretations of slowed RT could apply.

The observation that inflammation reduces flexibility may present a cognitive mechanism linking inflammation to vulnerability to psychopathology. Indeed, deficient flexible goal-directed behaviour and excessive habit formation has been shown to accompany several

psychiatric disorders including social anxiety disorder (Alvares, Balleine, & Guastella, 2014), stress (Soares et al., 2012), schizophrenia (Morris, Quail, Griffiths, Green, & Balleine, 2015), obsessive-compulsive disorder (Gillan & Robbins, 2014), eating disorders (Voon et al., 2015), and addiction (Everitt, Dickinson, & Robbins, 2001). Elevated inflammatory activity has been increasingly implicated in the etiology of most of these disorders (Glaser & Kiecolt-Glaser, 2005; Müller, Weidinger, Leitner, & Schwarz, 2015; Vogelzangs, Beekman, De Jonge, & Penninx, 2013). The present findings are in support of the hypothesis that inflammation may be an important biological factor that might increase the risk of reduced cognitive flexibility. Whether inflammation represents a biobehavioural pathway for reduced flexibility in psychiatric disorders deserved further scrutiny.

There may be several interpretations for the observed reduced rate of motivated learning. First, inflammation may have decreased the perceived value of the benefit (i.e., collecting points in exchange for money is less rewarding when inflammation). However, this explanation seems less likely as sensitivity to reward and loss was unaltered. Second, effort mobilisation is an implicit characteristic that supports initiation of actions (Matthews et al., 2010) and inflammation may have decreased the ability to mobilise the required amount of effort. In a similar vein to the explanation provided above for the slower adaptation of changing contingencies, initial acquisition of symbol-outcome associations requires more effort and develops slower than maintaining stable performance of learned symbol-outcome associations; which may explain why acquisition, but not maintenance, was impacted by inflammation. The observed slower adaptation to reward contingencies similarly suggests that inflammation reduced cognitively demanding learning behaviours. Thus, although speculative at this point, inflammation could have impaired mobilisation of high amounts of effort and/or the same behaviours may require greater investment of effort. Both explanations might apply and the current study did not allow to differentiate whether reduced motivated learning was secondary to task demand (recruited effort was insufficient) or to effort exertion (insufficient effort was recruited). Physiological measures of mental effort such as electroencephalogram (EEG) markers (see Chapter 7) or pupil dilation could be used to further address this matter. A third possible

explanation is that inflammation reduced motivated learning indirectly via impairing other cognitive processes that are required for optimal reinforcement learning, such as working memory capacity and attention. This explanation, however, seemed less likely since inflammation did not affect verbal or spatial working memory (see Supplementary Materials with this chapter) or overt attention processes (i.e., alerting, orienting, executive control) (data presented in Chapter 8).

The present study had a few limitations that warrant consideration. First, a notable feature of the typhoid model of inflammation is that it modestly raises IL-6 levels by about 4-fold. It may be argued, however, that the mild nature of typhoid vaccination has several advantages over more potent immune stimuli; the level of immune activation is akin to the low-grade inflammatory levels seen in depressed individuals, as well as in common medical conditions such as diabetes and atherosclerosis (Dowlati et al., 2010; Wegner et al., 2013). A second advantage is that the mild response minimises potentially confounding side effects such as physical malaise. A second limitation is that IL-6 primarily acted as an inflammation check and no causal assumptions about the role of IL-6 can be made. Future research may consider a larger panel of inflammatory mediators. Third, generalisation of the acute effects of vaccination to chronic inflammation and clinical populations remains to be determined

In sum, experimentally induced low-grade inflammation reduced the rate at which reward associations were acquired, behaviour became less flexible, and responses became slower overall. Other aspects of reinforcement learning were unaffected: mean accuracy and peak learning, stability of performance, and sensitivity to the valence or magnitude of the reinforcer. These results provided direct empirical evidence for a distinct pattern of inflammation-related motivational changes, whereby low-grade inflammation appears to reduce cognitively demanding aspects of reinforcement learning.

4.7 SUPPLEMENTARY MATERIALS

4.7.1 Injection order effect

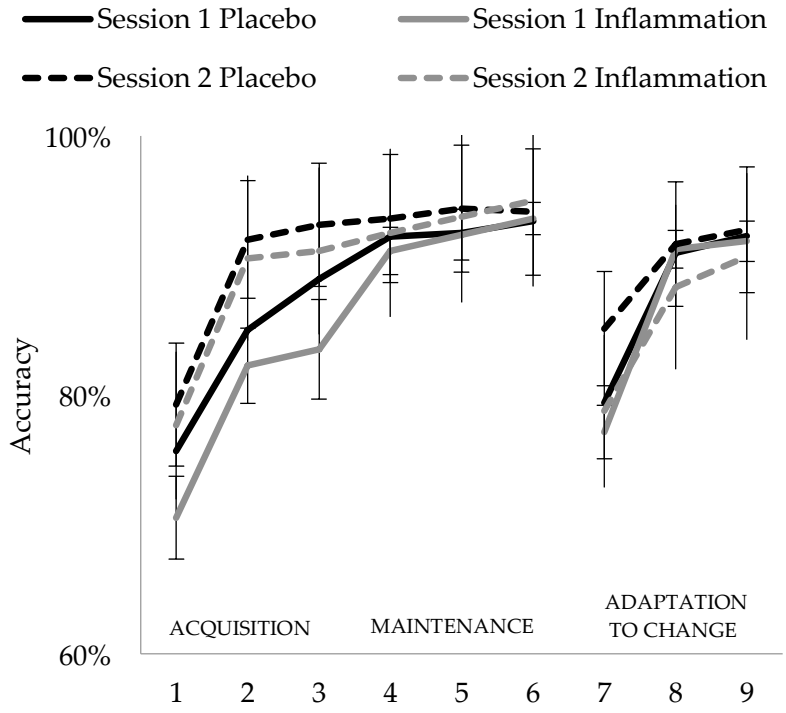

Figure S1. Accuracy learning curves and RTs for the acquisition (block 1-3), maintenance (block 4-6), and adaptation to change (block 7-9) phase separated by injection condition (black colour represents placebo, grey colour represents inflammation condition) and injection order (solid lines represent session 1, dashed lines represent session 2); error bars represent standard error of the mean. Note: accuracy scores for the adaptation to change phase also include outcome pairs that did not change (i.e., included also outcome pairs with +1 and -1).

4.7.2 *Between subjects analysis: rate of learning*

As shown in Figure S2, participants in the inflammation condition needed significantly more blocks to reach the learning criterion of 80% accuracy (rate of learning) (inflammation M = 2.2 blocks, SE = 0.3; placebo M = 1.4 blocks, SE = 0.1) ($t(37) = -2.40$, $p = .021$, $d = -0.770$; $BF_{10} = 2.8$).

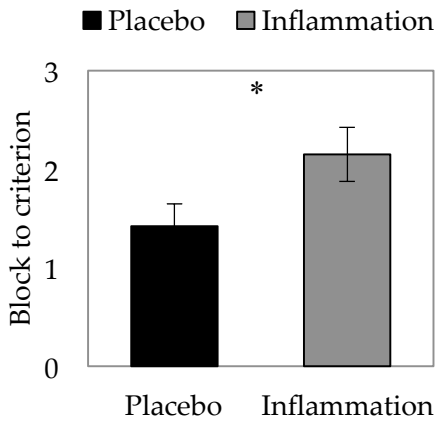

Figure S2. Rate of learning, i.e., number of blocks needed to reach 80% of the individual's peak learning score (analysis performed on test day 1 only, placebo N = 19, inflammation N = 20).

4.7.3 Working memory

MATERIALS

Spatial Working Memory. The spatial working memory (WM) test consisted of two parts. Part one assessed forward spatial WM capacity and part two backward spatial WM capacity. Each trial started with nine white boxes presented at random locations on a grey screen. One of the boxes briefly turned blue for 1 second upon which another box turned blue. The participant was asked to click the boxes that changed colour in the same order as presented. Three trials of each sequence were completed. Progressively more boxes changed colour after two out of three trials of each sequence were correctly tapped. If two or more errors at the same sequence length were made, the first part was terminated and participants were prompted with the instruction of the second part. Part two was similar to part one except that the participant was asked to click the boxes that changed colour in the reverse order as presented. If two or more errors at the same sequence length were made, the spatial WM test was terminated. Outcome measures were maximum spatial WM span forward and maximum spatial WM span backward. The spatial WM test was completed about 7h30 after injection.

Verbal Working Memory. Similar to the spatial WM test, the verbal WM test consisted of two parts. Part one assessed forward verbal WM capacity and part two backward verbal WM capacity. White digits were presented in the centre of a grey screen, one by one at the rate of 1/s. Digits were randomly sampled without replacement up to list lengths of 10, starting with a list length of two digits. Participants were instructed to repeat each digit out loud upon presentation. When the whole sequence was presented, the participant entered the digit in the same order as presented. Three trials of each sequence were completed. Progressively more digits were added after two out of three trials of each sequence were correctly reported. If two or more errors at the same sequence length were made, the first part was terminated and participants were prompted with the instruction of the second part. Part two was similar to part one except that the participant was asked to report the digits in the reverse order as presented. If two or more errors at the same sequence length were made, the verbal WM test was terminated. Outcome measures were maximum verbal span forward and maximum verbal span backward. The verbal WM test was completed about 6h30 after injection.

RESULTS

Working memory. Data points that exceeded 2.5 *SD* from means and maximum spans of 2 (starting sequence) were removed from the data (N = 0 for verbal WM; N = 3 for spatial WM (2 forward, 1 backward, all in the placebo condition). A repeated measures ANOVA was performed with WM dimension (spatial, verbal), WM type (forward, backward) and Injection Condition (placebo, inflammation) as within subjects factors. A main effect of WM dimension ($F(1, 35) = 31.57$, $p < .001$, $\eta_p^2 = .47$; $BF_{10} > 100$) and WM condition ($F(1, 35) = 23.24$, $p < .001$, $\eta_p^2 = .40$; $BF_{10} = 75.86$) indicated better performance on verbal relative to spatial WM and greater maximum forward span as compared to maximum backward span. See Table S1. No significant effect of injection condition ($F < 1$; $BF_{10} = 0.44$), or significant interactions between injection condition and WM dimension ($F < 1$; $BF_{10} = 0.17$), injection condtion and WM type ($F < 1$; $BF_{10} = 0.22$), or a three-way interaction ($F < 1$; $BF_{10} = 0.20$) was evident. Order of injection did not

significantly interact with any of the results. The current results suggest that spatial working memory and verbal working memory were not affected by experimentally induced low-grade inflammation.

Table S1. Mean forward and backward span separated by working memory (WM) dimension (spatial, verbal) and injection condition (placebo, inflammation) presented as means (SD).

		Placebo	Inflammation
Spatial WM	Forward	6.1 (1.4)	6.2 (0.9)
	Backward	5.5 (1.4)	5.7 (1.6)
Verbal WM	Forward	7.6 (1.4)	7.4 (1.3)
	Backward	6.9 (1.5)	6.8 (1.7)

CHAPTER 5

Inflammation, Motivated Learning, and Emotion Recognition – Associations with Age- and Body Weight-Related Inflammation

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Based on: "Balter, L. J. T., Bosch, J. A., Aldred, S., Raymond, J. E., & Higgs, S. (in preparation). Inflammation, Motivated Learning, and Emotion Recognition – Associations with Age- and Body Weight-Related Inflammation"

ABSTRACT

Experimental evidence showed that acute systemic inflammation negatively affects motivated learning and emotion recognition; behaviours that are key to human social interaction. The current study attempted a potential conceptual replication to test if such observations generalise to more naturalistic chronic low-grade inflammation, i.e., ageing- and obesity-related inflammation. Ninety young (*M* = 26 years, SD = 4) or older (*M* = 71 years, SD = 4) participants, who had either a low body mass index (BMI) (*M* = 22.4, SD = 2.3) or high BMI (*M* = 33.1, SD = 3.8; 80% obese), completed a reinforcement learning task and the Reading the Mind in the Eyes Test to assess motivated learning and emotion recognition, respectively. Ageing and interleukin (IL)-6 levels were associated with selective reductions in motivated learning. The associations with IL-6 were independent of age, BMI, demographics and health factors (e.g., sex, education, smoking status, alcohol intake, presence of illness symptoms, and medication intake). However, IL-6 did not mediate the age-related reductions in motivated learning. Elevated IL-6 level was also associated with lower emotion recognition, independent of demographic and health factors. Emotion recognition was associated with BMI in an age-dependent manner: young individuals with a high BMI showed worse emotion recognition as compared to their low BMI counterparts, while the opposite was found in older individuals. The present results replicated earlier observations that elevated low-grade inflammation is negatively related to motivated learning and emotion recognition. Somewhat unexpected, these associations appeared independent of age and BMI, although these factors showed the expected enhanced inflammatory activity. Protracted low-grade inflammation may thus present a biobehavioural pathway for altered motivated behaviours and emotion recognition across age and body weight categories.

5.1 INTRODUCTION

Systemic inflammation, via the effects of inflammatory cytokines, acts on neural substrates on which it may exert acute and long-term effects (Felger & Lotrich, 2013). For example, experimentally-induced inflammation rapidly modulates brain responses of reward-related areas (Brydon et al., 2008; Eisenberger et al., 2010; Harrison, Voon, et al., 2015), but chronic inflammatory activity may also exert long-lasting effects on cognitive and affective functioning via neuronal damage, demyelination, and blood brain barrier dysfunction (Elwood, Lim, Naveed, & Galea, 2017; Hernández-Espinosa, Massieu, Montiel, & Morán, 2019; Raz & Rodrigue, 2006). Specifically, experimental human and animal studies have identified acute inflammation as a powerful regulator of social and reward-motivated behaviours (Balter et al., 2018; Draper et al., 2017; Eisenberger et al., 2017; Felger & Treadway, 2016; Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015). In these studies, inflammation was induced in healthy young individuals through the administration of immune-activating agents, such as bacterial endotoxin or a vaccine against Salmonella typhi. Whether the behavioural effects of such acute inflammation inductions translate to chronic inflammation still remains to be determined.

Chronic elevated inflammation is common to obesity and ageing (Capuron et al., 2014; Shelton & Miller, 2010). Elevated inflammatory activity in individuals with a high body mass index (BMI) is mainly due to adipose cells and local immune cells that produce copious amounts of inflammatory cytokines (Cancello & Clément, 2006). In ageing, factors such as oxidative stress, immunosenescence (i.e., gradual deterioration of the immune system), declining levels of sex hormones and age-related conditions (e.g., atherosclerosis) all contribute to elevated inflammatory activity, a phenomenon denoted as "inflammageing" (Baylis, Bartlett, Patel, & Roberts, 2013; Chung et al., 2009; Franceschi et al., 2007). Increased inflammation with high BMI and ageing thus appear to underlie partly independent processes. In addition to being stably associated with elevated inflammation, age and high BMI represent physiological (e.g., non-disease-related) models of low-grade inflammation, and analyses of inflammation in these groups may thus present an ecologically valid method to gain insight into the relationship between cognitive function and persistent low-grade inflammation.

Ageing and high BMI exert adverse effects on selective cognitive domains (Dye, Boyle, Champ, & Lawton, 2017; Miller & Spencer, 2014), whereby some cognitive processes appear to be more vulnerable to the deleterious effects of ageing and high BMI than others. For example, autobiographical memory and verbal ability appear less affected by older age, whereas encoding of new memories, executive function, processing speed, and reasoning tend to be more prone to decline with age (Hedden & Gabrieli, 2004a). Similarly, it has been suggested that memory, executive functions, and speed of processing are negatively impacted in those with high BMI (Chan, Yan, & Gregory Payne, 2013). Many BMI-associated changes are related to physiological function, e.g., immune dysregulation (e.g., inflammation) and impaired metabolic control (e.g., insulin resistance) and these are similarly hallmarks of ageing (Pérez et al., 2016; Trim, Turner, & Thompson, 2018). Accordingly some authors have proposed that high BMI facilitates cognitive ageing and that high BMI superimposed on ageing may thus accelerate ageing-related effects. However, actually only a handful of studies have tested possible joint effects of age and BMI on cognitive function. Both synergistic effects (i.e., obesity-related cognitive deficits increase with age) (Stanek et al., 2013) and mere additive effects (i.e., relationships between cognition and BMI do not vary with age) have been reported for selective cognitive domains (Benito-León, Mitchell, Hernández-Gallego, & Bermejo-Pareja, 2013; Gunstad, Paul, Cohen, Tate, & Gordon, 2006; Gunstad et al., 2007). Occasionally studies even suggest protective effects of high BMI against cognitive decline in older age (e.g., visuospatial skills), a phenomenon referred to as the "obesity paradox" (Gunstad, Lhotsky, Wendell, Ferrucci, & Zonderman, 2010; Kuo et al., 2006).

Previously we reported that acute low-grade inflammation impairs aspects of motivated learning and emotion recognition. Corroborated by other research, reward-motivated and social behaviours have been thought to be specifically sensitive to the effects of inflammation (Eisenberger et al., 2017; Felger & Treadway, 2016). Therefore, the current study aimed to extend these findings, by testing the independent and possible interactive effects of inflammation in ageing and BMI on motivated learning and emotion recognition. Ample evidence shows that older adults learn more slowly from reward and punishment feedback as compared to young

adults (De Wit, Van De Vijver, & Ridderinkhof, 2014; Eppinger, Herbert, & Kray, 2010; Marschner et al., 2005; Mell et al., 2005; Van De Vijver, Ridderinkhof, & De Wit, 2015; Weiler, Bellebaum, & Daum, 2008). It is less clear how high BMI is associated with reinforcement-based learning. Several studies showed that obesity is associated with impaired monetary reinforcement-based associative learning (Coppin, Nolan-Poupart, Jones-Gotman, & Small, 2014; Kube et al., 2017). However, there are also studies that report no behavioural differences between lean and obese individuals in monetary reinforcement-based learning (Balodis et al., 2013; Kube et al., 2016), while Zhang et al (2014) found only a food-specific associative learning impairment in female individuals with obesity. A different pattern of results is evident for emotion recognition. Ageing is generally accompanied by reduced emotion recognition, although not for all emotions, i.e., recognition of disgust seems to be preserved with age (Ruffman, Henry, Livingstone, & Phillips, 2008). On the other hand, a meta-analysis found that there is no evidence for an impaired ability to recognise emotions with a high BMI (Fernandes, Ferreira-Santos, Miller, & Torres, 2018). A limitation is that studies that assessed effects of high BMI mostly tested young individuals, and it is unclear whether age and BMI interact to exert alterations in emotion recognition as well as in motivated learning.

As such, the aim of the present study was twofold. First, to assess possible independent and interactive effects of BMI and age on motivated learning and emotion recognition. Second, to assess the role of BMI- and age-associated low-grade inflammation in motivated learning and emotion recognition. Young and older adult groups with a low or high BMI completed measures of motivated learning (i.e., reinforcement-learning task) and emotion recognition (i.e., reading the mind in the eyes test).

5.2 METHOD

5.2.1 Participants

Ninety participants (60.7% male) with an age between 21 and 35 (M = 26.3, SD = 4.1; young) or 63 and 80 (old; M = 70.7, SD = 4.0) and a BMI between 17 and 25 (M BMI = 22.4, SD =

2.2; low BMI) or greater than 27 (*M* BMI = 33.2, *SD* = 3.8; high BMI) were recruited through a database of the University of Birmingham and via (online) advertisements. The groups did not differ in sex, age (BMI groups did not differ in age) and BMI (age groups did not differ in BMI). Individuals who reported a history of gastric banding, eating disorders, neurological or inflammatory disorders (e.g., rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, periodontitis) or use of anti-depressant, anti-histamine, or anti-inflammatory (e.g., antibiotics) medication during the past 7 days were excluded. Participants reported normal or corrected-to-normal vision and stable body weight for at least six months (i.e., fluctuations < 7.5 kg for high BMI individuals, < 5 kg for low BMI individuals). The participants were paid a maximum of £25 to reimburse travel expenses. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the University of Birmingham Research Ethics Committee.

5.2.2 Procedures

Test sessions started between 8:30 and 15:30 hours. Start times of test sessions were matched across groups to control for minor diurnal variations in IL-6 (Nilsonne et al., 2016). Time of day did not significantly affect any of the results and was therefore not included in the analysis (*F's* < 1.30, *p's* > .260). Written informed consent was obtained on arrival and it was verified whether participants had complied with instructions. Participants were instructed to have their breakfast/lunch as usual but avoid consumption of high-fat products (e.g., bacon, fries), because these foods may induce a short-lived inflammatory response (Herieka & Erridge, 2014), and to refrain from eating, drinking (except for water), and smoking for 1 hour before the start of the test session. Participants were also asked not to engage in strenuous physical exercise or consume alcohol within 12 hours before the test session, and reschedule their appointment if they had suspected infection symptoms on the day of testing. A blood sample was taken by venepuncture and questionnaires and cognitive tests were completed, including the reinforcement learning task and the reading the mind in the eyes test (see further below). Other

tests included measures of attention, psychomotor speed and memory. Lastly, a measure of height and body composition was taken.

5.3 MATERIALS

5.3.1 Reinforcement Learning Task

The reinforcement learning task comprises three learning phases; acquisition, maintenance, and adaptation to contingency change. In this task, participants associate abstract symbols with monetary reward or loss that differ in magnitude (large (+5 or -5) and small (+1 or -1)) or a neutral outcome (zero (0)). The primary goal of the task is to learn, by trial-and-error, the relationship between a symbol and an outcome (shown in Figure 1). The task was designed to assess: 1) the ability to learn (mean RT, mean accuracy, peak, and rate of learning); 2) stability of performance; 3) the ability to adapt to changes in reward contingencies (flexibility); 4) the relative impact of reinforcement value, i.e., sensitivity to the outcome: reward versus loss outcomes (valence) and large versus small outcomes (magnitude). For a detailed description see Section 5.3.3 below.

5.3.2 Procedure

The participant's aim was to collect as many points as possible. The participant was informed that the more points collected the more money would be paid out. To ensure that participants could easily distinguish the symbols and to determine the individual symbol presentation duration, the task started with a symbol discrimination phase in which participants were asked to indicate, as fast and accurate as possible, whether two symbols were a copy of each other (by pressing 's' on the keyboard) or different from each other (by pressing 'd' on the keyboard). Fifty percent of the individual's average response time was taken as symbol presentation duration during the experimental phase, with a minimum of 250 ms and a maximum of 1000ms. As expected, response times during the symbol discrimination phase were significantly slower for older (M = 808 ms, SE = 20 ms) relative to young individuals (M = 630

ms, SE = 22 ms) ($F(1, 80) = 36.33$, $p < .001$, $\eta_p^2 = .31$). Correspondingly, symbol presentation during phase 1, 2, and 3 was, on average, 178 ms longer for old relative to young adults.

Each trial began with a 750 ms fixation cross that was immediately followed by a 250-1000 ms pair of symbols (depending on the response times during the symbol discrimination phase). The participant chose the symbol yielding the better outcome by pressing the left and right key on the keyboard to indicate left or right, respectively. The relationship between the symbol and the outcome is learned by trial-and-error. Upon response, visual feedback for both symbols was presented for 500 ms, then the visual feedback corresponding to the selected character was presented at its location for a further 500 ms. Immediately following the visual feedback, the next trial began with a 750 ms fixation cross (see Figure 1 for two trial examples). Two practice blocks with different stimuli were completed before the experimental blocks started. In practice block 1, symbol presentation duration was set to 1000 ms, followed by another block with each individual's symbol presentation duration (50% of RT during the symbol discrimination phase).

The reinforcement learning task comprised three phases. In Phase 1 (Acquisition), participants completed three blocks of 40 trials each; Phase 2 (Maintenance) consisting of two blocks identical to phase 1; and Phase 3 (Adaptation to Contingency Change) began with 20 trials using the same symbol-outcome assignments as were used in Phase 1 and 2 (these extra trials were discarded), and then without interruption, another 40 trials were presented using new symbol-outcome pairings. Another two complete blocks (40 trials each) using the new rules were then completed. Each block was separated by a self-paced break. At the start of the task, participants were warned of a potential change in feedback contingency and reminded that the task was to maximize winning and minimize losing. Rule changes were confined to the large values and zero (i.e., Large Win, Large Loss and Zero), restricting potential rule changes to two possibilities that were counterbalanced across participants.

The procedure of the reinforcement learning task reported here differed in two ways from the reinforcement learning task presented in Chapter 4 (experimental study of acute mild low-grade inflammation). First, Phase 2 (Maintenance) comprised two instead of three blocks

and, second, symbol presentation duration was individually adjusted (with a minimum of 250 ms and a maximum of 1000 ms) instead of a fixed symbol presentation duration of 250 ms.

5.3.3 Assessment of motivated learning

The reinforcement-learning task was designed to distinguish between several aspects of reinforcement learning.

(1) Learning ability. Mean RT and mean accuracy scores were calculated for each block. The peak learning performance was the highest achieved accuracy score during one of the first 5 blocks of 40 trials each. The rate of learning was determined by calculating the number of blocks needed to reach 80% accuracy of an individual's highest achieved accuracy score across block 1 to 6 (i.e., peak learning performance).

(2) Stable performance. Stable performance was calculated as the number of blocks in which performance was above 80% of the peak learning performance after this was reached for the first time. The rate of learning was taken into account to control for possible differences in rate of learning: (rate of learning (in blocks to criterion) + stable performance blocks) / total blocks (5) x 100%). A higher value is indicative of stable performance.

(3) Flexibility. For flexibility, accuracy scores before (block 5) and after (block 6) the unannounced change in symbol-outcome mapping were compared. Only outcome pairs that changed reward contingency (+5, -5, 0)) were included. Negative values indicate inflexibility.

(4) Sensitivity to outcomes. Valence learning (reward vs. loss) was assessed via comparing accuracy scores for reward (+5 vs. 0, +1 vs. 0) and loss (-5 vs. 0, -1 vs. 0) outcome pairs. Magnitude learning (large vs. small) was calculated by comparing accuracy scores for large (+5 vs. 0, -5 vs. 0) and small (+1 vs. 0, -1 vs. 0) outcome pairs. The analyses for sensitivity to the outcomes were performed on the acquisition and maintenance phase.

5.3.4 Stimuli

Five different black Japanese hiragana symbols were presented in Arial Unicode MS Hiragana font (each subtending 1.4 x° X 1.8 y° of visual angle) on a grey field. During the

learning task, centre-to-centre distance along the horizontal meridian between symbols was 4 x° with a black 0.5 x° fixation cross at the midpoint. Two symbols were paired to winning points (+1, +5), two symbols to losing points (-1, -5) and one symbol led to 0 points.

Table 1. All ten possible outcome pairs.

Outcome pairs	
Large Win / Large Loss	5 / -5
Large Win / Zero	5 / 0
Large Win / Small Win	5 / 1
Large Win / Small Loss	5 / -1
Large Loss / Zero	-5 / 0
Large Loss / Small Win	-5 / 1
Large Loss / Small Loss	-5 / -1
Small Win / Small Loss	1 / -1
Small Win / Zero	1 / 0
Small Loss / Zero	-1 / 0

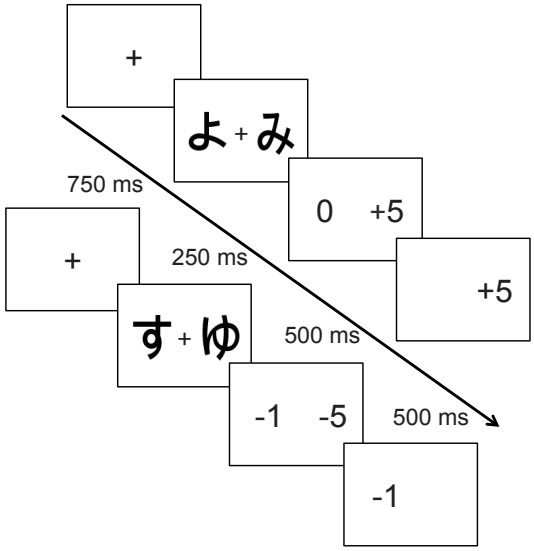


Figure 1. Successive screens of two typical trials. Stimulus presentation duration individually adjusted (between 250 and 1000ms)

5.3.5 Reading the Mind in the Eyes test (RMET)

The RMET is considered an advanced test of theory of mind involving mental state attribution and complex emotion recognition from photographs of the eye region of the face (Baron-Cohen, Wheelwright, Hill, et al., 2001; Oakley et al., 2016).

5.3.6 Procedure

The test display comprised a test eye image and four words placed in the centre of the screen. The participant was instructed to select the word that best described what the person in the test image was thinking or feeling by pressing one of four computer keys (Q, P, A, L) that

spatially corresponded to the position of each word. The correct (target) word had the same emotional valence as the accompanying three foil words. For example, the target word, 'panicked', was accompanied by 'arrogant', 'jealous' and 'hateful'. Target words were equally likely to appear in one of the four word locations on the screen. Each test display remained visible until a key response was made; the text test display was immediately presented thereafter. The test consisted of 36 different images, completed as one set. In line with previous studies, a glossary containing a definition of each word was available to the participant.

5.3.7 Stimuli

A grey-scale digital image (subtending 9 x° X 3.6 y° of visual angle) of the eye region of a face (including eyes and eyebrows) was presented in the middle of a grey field on a computer monitor. Four words describing mental states accompanied each test stimulus, presented in black Arial font (subtending 2.6 x° X 0.7 x° of visual angle).

5.3.8 Plasma IL-6

Blood (6 mL) was collected from an antecubital vein in the forearm into one vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500 x g for 10 min at 4 °C and plasma was aliquoted and stored at -80 °C for later assessment of interleukin-6 (IL-6), a marker of system low-grade inflammation. Plasma level of IL-6 was measured in duplicate using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The limits of detection of these assay was 0.11 pg/mL, with intra- and interassay coefficients of variation (CVs) of 0.69-11.6%.

5.3.9 Anthropomorphic measures

Participants were asked to remove footwear and coats and empty their pockets before a body composition measurement was taken using a TANITA BC-545N body composition

analyser (Tanita Europe, Amsterdam, The Netherlands). A stadiometer was used to measure height.

5.3.10 Questionnaires

To adjust for potential confounding factors, participants completed questionnaires about illness symptoms (modified version of SCQ; Sangha, Stucki, Liang, Fossel, & Katz, 2003), sleep quality (PSQI; Carpenter & Andrykowski, 1998), medication intake (number of medications), and demographic variables (i.e., age, sex, occupation status, education (education level (low-, middle-, high-educated), smoking (current smoker, ex-smoker, non-smoker), alcohol intake (units)).

5.4 STATISTICAL ANALYSIS

Data were examined and influential data points (i.e., which disproportionally biased estimates) were removed for each of the tasks separately using Cook's distance and data points that exceeded 2.5 *SD* from means. Non-learners were removed and defined as participants failing to show evidence of learning, i.e., accuracy score < 60% in the acquisition and maintenance phase of the reinforcement learning task (total $N = 4$ of which 2 young low BMI, 1 old low BMI, and 1 old high BMI) and total accuracy score < 30% for the RMET (total $N = 2$ young high BMI). Data were analysed using between-subjects analysis of variance (ANOVA) and t tests where appropriate. Age group (young, older) and BMI group (low BMI, high BMI) were entered as between-subjects factors to assess possible additive and interactive effects of age and BMI on motivated learning and emotion recognition. Since previous studies reported sex differences in reinforcement learning and emotion recognition (Evans & Hampson, 2015; Saylik, Raman, & Szameitat, 2018; Wingenbach, Ashwin, & Brosnan, 2018; Zhang et al., 2014), sex was also included as a between-subjects factor. However, the study was not designed to assess sex-dependent effects. Sex had no effect if not reported. Mean accuracy and mean response times were calculated per block and phase to assess performance on the reinforcement learning task. The other outcome measures were calculated as described in section 5.3.3. For the RMET, the

percentage of total correct responses was calculated. To assess the effect of emotional valence, percentage correct was calculated for each emotional valence (positive, neutral, and negative expressions) (Maurage et al. 2011). These variables were entered into ANOVAs with age group, BMI group and sex as between-subjects factors.

For IL-6 analysis, non-parametric tests were used or log transformation was applied because of the skewed distribution of raw IL-6 values. To assess effects of inflammation independent of age, BMI, demographic and health factors, multiple linear regression analysis was conducted: 1) a crude model with IL-6, and 2) an adjusted model correcting for age, BMI, demographic (i.e., sex, education level) and health variables (i.e., illness symptoms, smoking behaviour, alcohol intake, sleep quality, medication intake) previously shown to be associated with inflammation and/or cognitive function. The results of the multiple regression models are presented as standardised coefficient estimates (β) and *p*-values. Alpha values were set at .05 throughout. For all analyses where appropriate, Levene's test of Equality of Variances and Mauchly's Test of Sphericity were used to test for assumption violations; adjustments were made as needed using the Greenhouse-Geisser correction. Bonferroni corrections were applied to post-hoc pairwise comparisons (two-tailed unless stated otherwise) to control for type I error rate. The PROCESS macro (Hayes, 2013) was used to test possible mediating effects of IL-6 (Model 4 with 5000 bootstrap samples).

In addition to traditional null hypothesis significance testing, Bayes Factors were calculated for non-significant effects via Bayesian ANOVA using default prior probabilities in JASP version 0.9. Non-significant effects can be the result of absence of differences or because of a lack of statistical power to detect differences. Bayesian analyses can be used to distinguish between these two options (Dienes, 2014). Bayes factors provide relative evidence of both null (H_0) and alternative hypotheses (H_A), compared to the conclusions about the null hypothesis proffered by traditional null hypothesis significance testing. All statistical analyses were conducted using SPSS v.24.0 (IBM-SPSS Inc., Chicago, IL, USA) and JASP (Version 0.9; JASP Team 2019).

5.5 RESULTS

5.5.1 BMI, age and inflammation

Table 2 shows the summary statistics of included participants. As anticipated, ANOVA yielded significantly higher log IL-6 levels in the high versus low BMI group ($F(1, 83) = 34.64$, $p < .001$, $\eta_p^2 = .29$), as well as higher log IL-6 levels in the old versus young group although non-significant ($F(1, 83) = 2.47$, $p = .120$, $\eta_p^2 = .03$; $BF_{10} > 1.00$). There was no evidence for an age x BMI group interaction ($F(1, 83) = 0.01$, $p = .826$, $\eta_p^2 = .00$; $BF_{10} = 0.33$) (Also see Chapter 6 in this thesis).

Table 2. Descriptive statistics of included participants given as means ± SD unless otherwise stated. Significant main effects ($p < .05$) for age group and BMI group are indicated by ● and ◆, respectively. No significant age x BMI group interactions were evident.

	Young Low BMI	Young High BMI	Old Low BMI	Old High BMI
N	20	20	19	25
Age (years)				
Mean● ◆	25	27	72	70
Range	21 – 32	21 – 35	66 – 79	63 – 76
Sex (% Female)	45%	39%	40%	40%
IL-6 (pg/ml) ●	1.04 ± 0.44	2.11 ± .1.26	1.31 ± 0.68	2.29 ± 1.05
Range	0.3 – 2.1	0.9 – 5.6	0.4 – 3.2	0.8 – 6
Weight Status				
Current BMI (kg/m2)	21.7 ± 2.5	33.6 ± 3.7	22.8 ± 1.7	32.6 ± 3.9
BMI when young (21-35) (kg/m2)			21.8 ± 1.6	25.9 ± 4.5
Body fat %				
Females ●	27.9 ± 3.9	45.3 ± 4.8	33.6 ± 5.8	44.9 ± 4.8
Males ●	15.7 ± 4.4	28.9 ± 3.8	22.4 ± 5.0	31.6 ± 5.1

5.5.2 Reinforcement learning task

5.5.2.1 Ability to learn: mean RT, mean accuracy, peak, and rate of learning

Mean RT. As shown in Figure 2a, RT changed across blocks ($F(7, 560) = 10.87$, $p < .001$, $\eta_p^2 = .12$; $BF_{10} > 100$). RT decreased from 958 ms (*SE* = 40 ms) in block 1 to the 929 ms (*SE* = 31 ms) in block 3, stabilised in block 4 (*M* = 887 ms, *SE* = 28 ms) and block 5 (*M* = 878 ms, *SE* = 25 ms) and increased to 1020 ms (*SE* = 31 ms) after the contingency change in block 6 and decreased again to 930 ms (*SE* = 26 ms) in block 8. Participants in the older group were on average 127 ms (*SE* = 60 ms) slower as compared to the young group ($F(1, 80) = 5.03$ $p = .028$, $\eta_p^2 = .06$; $BF_{10} = 2.13$). No significant effect of BMI group ($F < 1$; $BF_{10} = 0.41$) or a significant age x BMI group interaction ($F < 1$; $BF_{10} = 0.57$) was evident. Including accuracy as a covariate did not alter these results.

Mean accuracy. Figure 2b presents, accuracy learning curves. Analysis of proportion correct scores verified that performance changed across blocks ($F(7, 560) = 67.54$, $p < .001$, $\eta_p^2 = .46$; $BF_{10} > 100$), which showed the expected pattern: In the acquisition phase, performance rose from 66.6% (SE = 1.3%) in block 1 to 82.2% (SE = 1.1%) in block 3, reflecting learning, and levelled off during the maintenance phase (blocks 4 and 5) (*M* = 84.7%, SE = 1.0%). After the change in symbol-outcome pairing in block 6, accuracy dropped to 67.7% (SE = 1.5%) in block 6 (after the change in symbol-outcome pairing) and recovered to 85.0% (SE = 1.3%) in block 8 (adaptation to change phase). A main effect of age group showed lower accuracy scores across all outcome pairs for older (*M* = 74.1%, SE = 1.1%) relative to young adults (*M* = 83.7%, SE = 1.2%) ($F(1, 80) = 17.91$, $p < .001$, $\eta_p^2 = .18$; $BF_{10} > 100$). No effect of BMI group ($F < 1$; $BF_{10} = 0.24$) or interactions between age and BMI group were evident ($F's < 1$; $BF_{10} = 0.27$).

Peak and rate of learning. As can also be seen in Figure 2b, peak learning performance (based on the block with the highest score) was lower in the older group (*M* = 84.9%, SE = 1.2%) compared to the young group (*M* = 92.5%, SE = 1.3%) ($F(1, 82) = 18.45$, $p < .001$, $\eta_p^2 = .18$; $BF_{10} > 100$). Moreover, older (*M* = 1.9, SE = 0.1) compared to young adults (*M* = 1.6, SE = 0.1) needed significantly more blocks to reach their individually set learning criterion (i.e., 80% accuracy of personal peak learning score) ($F(1, 80) = 6.81$, $p = .011$, $\eta_p^2 = .09$; $BF_{10} = 2.52$), suggesting a slower

rate of acquisition (see Figure 3a). No effects of BMI group (peak learning: F = 2.53; BF_{10} = 0.82; rate of learning: F = 2.37; BF_{10} = 0.52) or interactions between age and BMI group were evident (peak learning: F < 1; BF_{10} = 0.31; rate of learning: F = 2.37; BF_{10} = 0.73).

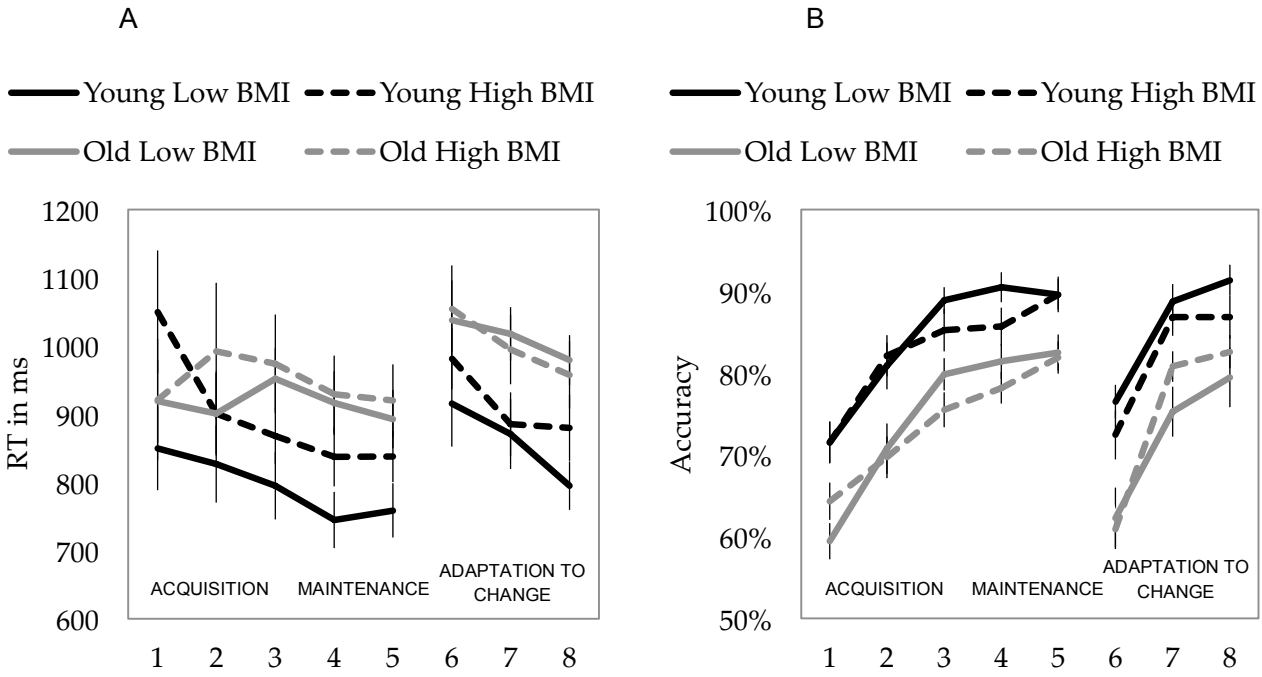


Figure 2. RT (A) and accuracy (B) learning curves for the acquisition (block 1-3), maintenance (block 5-6), and adaptation to change (block 7-9) phase for each age and BMI group; lines indicate means, error bars represent standard error of the mean. Note: accuracy scores for the adaptation to change phase also include outcome pairs that did not change (i.e., outcome pairs with small win and small loss).

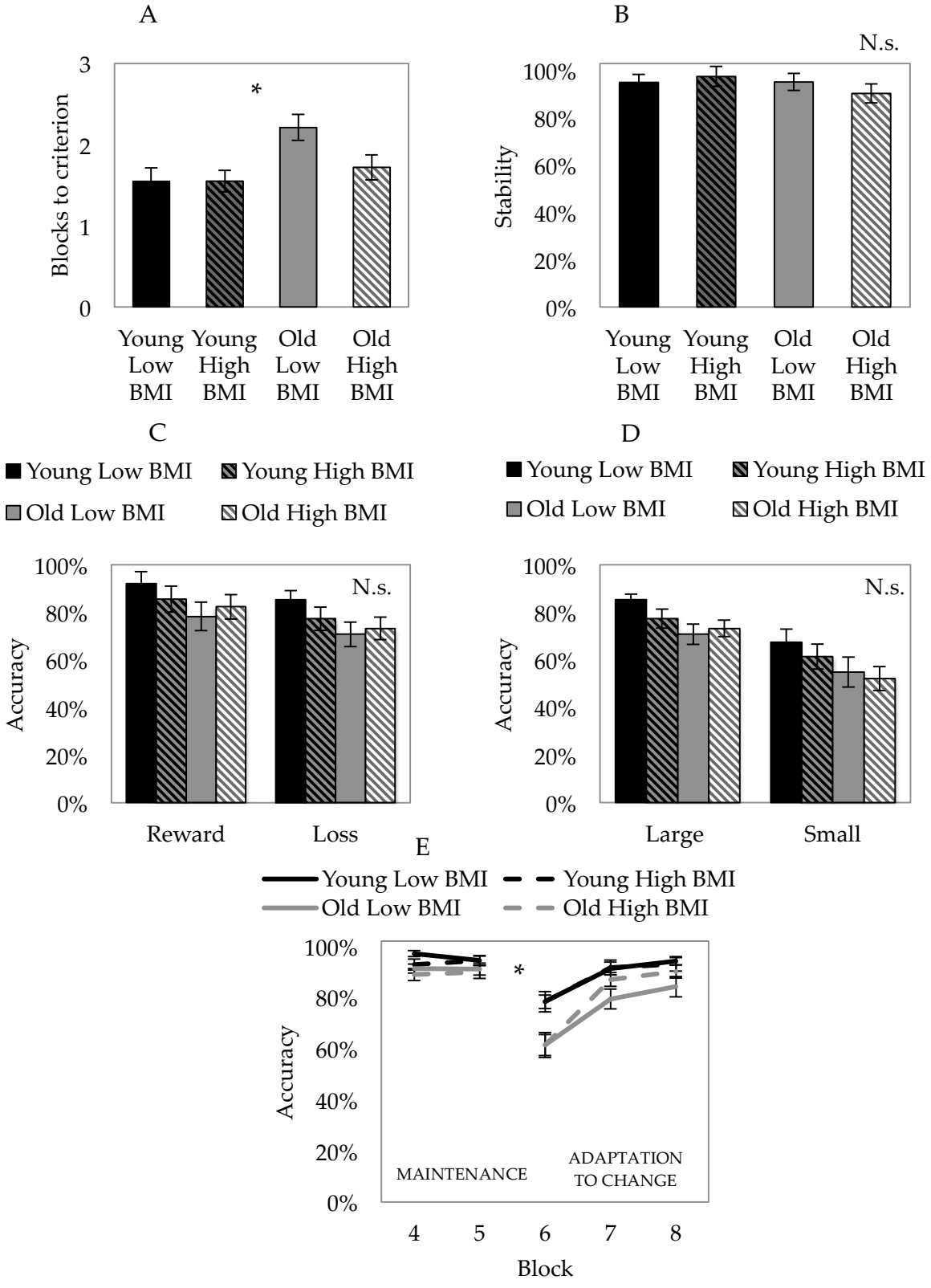
5.5.2.2 Stable performance and flexibility

As can been seen from Figure 3b, stability of response behaviour was not different between the age (F < 1; BF_{10} = 0.34) and BMI groups (F < 1; BF_{10} = 0.24) and no age x BMI group interaction was evident (F < 1; BF_{10} = 0.43). Next, analysis tested flexible adaptation to unannounced changes in the reward environment. As shown in Figure 3e, an age group x block (5: before change, 6: after change) interaction indicated that the drop in performance after the

contingency change was significantly larger in the older (M = -20.7%, SE = 2.8%) relative to the young (M = -10.6%, SE = 2.8%) age group ($F(1, 80) = 9.71$, $p = .003$, $\eta_p^2 = .05$; $BF_{10} = 10.57$), providing evidence for reduced flexibility with older age. Age group did not interact with outcome pair (+5 vs. 0, -5 vs. 0, +5 vs. -5) ($F < 1$; $BF_{10} = 0.24$). No significant effect of BMI group ($F < 1$; $BF_{10} = 0.23$) or a significant age x BMI group interaction was found for flexibility ($F < 1$; $BF_{10} = 0.32$).

5.5.2.3 Sensitivity to valance and magnitude

To test if learning was differentially impacted by valence (i.e., gain or loss) or magnitude of the outcome, a RM ANOVA with valence (reward vs. zero, loss vs. zero), magnitude (large vs. zero, small vs. zero), phase (acquisition, maintenance, adaptation to change) and age and BMI group was performed. Across groups, accuracy was 5.4% (SE = 2.3%) better for rewards as compared to loss outcome pairs ($F(1, 80) = 5.50$, $p = .021$, $\eta_p^2 = .06$; $BF_{10} = 1.12$) and 19.7% (SE = 1.2%) better for large as compared to small outcome pairs ($F(1, 80) = 250$, $p < .001$, $\eta_p^2 = .76$; $BF_{10} > 100$). Age or BMI group did not interact with magnitude or valence, and no three-way interactions were evident (F's < 2.87; $BF_{10} < 1$). Results are presented in Figure 3c and 3d.


A
Blocks to criterion
3
2
1
0
*
Young Low BMI
Young High BMI
Old Low BMI
Old High BMI
B
N.s.
Stability
100%
80%
60%
40%
20%
0%
Young Low BMI
Young High BMI
Old Low BMI
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Young High BMI
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Old High BMI
Accuracy
100%
80%
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40%
20%
0%
*
MAINTENANCE
ADAPTATION TO CHANGE
4
5
6
7
8
Block

*Figure 3. Rate of learning, i.e., number of blocks needed to reach 80% of the individual's highest achieved score (A); Stable performance (higher percentage indicates stable performance), i.e., number of blocks in which performance was maintained (i.e., above 80% of the individual's highest achieved score), adjusted for rate of learning (B); Sensitivity to valence (reward, loss) and magnitude (large, small), i.e., accuracy for reward and loss (C) and for large and small (D) outcome pairs; Flexibility, i.e., drop in accuracy before and after the change in symbol-outcome pairing. Only the outcome pairs that changed were included: +5 vs. 0, -5 vs. 0, and +5 vs. -5 (E); Error bars indicate standard error of the mean; * indicates age group effect p < .05; N.s. = No significant effect of age or BMI group.*

5.5.3 Motivated learning and inflammation

Associations with inflammation were tested in two ways. First, multiple regression analysis was conducted to assess effects of IL-6, as a marker of systemic inflammation, independent of age, BMI, further adjusting for demographic (sex, education level) and health-related variables (comorbid medical symptoms, smoking, alcohol intake, sleep quality, and medication intake). Second, mediation analyses were performed to test if inflammation accounted for any of the BMI and age-related effects.

Significant negative correlations were found between log IL-6 levels and accuracy scores during the acquisition ($r(80) = -.233$, $p = .037$; $BF_{10} = 1.18$), maintenance phase ($r(80) = -.314$ $p = .005$; $BF_{10} = 7.16$), and peak learning performance ($r(80) = -.296$, $p = .008$; $BF_{10} = 4.60$). Mean RT ($BF_{10} = 0.16$), stability ($BF_{10} = 0.14$), and flexibility ($BF_{10} = 0.16$) were not significantly correlated with log IL-6. As shown in Table 3, the effect sizes of log IL-6 showed modest attenuation after full adjustment for age, BMI and demographic and health variables but remained (marginally) significant for most outcome variables. Rate of acquisition was marginally correlated with log IL-6 after adjustment. Overall, higher log IL-6 levels, older age, and lower education predicted lower performance. For each significant effect, PROCESS software (Hayes, 2013) was used to test mediation effects of inflammation (Model 4 with 5000 bootstrap samples). Inflammation had no mediating role in any of the significant age effects (results shown in Supplementary Materials 5.7.1.1).

*Table 3. Multiple linear regression analysis of the relationship of inflammation (IL-6) with motivated learning behaviours adjusted for influences of age, BMI, health- and demographic variables (adjusted model). Age group: 1 = young, 2 = older; BMI group: 1 = low BMI, 2 = high BMI; Sex: 1 = Female; 2 = Male; Education: higher score is higher educated; Smoke: 0 = never, 1 = ex-smoker, 2 = smoker; Alcohol intake in units; Illness symptoms = number of symptoms; Sleep quality: higher score is lower quality of sleep; Medication intake = number of medications; *** p < .001, ** p < .01, * p < .05, # p < 0.07.*

	Mean RT	Acquisition phase	Maintenance phase	Peak learning	Rate of learning	Stability	Flexibility
Model 1	R^2 = .00	R^2 = .05*	R^2 = .10*	R^2 = .09*	R^2 = .02	R^2 = .00	R^2 = .00
Adjusted model	R^2 = .21	R^2 = .32**	R^2 = .34***	R^2 = .35***	R^2 = .35*	R^2 = .10	R^2 = .17
	β	β	β	β	β	β	β
Model 1							
IL-6	.065	-.233*	-.314*	-.296**	.125	-.005	.064
Adjusted model							
IL-6	-.037	-.191	-.299*	-.270#	.261#	.039	.129
Age group	.445**	-.356**	-.305*	-.270*	.213	-.200	-.397**
BMI group	.183	.080	.061	-.002	-.243	-.092	-.067
Sex	-.081	-.043	-.179	-.090	.121	.061	.069
Education	.190	.249*	.263*	.283*	-.031	.122	-.197
Smoking	-.030	.033	-.102	-.089	-.088	-.075	-.138
Alcohol	-.198	-.137	-.026	-.019	.168	.041	.087
Illness symptoms	.003	.092	.081	.141	.041	.376#	-.085
Sleep quality	.125	.020	.103	.076	-.009	.077	.027
Medication intake	-.226	-.080	-.025	-.207	-.212	-.188	-.107

5.5.4 Reading the Mind in the Eyes Test (RMET)

As can be seen in Figure 4a, an age x BMI group interaction ($F(1, 83) = 10.34$, $p = .002$, $\eta_p^2 = .11$; $BF_{10} = 20.32$) showed that young individuals with a high BMI performed significantly worse as compared to their low BMI counterparts (M difference = 10.0%, SE = 3.9%) ($t(38) = 2.54$, $p = .015$, $d = .80$; $BF_{10} = 3.56$). In the older group, a main effect of BMI group ($F(1, 43) = 4.46$, $p = .040$, $\eta_p^2 = .09$; $BF_{10} = 1.34$) indicated that individuals with a high BMI (M = 73.0%, SE = 2.1%) outperformed individuals with a low BMI (M = 66.3%, SE = 2.7%). No main effect of Age group ($F = 0.36$; $BF_{10} = 0.28$) or BMI group ($F = 0.40$; $BF_{10} = 0.24$) were evident.

In light of prior research showing sex effects on emotion recognition, exploratory analyses were performed, confirming a significant effect of sex (males: M = 64.8%, SE = 2.0%; females: M = 71.2%, SE = 1.6%), and therefore the next analyses will incorporate this factor ($F(1, 79) = 6.27$, $p = .014$, $\eta_p^2 = .06$; $BF_{10} = 1.28$). A BMI group x sex interaction ($F(1, 79) = 4.02$, $p = .048$, $\eta_p^2 = .04$; $BF_{10} = 0.44$) indicated that the reduced emotion recognition in young high BMI individuals was driven by lower performance of males (M = 53.2%, SE = 5.6%) (Females: M = 68.4%, SE = 4.6%) ($F(1, 36) = 5.14$, $p = .029$, $\eta_p^2 = .13$; $BF_{10} = 2.07$). Including sex as a covariate yielded similar results (M difference = 10.6%, SE = 3.7) (BMI effect on emotion recognition in young individuals corrected for sex: $F(1, 37) = 7.49$, $p = .010$, $\eta_p^2 = .17$). Sex had no significant effect in the older group ($F(1, 43) = 3.12$, $p = .085$, $\eta_p^2 = .07$; $BF_{10} = 0.81$) and also no interaction of sex x BMI group was evident ($F < 1$; $BF_{10} = 0.39$). Including sex as a covariate did not alter these results (i.e., BMI effect in the older group remained significant: $F(1, 44) = 4.30$, $p = .044$, $\eta_p^2 = .09$; $BF_{10} = 1.34$).

5.5.4.1 Associations with valence type

Exploratory analyses tested if stimuli selected on valence (negative, neutral, positive) showed that performance was best on positive expressions (M = 78.3%, SE = 1.9%), followed by neutral (M = 69.0%, SE = 1.5%) and negative expressions (M = 63.1%, SE = 1.9%), with significant differences between all three valence types ($F(2, 166) = 25$, $p < .001$, $\eta_p^2 = .23$; $BF_{10} > 100$). As

shown in Figure 4b, the data provided no evidence for a selective impact of age or BMI group on the different valence types (age: $F < 1$; $BF_{10} = 0.20$, BMI: $F < 1$; $BF_{10} = 0.22$).

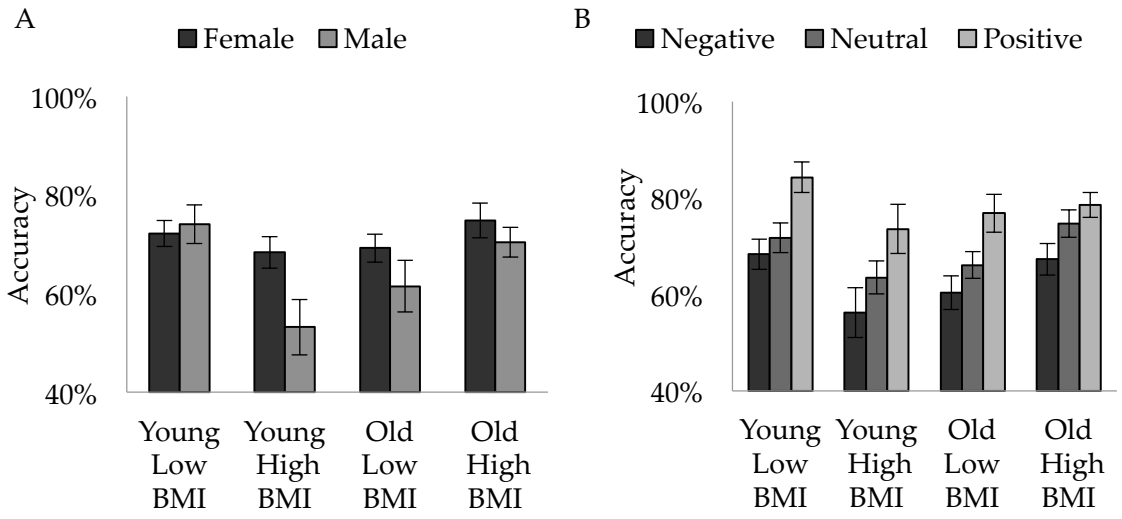

Figure 4. Mean accuracy scores for RMET total score for each age and BMI group separated by sex (A), and for each emotional valence separated by age and BMI group (B); Error bars indicated standard error of the mean. Please note that the y-axes start at 40% accuracy.

5.5.5 Emotion recognition and inflammation

Performance on the RMET was significantly correlated with log IL-6 ($r(82) = -.239$, $p = .030$; $BF_{10} = 1.40$). Accuracy on positive stimuli was also negatively correlated with log IL-6 ($r(82) = -.298$, $p = .006$; $BF_{10} = 5.37$). Multiple regression analysis showed that IL-6 remained a significant predictor of overall accuracy and accuracy scores for positive expressions when adjusting for age and BMI group and demographic and health variables (adjusted model) (see Table 4). Inflammation had no mediating role in any of the significant effects (results shown in Supplementary Materials 5.7.1.2).

*Table 4. Multiple regression analysis of the relationship of inflammation (IL-6) with emotion recognition adjusted for influences of age, BMI, demographic and health variables (adjusted model). Age group: 1 = young, 2 = older; BMI group: 1 = low BMI, 2 = high BMI; Sex: 1 = Female; 2 = Male; Smoke: 0 = never, 1 = ex-smoker, 2 = smoker; Alcohol intake in units; Illness symptoms = number of symptoms; Sleep quality: higher score is lower quality of sleep; Medication intake = number of medications; *** p < .001, ** p < .01, * p < .05, # p < .07.*

	Emotion recognition	Positive expression
Model 1	R^2 = .06*	R^2 = .09**
Adjusted model	R^2 = .22	R^2 = .17
	β	β
Model 1		
IL-6	-.239*	-.298**
Adjusted model		
IL-6	-.338**	-.367**
Age group	-.010	.047
BMI group	.026	-.047
Sex	-.145	-.148
Education	.037	-.147
Smoking	.087	.070
Alcohol	-.089	-.084
Illness symptoms	.465*	.012
Sleep quality	.014	.031
Medication intake	-.229	.030

5.6 DISCUSSION

Previously we showed that experimentally induced low-grade inflammation reduces motivated learning (Chapter 4) and emotion recognition (Chapter 2; Balter et al., 2018). The present study aimed to extend these findings by testing if age- and BMI-related inflammation was likewise associated with motivated learning and emotion recognition. Here we examined the effects of inflammation, age and BMI on motivated learning and emotion recognition. Regarding motivated learning, age and inflammation were independently associated with reductions in aspects of motivated learning. Adjustment for age, BMI and other potential confounders (e.g., sex, education level, medication intake) only marginally attenuated the effect of IL-6 on motivated learning. Using the reading the mind in the eyes test as a test of emotion recognition, the link appeared more complicated whereby we observed age-dependent associations of BMI with emotion recognition; young individuals with a high BMI performed worse as compared to their low BMI counterparts, whereas in older adults individuals with a high BMI outperformed those with a low BMI. Replicating findings of our vaccination model (Balter et al., 2018), elevated IL-6 was again independently associated with reduced emotion recognition and withstood full adjustment for demographic and health variables.

Notably, the present pattern of results showed a striking parallel with the results found with experimentally induced inflammation (Balter et al., 2018; also see Chapter 2 and 4 in this thesis): both acute inflammation and older age was associated with a slower pace of acquisition, reduced flexibility and intact stability. Based on these findings one may tentatively suggest that mild inflammation is transiently associated with ageing-like effects on motivated learning behaviours.

There are mechanistic parallels between ageing and low-grade inflammation that may help explain the overlapping effects of inflammation and ageing on motivated learning behaviours. Others, and we, have found that inflammation partially mediates age-related decline in short-term memory and processing and psychomotor speed (Lin et al., 2018; Balter et al., unpublished see Chapter 6 in this thesis). The current correlation analyses could not confirm that chronic low-grade inflammation (i.e., IL-6 levels) mediated the link between age and

impairments in motivated learning. A putative common pathway by which both age and acute low-grade inflammation might exert changes in motivated learning is through modulation of dopamine pathways (Felger & Treadway, 2016). The nigrostriatal dopamine pathway is pivotal in motivated behaviours, and it has been demonstrated that inflammation modulates this system (Brydon et al., 2008; Eisenberger et al., 2010; Harrison, Voon, et al., 2015). The dopamine system has shown to be particularly vulnerable to ageing too (Volkow et al., 2000) and lower levels of striatal dopamine transporter and reduced striatal activity in older adults are associated with impaired reward learning (Dreher, Meyer-Lindenberg, Kohn, & Berman, 2008; Marschner et al., 2005).

In the current study, no evidence was found in support of the notion that high BMI negatively affects monetary reinforcement-based learning. Moreover, Bayesian analysis did not (or only weakly) provide support for absence of BMI effects on motivated learning. This observation regretfully adds to the inconsistent findings concerning the relationship between BMI and motivated learning; several previous studies similarly reported no effect of BMI on monetary reinforcement-based learning (Balodis et al., 2013; Kube et al., 2016), however, also BMI-related reductions in reinforcement-based associative learning have been reported (Coppin et al., 2014; Kube et al., 2017). Zhang et al (2014) suggest a food-specific associative learning impairment in female individuals with obesity. The complexity of the relationship between BMI and motivated learning may possibly in part be attributed to the heterogeneity of the obese phenotype. For example, Balodis et al (2013) showed large heterogeneity among obese individuals with respect to the neural correlates of reward processing. Factors related to high BMI, but that were not assessed in the current study, may potentially explain individual variation in motivated learning, such as leptin levels (Jastreboff et al., 2014) and functioning of the dopaminergic system (Wang, Volkow, Thanos, & Fowler, 2009), but could also involve number of years having overweight and previous weight-loss attempts (Teixeira et al., 2004).

To the best of our knowledge, this is the first study to assess the effect of BMI and age on emotion recognition. Results showed that high BMI was negatively associated with emotion recognition in young individuals while older adults with a high BMI outperformed those with a

low BMI. A growing body of evidence suggest that some cognitive effects of high BMI may be less detrimental in older age (Noh et al., 2017; Sabia, Kivimaki, Shipley, Marmot, & Singh-Manoux, 2009; Smith, Hay, Campbell, & Trollor, 2011; Yoon et al., 2012). However, these studies mostly used coarse measures of cognition such as the Mini Mental State Examination Test. The current results suggest that an age-dependent effect may apply also to emotion recognition.

Currently it is still unknown why older individuals may be (selectively) spared for BMI-related cognitive deficits as the bulk of available data is mostly pointing towards negative effects of high BMI. As such, in the obesity paradox debate, age-related effects of BMI have been attributed by some to artefacts or methodological limitations, and those may possibly also apply be the current study (Monda et al., 2017; Robinson, Furberg, & Banack, 2014). For example, caution is needed because BMI may not represent a reliable index of adiposity in elderly. The ageing process is associated with a decrease in lean body mass (e.g., muscle mass), while adipose tissue increases without weight gain (Jackson, Janssen, Sui, Church, & Blair, 2012). BMI may thus underestimate adiposity in older adults and alternative tools that have been proposed as better adiposity markers in old age include waist circumference and waist-to-hip ratio (Bischof & Park, 2015; Hartanto & Yong, 2018; Lindqvist et al., 2012). However, arguing against this explanation, separating BMI groups on age- and gender-specific body fat percentages (see Pasco et al., 2014) did not alter the direction of the BMI group results of the current study (effect of body fat percentage on emotion recognition in young individuals: M = -11.2%, SE = 2.7% and old individuals: M = +1.6%, SE = 2.7%). A second potential explanation for absence of BMI effects in older age is selection bias. I.e., one may speculate that the old high BMI group may represent a healthier sample of high BMI adults as heavier individuals may experience more overweight-related diseases that prevented them from taking part in this research including because of mortality (i.e., those available for research are the relatively healthy survivors) (Banack & Kaufman, 2013).

A number of meta-analyses and literature reviews have shown that females generally outperform males in facial emotion recognition (Kret & De Gelder, 2012; Thompson & Voyer, 2014). Hence post-hoc analyses were performed to test for such sex differences, and were

confirmed. Because the current study was not designed to assess sex effects or interactions between sex, age and BMI, these exploratory results will only be briefly discussed. BMI-related sex-differences were apparent in young adults but not in older adults. Factors that influence sex differences in obesity and ageing, such as insulin resistance, could be considered in future research. Further research with better-powered studies specifically designed to address such issues seems warranted.

To further expand on the relevance of the current results, motivated learning and emotion recognition are potential trans-diagnostic endophenotypes that cut across traditional mental health diagnostic categories. There is substantial interest in endophenotypes for major depressive disorder (MDD); i.e., processes and measures that index susceptibility for the disorder (Goldstein & Klein, 2014). Social-cognitive and motivational disruptions are a characteristic of MDD, and, in parallel, MDD has been linked to elevated inflammation, which is consistent with the proposal that inflammation may present a common biological pathway (Dalili, Penton-Voak, Harmer, & Munafò, 2015; Dowlati et al., 2010; Treadway et al., 2012). Research on the role of inflammation in MDD has been stymied by between-subject variability, i.e., only distinct subtypes of MDD appear to show increased inflammation (Miller, Haroon, & Felger, 2017). Motivated learning and emotion recognition could be potentially interesting cognitive endophenotypes that may be more specifically tied to depression phenotypes linked with inflammation. To verify this idea, testing large clinical populations is needed.

The current results need to be seen in the light of several limitations. First, this study was conducted with a moderate sample size and null-findings, including the lack of mediation, should thus be interpreted with some caution. Second, assessment of IL-6 merely acted as an inflammation check and no causal assumptions about the role of IL-6 can be made. Future research may also consider a larger panel of inflammatory mediators. Third, only one test for emotion recognition was used, which does not fully capture the breadth of this concept. Moreover, while it was shown that accuracy on the reading the mind in the eyes was reduced with inflammation and higher BMI, this test did not provide any clues on underlying

mechanisms. For example, eye-tracking methodology could be used to assess whether inflammation changes visual scanning behaviour.

In sum, in line with earlier experimental research, the current research supports the notion that low-grade inflammation and old age adversely influenced aspects of motivated learning. Emotion recognition was likewise affected by low-grade inflammation, and by BMI in an age-dependent manner. Inflammation may be an independent biobehavioural pathway linked to motivation and social cognition.

5.7 SUPPLEMENTARY MATERIALS

5.7.1 Mediation analysis results

A mediating variable is a variable that is part of the pathway by which an independent variable affects a dependent variable. The main requirement for mediation is that the *indirect effect* of the independent variable (e.g., Age group) through the mediator (e.g., IL-6) on the dependent variable (e.g., accuracy) is significant. Mediation indirect effects can be interpreted as the strength of the relationship between the independent variable (Age group) and dependent variable (accuracy) when accounting for the mediating pathway (IL-6) (Hayes, 2009). Each outcome measure that was significantly affected by Age or BMI group was used as a dependent variable in separate mediation analyses. Variables were Z-transformed before analysis yielding standardised regression coefficients.

5.7.1.1 Reinforcement Learning Task

Performance acquisition phase. Older age (controlling for BMI group) was significantly associated with lower performance in the acquisition phase (β = -.493, SE = .097, 95% CI = -.686, -.310, p < .001) but not with IL-6 (β = .155, SE = .091, 95% CI = -.031, .332, p = .103). IL-6 was not significantly associated with performance when adjusting for age group and BMI group (β = -.117, SE = .118, 95% CI = -.351, .117, p = .323) while age group remained a significant predictor of performance when adjusting for IL-6 and BMI group (β = -.476, SE = .098, 95% CI = -.672, -.280, p < .001). The indirect effect of IL-6 on the association between age group and performance was not significant (β = -.018, 95% CI = -.083 – .017), suggesting that IL-6 did not mediate the relationship between age group and performance in the acquisition phase.

Performance maintenance phase. Older age (controlling for BMI group) was significantly associated with lower performance in the maintenance phase (β = -.404, SE = .101, 95% CI = -.605, -.204, p < .001) but not with IL-6 (β = .155, SE = .091, 95% CI = -.031, .332, p = .103). IL-6 was not significantly associated with performance when adjusting for age group and BMI group (β = -.212, SE = .121, 95% CI = -.453, .029, p = .084) while age group remained a significant predictor of

performance when adjusting for IL-6 and BMI group (β = -.372, SE = .101, 95% CI = -.574, -.171, p < .001). The indirect effect of IL-6 on the association between age group and performance was not significant (β = -.032, 95% CI = -.107, .007), suggesting that IL-6 did not mediate the relationship between age group and performance in the maintenance phase.

Peak learning performance. Older age (controlling for BMI group) was significantly associated with a lower peak learning performance (β = -.416, SE = .099, 95% CI = -.613, -.218, p < .001) but not with IL-6 (β = .044, SE = .027, 95% CI = -.009, .098, p = .103). IL-6 was not significantly associated with performance when adjusting for age group and BMI group (β = -.194, SE = .119, 95% CI = -.432, .043, p = .108) while age group remained a significant predictor of performance when adjusting for IL-6 and BMI group (β = -.386, SE = .100, 95% CI = -.585, -.188, p < .001). The indirect effect of IL-6 on the association between age group and performance was not significant (β = -.029, 95% CI = -.102, .006), suggesting that IL-6 did not mediate the relationship between age group and peak learning performance.

Rate of learning. Older age (controlling for BMI group) was significantly associated with a slower rate of learning (β = .264, SE = .106, 95% CI = .053, .476, p = .015) but not with IL-6 (β = .044, SE = .027, 95% CI = -.009, .098, p = .103). IL-6 was not significantly associated with rate of learning when adjusting for age group and BMI group (β = .107, SE = .129, 95% CI = -.151, .364, p = .411) while age group remained a significant predictor of rate fo acquisition when adjusting for IL-6 and BMI group (β = .248, SE = .108, 95% CI = .033, .463, p = .024). The indirect effect of IL-6 on the association between age group and performance was not significant (β = .016, 95% CI = -.033, .082), suggesting that IL-6 did mediate the relationship between age group and rate of learning.

Flexibility. Older age (controlling for BMI group) was significantly associated with reduced flexibility (β = -.330, SE = .105, 95% CI = -.539, -.120, p = .002) but not with IL-6 (β = .132, SE = .128, 95% CI = -.123, .386, p = .306). IL-6 was not significantly associated with flexibility when adjusting for age group and BMI group (β = .132, SE = .128, 95% CI = -.123, .386, p = .306) while age group remained a significant predictor of flexibility when adjusting for IL-6 and BMI group (β = -.350, SE = .107, 95% CI = -.562, -.137, p = .002). The indirect effect of IL-6 on the

association between age group and flexibility was not significant (β = .020, 95% CI = -.015, .079), suggesting that IL-6 did not mediate the relationship between age group and flexibility.

5.7.1.2 Reading the Mind in the Eyes Test

Mean accuracy. Higher BMI in the young group (controlling for sex) was associated with lower performance (β = -.016, SE = .007, 95% CI = -.031, -.001, p = .035) and with higher IL-6 (β = .621, SE = .118, 95% CI = .381, .862, p < .001). IL-6 was not significantly associated with performance when adjusting for BMI group and sex (β = -.007, SE = .012, 95% CI = -.029, .015, p = .504) and BMI group was not a significant predictor of performance anymore when adjusting for IL-6 and sex (β = -.012, SE = .010, 95% CI = -.032, .009, p = .253). The indirect effect of IL-6 on the association between BMI group and performance was not significant (β = -.005, 95% CI = -.020, .011), suggesting that IL-6 did not have a mediating role.

CHAPTER 6

Inflammation Mediates Body Weight and Ageing Effects on Psychomotor Slowing

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Based on: "Balter, L. J. T., Higgs, S., Aldred, S., Bosch, J. A., & Raymond, J. E., (under review). Inflammation Mediates Body Weight and Ageing Effects on Psychomotor Slowing"

ABSTRACT

Inflammation (immune system activation) affects neuronal function and may have consequences for the efficiency and speed of functional brain processes. Indeed, unusually slow psychomotor speed, a measure predictive of behavioural performance and health outcomes, is found with obesity and ageing, two conditions also associated with chronic inflammation. Yet whether inflammation is the mediating factor remains unclear. Here, we assessed inflammation by indexing interleukin-6 level in blood and measured psychomotor speed as well as indices of selective visual attention in young (mean = 26 years) or old (mean = 71 years) adults (N = 83) who were either lean or currently significantly overweight (mean body mass index = 22.4 and 33.8, respectively). Inflammation was positively and significantly correlated with psychomotor speed, age, and body mass index but not with attention measures. Using mediation analyses we show for the first time that inflammation fully accounts for the significant psychomotor slowing found in those with high BMI. Moreover, we further show that age-related psychomotor slowing is partially mediated by inflammation. These findings support the proposal that reducing inflammation may mitigate weight- and age-related cognitive decline and thereby improve performance on daily tasks and health outcomes more generally.

6.1 INTRODUCTION

Inflammation, a biological process that results from immune system activation, is a well-established characteristic of advanced age (Capuron et al., 2014; Singh & Newman, 2011) as well as a feature of obesity (Nguyen et al., 2014). At the cellular level, inflammation is known to cause neuronal dysfunction via alterations to microglia (Cope et al., 2018; Herz, Filiano, Smith, Yogev, & Kipnis, 2017; Marin & Kipnis, 2017) but how such alterations affect overt brain function remains less well studied. Nevertheless, emerging evidence tentatively links inflammatory states with subtle cognitive deficits, including slowed psychomotor speed, i.e., the minimum time required to make accurate, well-learned motor responses to obvious sensory stimuli (Brydon et al., 2008), slowed decision processes (Lin et al., 2018), and memory deficits (Cohen et al., 2003; Marsland et al., 2015 but see Bourassa & Sbarra, 2016, also Grigoleit et al., 2010). Many of these same behavioural deficits are also found in individuals with obesity (Gunstad et al., 2006; Prickett, Brennan, & Stolwyk, 2015) as well as in those in advanced age (Salthouse, 1996). Taken together, the parallel relationships of both age and weight with cognitive function and inflammation raise the possibility that inflammation may be the underlying mechanism by which advanced age and excessive body weight lead to suboptimal cognitive capacity. Yet no study to date has examined how inflammation, age, and obesity interact to determine human cognitive performance using a participant sample that varies substantially on these dimensions. Not only does it remain unclear whether age and body weight are additive or multiplicative in their impact on performance, the hypothesis that inflammation may be the mediating mechanism remains untested. To address this shortfall, we measured psychomotor speed and selective visual attention function in four groups of adults characterised by each possible combination of young versus old age and low versus high body mass index (BMI), and assessed individuals levels of inflammatory cytokines (specifically Interleukin-6, IL-6) in blood plasma on the day of behavioural testing to index inflammation. Mediation and other statistical analyses were used to assess the contribution of inflammation to behavioural effects.

A fundamental brain function that underpins many everyday activities is the capacity to respond rapidly and appropriately to new or changing visual information in a manner consistent with ongoing and future goals (Posner & Petersen, 1990). This requires appropriate selection of relevant information from the everchanging complex visual sensory environment, as well as the capacity to quickly activate appropriate motor responses as soon as cognitive decisions, e.g., object identification, have been reached. The former set of processes are known as selective visual attention (Broadbent, 1966) and the latter as psychomotor speed (e.g., Devita et al., 2017). A typical example of the importance of these functions is fast braking in the face of a sudden, unexpected road obstacle. The obstacle must be selected from the road scene, identified as an obstacle, and then the brake pedal must be pressed. Importantly, selective attention and psychomotor speed are not only necessary for rapidly changing situations like driving, but are also essential for fluent action and decision-making in more mundane tasks such as social discourse, cooking, or using digital media. Indeed, deficits in these functions are associated with poor mental health and low emotional well-being (Lee, Hermens, Porter, & Redoblado-Hodge, 2012). Selective attention may be viewed as a set of discrete cognitive sub-functions that ultimately facilitate efficient selection of relevant information (targets) from the sensory array, with each sub-function subserved by different but overlapping brain networks (Fan, McCandliss, Fossella, Flombaum, & Posner, 2005; Fan et al., 2002; Ishigami, Fisk, Wojtowicz, & Klein, 2010; Posner & Petersen, 1990). One network function, referred to as Alerting, promotes selection by reacting to sensory cues that signal *when* a target might appear; another, Orienting, reacts to cues signalling *where* a target might appear; and a third, Executive Control, promotes high level processing of specifically task relevant information (e.g., roads signs) and suppresses processing of concurrent distracting information (e.g., billboards). Such functions can be assessed using the Attention Network Test (ANT) which requires participants to respond as quickly and accurately as possible to a series of simple computer-based trials, each comprised of a cue display that is quickly followed by a target array. See Fig. 1. The speed and accuracy of key press responses to targets are measured and compared across different cue-target conditions (Fan et al., 2002; Ishigami et al., 2010; Posner & Petersen, 1990). In addition to comprehensively

assessing attention, this test provides a robust measure of psychomotor speed (overall mean response time, RT, across all cue conditions). Although psychomotor speed in everyday scenarios depends on the nature of the information being presented and the complexity of the action decisions required (Cepeda, Blackwell, & Munakata, 2013), its measurement when based on simple manual motor responses, such as required in the ANT, is nevertheless predictive of a range of performance outcomes, e.g., driving (Niewoehner et al., 2012), health outcomes, e.g., response to depression treatment (Frank et al., 2011), and even mortality risk (Tabue-Teguo et al., 2015).

Decline in attention and slowing of psychomotor speed has long been linked to ageing (Salthouse, 2009) and has been well-studied using the ANT (Gamboz, Zamarian, & Cavallero, 2010; Ishigami et al., 2010; Jennings, Dagenbach, Engle, & Funke, 2007; Noh, Larcom, Liu, & Isaacowitz, 2012; Salthouse, 2009; Zhou, Fan, Lee, Wang, & Wang, 2011). Such deficits are thought to contribute to frailty, risk of falling, depression, and poor health (Beheydt et al., 2015; Feil, Zhu, & Sultzer, 2012; Rosano, Newman, Katz, Hirsch, & Kuller, 2008; Turcu et al., 2004). Several studies report weaker alerting benefits and/or diminished distractor suppression, an index of executive control (Gamboz et al., 2010; Jennings et al., 2007; Noh et al., 2012; Zhou et al., 2011). However, after adjusting for the effects of generalised psychomotor slowing (Salthouse, 2009), age-related attentional deficits disappear or become weaker (Gamboz et al., 2010; Jennings et al., 2007; Williams et al., 2016). Hence, ageing may have only modest effects on attention beyond generalised slowing, an issue we probe here.

The notion that age-related cognitive decline may be mediated by inflammation (Marsland, Gianaros, Abramowitch, Manuck, & Hariri, 2008; Schram et al., 2007) has received modest attention. A recent study by Lin et al. (2018) showed that showed that for lean adults, inflammation only partially mediated the association between age and complex cognitive processing speed (on a digit-symbol substitution task, DSST) and that inflammation did not significantly mediate links between age and short-term memory. Other studies show clear negative correlations between complex cognitive performance (on DSST, Stroop, and memory tasks) and inflammation in midlife (Marsland et al., 2006; Marsland et al., 2015) or in older

participants (Schram et al., 2007), but did not conduct mediation analyses nor consider the role of body weight.

Indeed, recent evidence provides strong support for the contention that age-related cognitive decline may be accelerated by excessive body weight, causing pre-senescent cognitive deficits (Dahl et al., 2010; Walther, Birdsill, Glisky, & Ryan, 2009). Indeed, a high BMI across the life span is associated with lower performance across several cognitive domains that are characteristically degraded in ageing, including verbal memory (Isaac et al., 2011; Prickett, Stolwyk, O'Brien, & Brennan, 2018), executive functioning (Higgs & Spetter, 2018; Smith et al., 2011; Walther et al., 2009) and, complex visual-motor coordination (Waldstein & Katzel, 2006). However, studies on the effects of excessive body weight on psychomotor slowing and selective visual attention are less clear. Some report weight-related deficits (Benito-León et al., 2013; Prickett et al., 2018; Tsai, Huang, & Tsai, 2017), others report no effect of BMI (Gonzales et al., 2010; Gunstad et al., 2007; Walther et al., 2009), and one found better performance with higher BMI (Gunstad et al., 2010). Complicating this picture is evidence that depression and poor cardiovascular health, conditions that are co-morbid with obesity, may themselves contribute to reduced cognitive function (Anstey, Cherbuin, Budge, & Young, 2011; Restivo et al., 2017). Indeed, Prickett et al., (2015) reviewed findings that linked obesity with cognitive deficit and concluded that evidence was equivocal as to whether cognitive problems stem from obesity or from other co-morbid health and demographic factors. Nevertheless, using a very large sample of exclusively older adults and adjusting for other co-morbid factors in a mediation analysis, Bourassa & Sbarra (2016) report support for a causal role of inflammation in weight-associated deficits on a delayed recall measure of memory. Although consistent with the hypothesis that inflammation may mediate both age and body-weight effects on cognitive performance, without inclusion of younger participants, the role of age *per se* cannot be determined from this study and conclusions are limited to memory function. As such the role of inflammation on the combined effects of age and body weight on psychomotor speed or selective visual attention remain unknown.

Support for the possibility that inflammation may be a driving mechanism of psychomotor slowing is found in studies that acutely or experimentally induced inflammation and measured cognitive function with and without inflammation. For example, the common cold, a condition that produces a potent acute inflammatory response, leads to psychomotor slowing (Bucks et al., 2008; Matthews, Warm, Dember, Mizoguchi, & Smith, 2001; Smith, 2012) and translates to slower responses in a simulated driving task (Smith, 2013; Smith & Jamson, 2012). Experimentally induced inflammation via administration of immune-activating agents, such as bacterial endotoxin or vaccination, has also been shown to cause psychomotor slowing (Brydon et al., 2008), memory deficits (Reichenberg et al., 2001) and alterations of social cognition (Balter et al., 2018; Harrison et al., 2009). However, the extant literature using experimental induction of inflammation so far provides little evidence of degraded selective attention (reviewed in Bollen et al., 2017), suggesting the possibility of domain-specificity in the inflammation-cognition link.

Taken together these findings support the notion that psychomotor slowing, shown clearly to be associated with advanced age and possibly with excessive body weight, may be mediated by chronic inflammation. The aim of the current study was to test this hypothesis by determining whether inflammation (as indexed by IL-6) is a mediator of age- and BMI-related psychomotor slowing. Additionally, we aimed to determine whether age and body weight are independent (Gunstad et al., 2006, 2007) or interactive (Gunstad et al., 2010) predictors of psychomotor speed and visual attention. We predicted that individuals with high BMI and advanced age would show higher levels of inflammation and slower psychomotor speed compared to their leaner and younger counterparts. The association between BMI, age and psychomotor speed was expected to be at least partially mediated by inflammation. Furthermore, attentional processing was not expected to be reduced by either older age or high BMI when psychomotor speed was taken into account (e.g., Jennings et al., 2007).

6.2 METHOD

6.2.1 Participants

Eighty-nine participants with a BMI (weight(kg)/height(m)^2) between 17 and 25 (low BMI) or greater than 27 (high BMI) and aged between 21 and 35 years (young) or between 63 and 80 years (old) were recruited through a database held by the University of Birmingham and via (online) advertisements. Individuals who reported a history of gastric banding, eating disorders, neurological or inflammatory disorders (e.g., rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, periodontitis) or use of anti-depressant, anti-histamine, or anti-inflammatory (e.g., antibiotics) medication during the past 7 days were excluded. Participants reported normal or corrected-to-normal vision and stable body weight for at least six months (i.e., fluctuations < 7.5 kg for individuals with high BMI, < 5 kg for low BMI individuals). All data from 6 individuals were excluded from all analyses as their overall behavioural accuracy was more than 2.5 s.d.'s below the group average (N = 2) or because their overall RT was identified by Cook's distance as being influential (N = 4). Table 1 shows descriptive statistics of the remaining participants' characteristics. Power analysis calculated using a power of 0.8 and an alpha of 0.05 suggests that a total sample size of 74 is adequate to detect medium to large effect sizes (d = 0.6) (Williams et al., 2016; Zhou et al., 2011). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the University of Birmingham Research Ethics Committee.

6.2.2 Procedures

Test sessions started between 8:30 and 15:30 hours. Start times of test sessions were matched across groups to control for minor diurnal variations in IL-6 (Nilsonne, Lekander, Åkerstedt, Axelsson, & Ingre, 2016). However, such variations were not observed in the current study (Spearman's correlation of IL-6 and time of day ($r_s(81)$ = .044, p = .696). Participants were instructed to have breakfast/lunch as usual but avoid consumption of high-fat products (e.g., bacon, fries), because these foods may induce a short-lived inflammatory response (Herieka &

Erridge, 2014), and to refrain from eating, drinking (except for water), and smoking for 1 hour before the start of the test session. Participants were also asked not to engage in strenuous physical exercise or consume alcohol within 12 hours before the test session, and reschedule their appointment if they had suspected infection symptoms on the day of testing. On arrival, written informed consent was obtained and it was verified via self-report whether all had complied with instructions. A blood sample was taken by venipuncture and questionnaires and cognitive tests including the ANT were completed (see further below). Other tests included measures of memory, reinforcement learning and emotion recognition (results not reported here). Lastly, a measure of height and body composition was taken.

6.3 MATERIALS

Questionnaires were completed on a touch screen tablet. A desktop running PsychoPy v1.83.01 (Peirce, 2007) was used to record data and present cognitive tests including the ANT.

6.3.1 Attention Network Test

6.3.1.1 Stimuli

A fixation cross (1° of visual angle in diameter) was continuously present at the screen's centre. Warning cues comprised one or two asterisks, each 0.6° in diameter, that appeared either 2.3° left and/or right of the central fixation cross. The test display comprised of a vertical row of five arrows centred horizontally within the screen and appearing either 2.3° left or right of the fixation. The entire row of arrows subtended 3.3° of visual angle; each individual arrow was identical in size (0.6° tall X 1.7° wide) with the space between each being 0.1°. Each arrow could point up or down. In all conditions, the target stimulus was the middle arrow. The two arrows appearing above and below the target (flankers; four in total) always had the same orientation but this could differ from the orientation of the target.

6.3.1.2 Procedure

Each trial began with one of four different warning cues (asterisks; (1) no cue, (2) double cue, (3) central alerting cue, (4) spatial cue), each lasting 100 ms. Regardless of warning cue condition, the cue's offset preceded the target display by a variable and randomly determined interval of 500–1000 ms. This display comprised the target and flanker arrows; it disappeared immediately upon response or within 2000 ms. The participant's task was to indicate the direction of the arrow in the centre of the target display by pressing a corresponding key on the keyboard using the index and middle finger of the dominant hand as quickly and accurately as possible. Response time (RT) was defined as the interval between the onset of the arrow display and the associated keyboard response (in ms). There were 50 trials per participant per warning cue condition; half of all trials per warning cue condition had congruent flankers and half had incongruent flankers. For the spatial cue condition, 80% of the trials were valid and 20% invalid.

Every combination of target orientation (up/down) and location (left/right) and distractor compatibility (congruent, incongruent) was equally likely for each warning cue condition. The order of all the various trial types was pseudo-randomly determined for each participant. Participants performed one practice block of 15 trials followed by one experimental block of 200 trials.

6.3.2 Blood sampling

Blood was collected into one 6ml vacutainer containing EDTA as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500 x g for 10min at 4 °C and plasma was aliquoted and stored at -80 °C for later assessment of IL-6, a marker of system low-grade inflammation. Plasma level of IL-6 was measured in duplicate using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The limit of detection of these assays was .11 pg/mL, with intra- and interassay coefficients of variation (CVs) of 0.7–11.6%. IL-6 was used as the primary index of inflammation but no causal

assumptions about the role of IL-6 in the observed effects can be made. No other inflammatory markers were assessed.

6.3.3 Questionnaires and Anthropomorphic measures

Participants completed health and demographic questionnaires for two reasons. Firstly, to describe the groups and secondly, to adjust for health and demographic factors previously shown to be associated with inflammation, i.e., illness symptoms (Self-Administered Comorbidity Questionnaire (SCQ); (Sangha, Stucki, Liang, Fossel, & Katz, 2003), depression, anxiety and stress (Depression Anxiety and Stress-21 (DASS-21); (Henry & Crawford, 2005)), loneliness (UCLA Loneliness Scale; (Russell, 1996)) and demographic variables, i.e., age, education level (low-, middle-, high-educated), perceived health status (rated from '1 very poor' to '10 excellent'), alcohol intake (units per week) and smoking status (never-, ex-, current-smoker).

6.4 STATISTICAL ANALYSIS

6.4.1 RT Data

As is conventional (e.g., Fan et al., 2002) RTs were excluded from the statistical analyses if the response was incorrect or too fast (RT <150 ms), accounting for 3.6% of the data. Also excluded were data from participants who disproportionally bias mean estimates (N = 4, 2 young high BMI, 1 old low BMI, 1 old high BMI; using Cook's distance) and those who had accuracy scores 2.5 s.d.'s lower than the mean (N = 2; 2 young high BMI), indicative of poor engagement or inadequate understanding of the task. Individual condition mean RTs and accuracy scores were then subjected to a mixed design repeated measures ANOVA using cue condition (no, double, valid, invalid, and centre cue) and target flanker (congruent and incongruent) as within-subjects factor and Age group and BMI group as between-subjects factors. Psychomotor speed (overall RT) data were then analysed using ANCOVA with age group and BMI group as between-subjects factors and mutual adjustment for age and BMI was

applied (i.e., BMI adjusted for age, and age adjusted for BMI). For these analyses and all subsequent analyses where appropriate, Levene's test of Equality of Variances and Mauchly's Test of Sphericity were used to test for assumption violations; adjustments were made as needed using the Greenhouse-Geisser correction. Spearman correlations were used to test relationships between age, BMI, IL-6 and RT. Alpha values were set at .05 throughout. All analyses were conducted using SPSS v.24.0 (IBM-SPSS Inc., Chicago, IL, USA).

To assess effects of age, BMI and inflammation on attention independent of psychomotor slowing, RT cue and target flanker conditions were *z*-score transformed by taking the individual's mean score for the condition of interest (e.g., RT double cue trials), subtracting his/her grand average (of all cue and target flanker conditions), and dividing this difference by the standard deviation associated with his/her grand average.

In addition to traditional null hypothesis significance testing, Bayes Factors were calculated for non-significant effects via Bayesian ANOVA using default prior probabilities in JASP version 0.9. Non-significant effects can be the result of absence of differences or because of a lack of statistical power to detect differences. Bayesian analyses can be used to distinguish between these two options (Dienes, 2014). Bayes factors provide relative evidence of both null (H_0) and alternative hypotheses (H_A), compared to the conclusions about the null hypothesis proffered by traditional null hypothesis significance testing. To allow for clear interpretation, the approximate classification scheme of (Wagenmakers et al., 2017) was used which states that an estimated Bayes Factor (BF_{10}; H_0/ H_A) value <1 supports evidence in favour of the H_0. For example, a BF_{10} of 0.25 indicates that the H_0 is 4 times (1:0.25) more likely than the H_A. Values close to 1 are not informative and a BF_{10} between 1 and 3 provides anecdotal evidence for the H_A. A BF_{10} between 1 and 0.33 provides anecdotal evidence for the H_0 (e.g., 1:3 probability in favour of H_0) and a BF_{10} between 0.33 and 0.10 provides moderate evidence for H_0. A $BF_{10} < 0.10$ provides strong evidence for H_0.

6.4.2 Mediation effects

PROCESS software (Hayes, 2013) was used to test mediation effects of inflammation (Model 4 with 5000 bootstrap samples). Sex, education level (low-, middle-, high-educated), perceived health status (self-report on a scale from 1-10), depression, anxiety, stress (DASS-21), illness symptoms (SCQ), smoking status (never-, ex-, current-smoker) and alcohol intake (units per week) were included as covariates and yielded virtually identical results. Variables were Z-transformed before analysis yielding standardised regression coefficients.

IL-6 Analysis

A $\log_{10}$ transformation was applied to IL-6 data due to the skewed distribution of raw values; correlational and partial correlation analyses were used to adjust for effects of other variables (for example, when assessing the relation between BMI and inflammation, we adjusted for age). Missing values (N = 5, 4 = young high BMI, 1 = old high BMI) and extreme plasma IL-6 concentrations (> 6 pg/ml) (N = 4, 3 = old low BMI, 1 = young high BMI) were replaced by group mean values. Repeating the main IL-6 analyses (Spearman correlations and ANOVAs) without replacement of IL-6 values produced similar results (see Supplementary information Table S1).

6.5 RESULTS

6.5.1 Inflammation

The study was conducted on four participant groups who differed in age (young, old) and BMI (low, high), but not in education level. The sample characteristics are presented in Table 1. To assess the effects of age and BMI on inflammation, an analysis of variance (ANOVA) was conducted on IL-6. Confirming expectations, IL-6 levels were higher in the high versus low BMI groups ($F(1, 79) = 49.25$, $p < .001$, $\eta_p^2 = .37$), and also higher in the old versus young groups ($F(1, 79) = 4.55$, $p = .036$, $\eta_p^2 = .03$). The interaction of age and BMI was non-significant ($F(1, 79) = 1.62$, $p = .207$, $\eta_p^2 = .01$). Mutual adjustment (i.e., BMI adjusted for age ($F(1, 80) = 49.00$, $p < .001$, $\eta_p^2 = .38$), and age adjusted for BMI ($F(1, 80) = 4.06$, $p = .047$, $\eta_p^2 = .05$) yielded a comparable

pattern of results. IL-6 significantly correlated with BMI ($r_S(81) = .655$, $p < .001$) and with age ($r_S(81) = .320$, $p = .003$). See Fig. 2a.

*Table 1. Descriptive statistics of participant characteristics. Numbers in parenthesis indicate s.d.; Statistical significance of main effects are indicated as follows; * p < .05, ** p < .01, *** p < .001. All interactions involving Age or BMI group were non-significant.*

Characteristic	Young Low BMI	Young High BMI	Old Low BMI	Old High BMI	Main effects Age	Main effects BMI
N	20	18	20	25		
Age (years)						
Mean	24.9	27.3	72.2	69.5	Age***	
Range	21 – 32	21 – 35	66 – 79	63 – 76		
Sex (% Female)	45	39	40	40		
IL-6 (pg/ml)	1.04 (0.44)	2.13 (0.67)	1.44 (0.61)	2.32 (1.01)	Age*	BMI***
Range	0.3 – 2.1	1.1 – 6.0	0.8 – 3.2	1.1 – 6.0		
Weight Status						
BMI (kg/m2)	21.7 (2.5)	33.2 (3.7)	23.1 (1.8)	32.5 (3.9)		BMI***
BMI when young (21- 35) (kg/m2)			21.8 (1.6)	25.9 (4.5)		
Body fat %						
Females	27.9 (3.9)	45.3 (4.8)	33.6 (5.8)	44.9 (4.8)		BMI***
Males	15.7 (4.4)	28.9 (3.8)	22.4 (5.0)	31.6 (5.1)		BMI***
Time of day tested (start time)	11h34 (1h45)	11h16 (1h27)	11h31 (1h51)	11h22 (1h15)		
Education level %						
Higher	100	72	75	72		
Middle	0	17	25	28		
Lower	0	11	0	0		
Self-reported health (scale 1-10)	8.4 (0.9)	7.6 (1.4)	8.7 (0.9)	8.0 (1.2)		BMI***
Illness symptoms (SCQ)	0.7 (1.0)	1.3 (2.0)	2.7 (2.0)	4.9 (3.2)	Age***	BMI**
History of illness symptoms (SCQ)			2 (1.7)	2.6 (3.2)		
Sum of medication	0.1 (0.3)	0 (0)	0.9 (1.4)	1.7 (1.6)	Age**	
Range	0-1	0-1	0-4	0-5		

Table 1. Continued

Characteristic	Young Low BMI	Young High BMI	Old Low BMI	Old High BMI	Main effects Age	Main effects BMI
Occupation status %						
Employed	10	22	10	0		
Student	90	50	0	0	Age***	BMI*
Retired	0	0	85	100	Age***	
Voluntary work	0 (0)	(0)	10 (2)	20 (5)	Age*	
Unemployed	0	28	5	0		
Smoking %						
Current smoker	0	6	0	0		
Ex-smoker	5	18	25	44	Age*	
Never smoker	95	76	75	56		BMI**
Alcohol intake in units	1.6 (3.3)	1.7 (3.3)	6.3 (4.5)	7.7 (10.2)	Age***	
Emotional health						
Depression	4.7 (6.1)	7.4 (7.5)	1.1 (2.0)	2.8 (3.7)	Age***	
Anxiety	4.7 (5.0)	6.0 (5.8)	0.6 (1.3)	1.5 (2.0)	Age***	
Stress	10.3 (7.5)	10.4 (7.0)	4.8 (4.5)	5 (4.8)	Age***	
Loneliness	37.2 (7.9)	37.4 (7.5)	35.3 (6.0)	36.5 (6.5)		

6.5.2 Behavioural measures

The ANT task was used to measure psychomotor speed (overall RT computed using all task conditions) and visual attention. Participants were shown one of four warning cues followed by a target arrow flanked by four similar arrows pointing in the same (congruent) or opposite (incongruent) direction as the target arrow. As illustrated in Fig. 1, the participant's task was to report the direction of the central target as quickly and accurately as possible. The four different warning cue conditions were: (1) no cue, (2) double alerting cue (one cue appearing simultaneously at each possible target location), (3) central alerting cue (appearing in place of the fixation cross), and (4) spatial cue (a single cue appearing at one of the two possible

target locations). The spatial cue predicted the target location correctly on 80% of trials (valid trials) and incorrectly on 20% of trials (invalid trials). Differences in RT for the different cue and target-flanker conditions were used to index the efficiency of separate components of attention in each individual. As is conventional (Ishigami et al., 2010), the alerting network was assessed by comparing performance on double-cue versus no-cue trials; the orienting network was assessed by comparing performance on valid spatial cue trials versus double-cue trials; validity effects were indexed by comparing performance on valid versus invalid spatial cue trials; and lastly, the executive control network was assessed by differences in performance for congruent versus incongruent flanker trials, regardless of cue condition.

ANOVA of individual condition mean RTs obtained in the ANT task revealed main effects of Age group ($F(1, 79) = 68.36$, $p < .001$, $\eta_p^2 = .46$), BMI group ($F(1, 79) = 6.62$, $p = .012$, $\eta_p^2 = .08$), Target Flanker condition ($F(1, 79) = 89.42$, $p < .001$, $\eta_p^2 = .53$), and Cue condition ($F(4, 316) = 20.08$, $p < .001$, $\eta_p^2 = .20$). As expected the interaction of Cue x Target Flanker condition was also significant ($F(4, 316) = 2.69$, $p = .031$, $\eta_p^2 = .03$), but neither this effect nor any other effect of target flanker or cue condition interacted with Age or BMI group (all F's < 1.2). Critically, Age and BMI group did not significantly interact ($F(1, 79) = .68$, $p = .412$, $\eta_p^2 = .01$). The group mean RTs and accuracy for each target flanker and warning cue condition for each age and BMI group are shown in Tables 2 and 3.

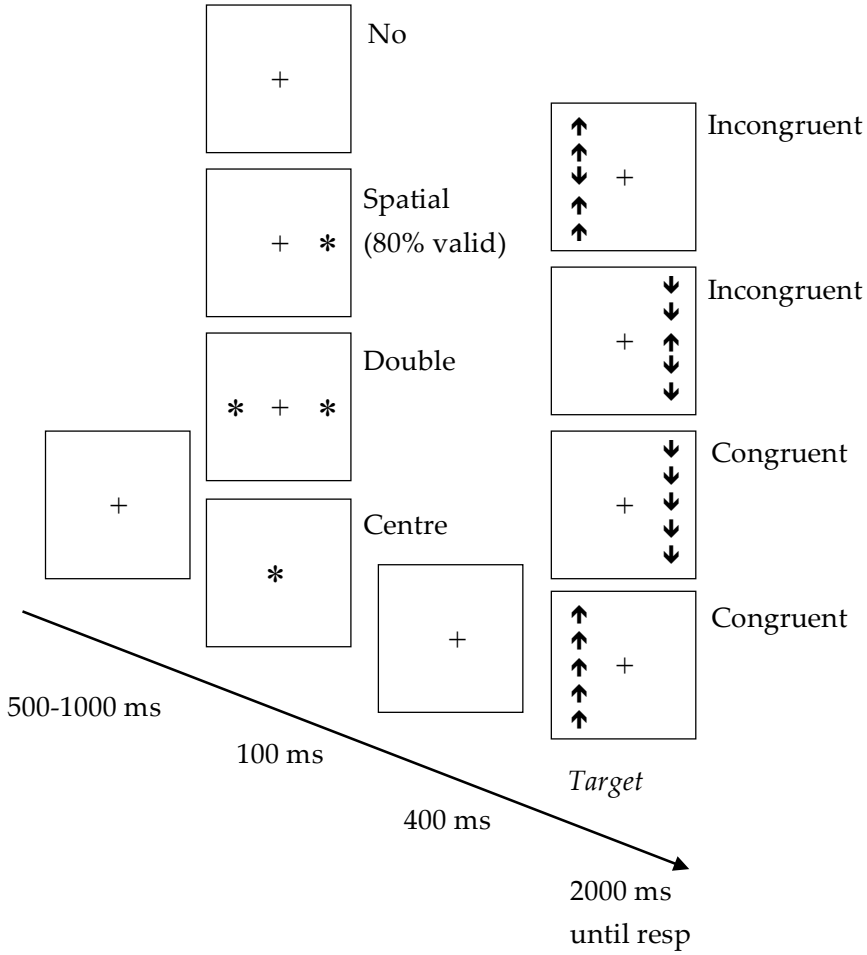

Figure 1. The sequence of displays presented in each trial of the ANT task. The cue display had four equally likely conditions: no cue, spatial cue (80% valid), double cue, and centre cue. The target array comprised a central arrow and four identical flankers that were either congruent or incongruent with the target arrow. The task was to report the direction (up, down) of the centre target arrow as quickly and accurately as possible.

Psychomotor Speed (overall RT).

Main effects of Age and BMI describe group effects on psychomotor slowing (overall RT), a point of primary interest for the current study, and therefore merit further examination. Contrasting with some previous studies (Walther et al., 2009), we found that individuals with high BMI were 53 ms (SE = 20 ms) slower than lean individuals (adjusted for age: $F(1, 79) = 6.90$,

p = .010, η_p^2 = .08) and that BMI was positively correlated with overall RT (r_S(81) = .334, p = .002). See Fig 2b. Confirming previous reports (Salthouse, 2009), psychomotor speed for older adults was on average 169 ms (SE = 20 ms) slower than that for young adults (adjusted for weight: F(1, 79) = 70.98, p < .001, η_p^2 = .47) and age and overall RT were highly correlated (r_S(81) = .659, p < .001) (see Fig. 2). See Table 2 for group mean RTs for each target flanker and warning cue condition. Adjustments for relevant demographic and health variables listed in Table 1 did not alter any of these results related to psychomotor speed or attention (all F's < 1).

As shown in Table 3, accuracy across all groups was high (M = 97.7%, SE = 0.3%), although accuracy was 1.8 percentage points better for old versus young participants (F(1, 78) = 20.52, p = .002, η_p^2 = .12), suggesting that speed accuracy trade-offs may have contributed to age-related slowing. Accuracy did not differ between the two BMI groups (F < 1). As shown in Fig. 3a,b,c,d, psychomotor speed was significantly correlated with inflammation for the low (r_S(40) = .304, p = .047) and high BMI (r_S(43) = .422, p = .007) groups; a similar effect was also found for the young group (r_S(38) = .612, p < .001) but was absent in the old group (r_S(45) = .179, p = .238). This supports the idea that performance in ageing is affected by multiple factors. To investigate the hypothesis that inflammation mediated the relationship between overall RT and either BMI or age, mediation analyses were performed on overall RT scores. As shown in Fig. 3e, when IL-6 was entered as a mediating variable, BMI group was no longer a significant predictor of RT (β = .00, 95% CI = -.26 – .25) indicating that inflammation level fully mediated the relationship between BMI and RT (β = .26, 95% CI = .09 – .46). As a further test, individuals were divided into two groups based solely on inflammation level (using a median split), and the group's overall RTs were compared. The high inflammation group was on average 90 ms (SE = 2.7 ms) slower compared to the low inflammation group (t(81) = -3.40, p = .001, independent 2-tailed).

The mediation analysis also showed a significant indirect effect of inflammation on the association between age and overall RT (β = .06, 95% CI = .01 – .15). However, age remained a significant predictor of RT after accounting for the mediating effect of IL-6 (β = .62, 95% CI = .46 – .77), indicating that IL-6 only partially mediated the effect of age on RT (see Fig. 3f). Adjusting for sex, education level, time of day, perceived health status, depression, anxiety, stress, illness

symptoms, smoking status and alcohol intake and repeating the above mediation analyses using hierarchical regression analysis did not significantly alter results (see Supplementary Materials Table S1 and S2).

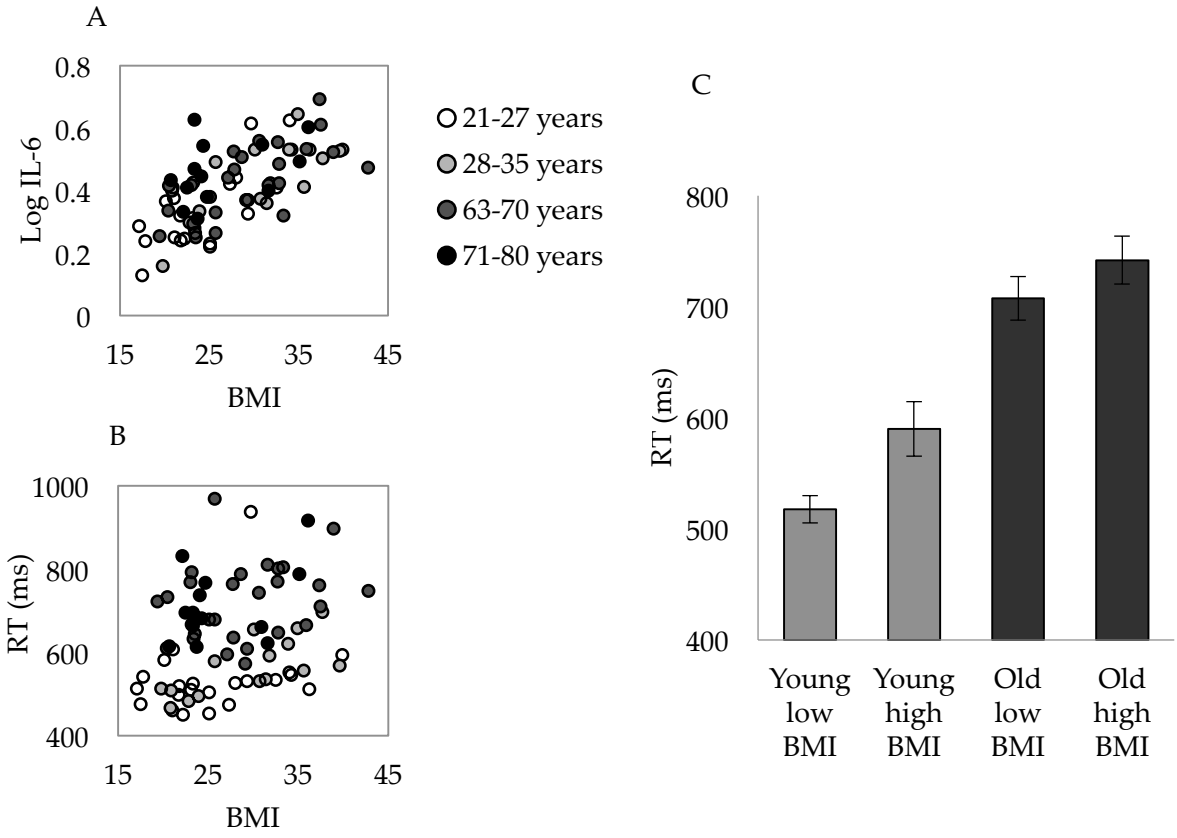

Figure 2. (A) Individual Log IL-6 level or (B) psychomotor speed plotted as a function of BMI. Each dot represents a single participant and dot grey scale reflects the participant's age bracket. Log IL-6 significantly correlated with BMI ($r_s(81) = .655$, $p < .001$) and Age ($r_s(81) = .320$, $p = .003$). Overall RT significantly correlated with BMI ($r_s(81) = .334$, $p = .002$) and Age ($r_s(81) = .659$, $p < .001$) (C). Mean psychomotor speed for each group. Error bars represent standard error of the mean (SE.).

Table 2. Mean RTs (SE) as a function of target flanker condition and warning cue condition for each Age and BMI group.

	Target Flanker condition		Warning Cue condition					
	Congruent	Incongruent	No	Centre	Double	Valid	Invalid	Mean-RT
Young	490	550	524	519	523	502	552	518
Low BMI	(11)	(14)	(12)	(11)	(14)	(12)	(17)	(12)
Young	556	625	601	591	602	571	606	590
High BMI	(18)	(31)	(22)	(24)	(29)	(26)	(19)	(25)
Old	678	740	708	705	723	693	734	708
Low BMI	(15)	(29)	(17)	(25)	(20)	(19)	(23)	(20)
Old	707	778	749	739	753	722	787	742
High BMI	(21)	(23)	(22)	(21)	(22)	(21)	(33)	(21)

Table 3. Mean accuracy scores in % (SE) as a function of target flanker condition and warning cue condition for each Age and BMI group.

	Target Flanker condition		Warning Cue condition					
	Congruent	Incongruent	No	Centre	Double	Valid	Invalid	Mean-%
Young	98.1	93.8	95.9	95.5	95.6	96.7	94.7	95.9
Low BMI	(0.6)	(1.5)	(1.4)	(1.1)	(1.1)	(0.9)	(2.1)	(1.0)
Young	98.6	94.8	96.9	96.3	95.8	97.4	96.5	96.7
High BMI	(0.4)	(1.3)	(1.0)	(1.1)	(1.1)	(0.6)	(1.4)	(0.8)
Old	99.0	97.7	98.1	98.0	98.5	98.6	98.1	98.3
Low BMI	(0.3)	(0.8)	(0.9)	(0.5)	(0.6)	(0.4)	(0.7)	(0.4)
Old	99.0	98.1	98.1	99.0	98.6	98.9	97.5	98.6
High BMI	(0.4)	(0.6)	(0.7)	(0.4)	(0.5)	(0.5)	(1.0)	(0.4)

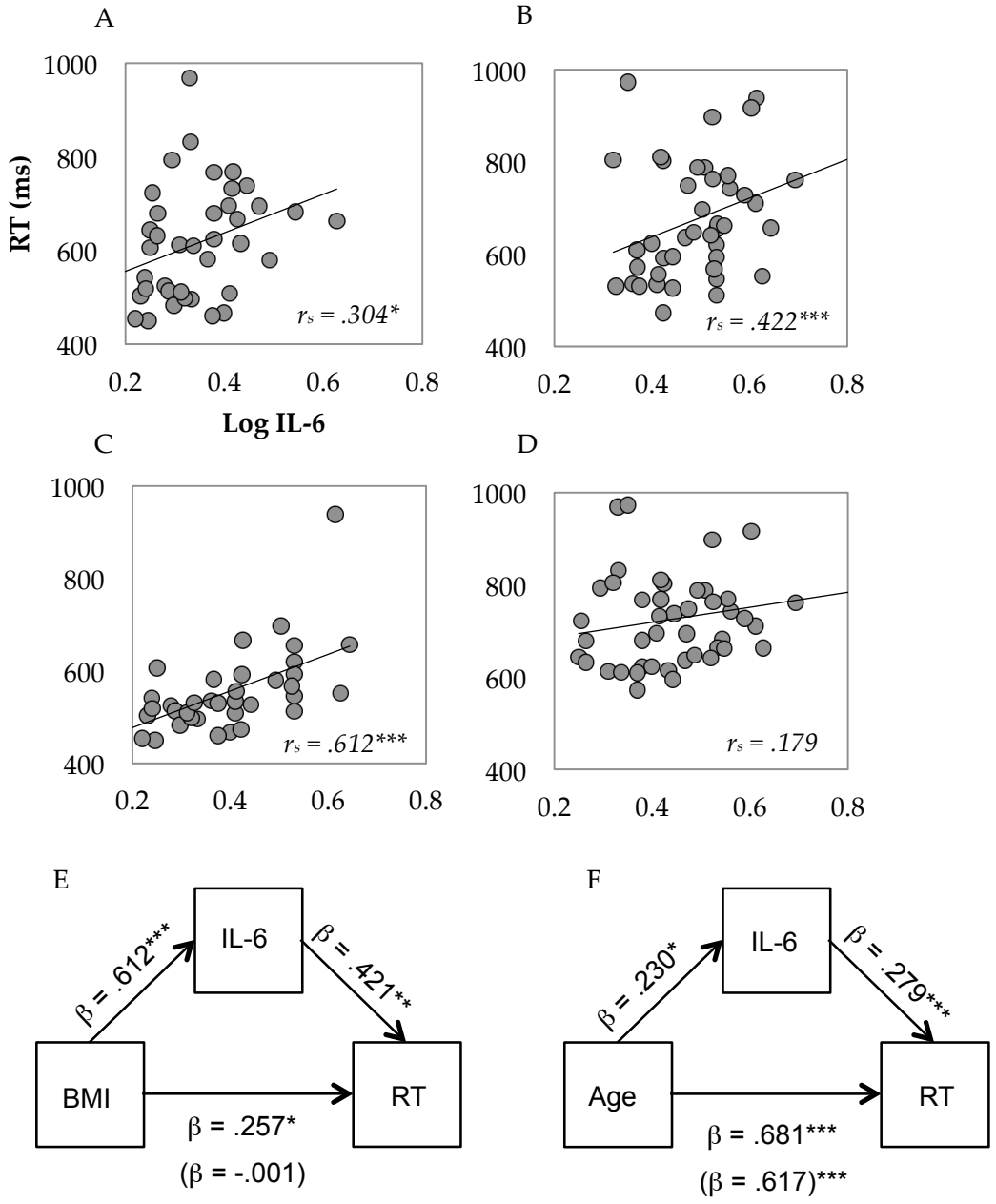

Figure 3. Spearman's correlation between IL-6 levels and psychomotor speed (RT) in the low BMI (A), high BMI (B), young (C), and old (D) groups. Standardised regression coefficients of the mediation analyses for the BMI and age groups are shown in (E) and (F), respectively. Age and BMI group significantly predicted RT and IL-6 level. IL-6 level still significantly predicted RT once the effect of BMI/Age on RT was taken into account. However, the effect between BMI and RT became non-significant after accounting for differences in IL-6, suggesting that IL-6 fully mediates the effect between BMI and RT. The effect between Age and RT became weaker but remained significant after taking into account IL-

*6, suggesting that IL-6 (only) partly mediates the effect of Age on RT. Indirect effects after taking mediation into account are presented in parentheses. * p < .05, ** p < .01, *** p < .001.*

Visual attention

Results of the main ANOVA showed only main effects of Age and BMI group on RT. Although neither Age nor BMI group significantly interacted with cue or target flanker condition, we redid the analysis using Z-transformed RT data (Jennings et al., 2007) to assess age, BMI and inflammation effects on visual attention, independently of psychomotor slowing (RT) (see the Statistical methods).

The group mean Z-scores for each network comparison are shown in Table 4. Analyses revealed that alerting (double) cues enhanced performance relative to the no cue condition more in young (M = 0.14, SE = 0.17) than in old (M = -0.40, SE = 0.15) adults ($F(1, 79) = 5.73$, $p = .019$, $\eta_p^2 = .07$). Young adults were more distracted by incongruent trials (M = 2.30, SE = 0.15) compared to old adults (M = 1.80, SE = 0.14; $F(1, 79) = 6.44$, $p = .013$, $\eta_p^2 = .08$). No significant age effects were observed for orienting or validity measures (F's < 1). Effects of BMI on all attention measure were non-significant (F's < 1, except for Conflicting, $F = 1.53$, $p = .220$). Furthermore, no correlation between any network measure and IL-6 reached significance (p's > 0.5). Hence, no further analyses were performed to test possible mediating effects of IL-6.

Bayesian correlation analyses showed moderate evidence for H_0 for a correlation between IL-6 and Z-score alerting (BF_{10} = 0.16), orienting (BF_{10} = 0.17), executive control (BF_{10} = 0.14) and validity effects (BF_{10} = 0.14). Furthermore, Bayesian ANOVAs showed moderate evidence for the null hypothesis (H_0) against the alternative hypothesis (H_A) for Z-scores alerting (BMI BF_{10} = 0.32), orienting (BMI BF_{10} = 0.25; ageing BF_{10} = 0.29), and validity (BMI BF_{10} = 0.24) and anecdotal evidence for the H_0 for executive control in BMI (BF_{10} = 0.47) and validity effect in ageing (BF_{10} = 0.36). The non-significant interactions between injection condition and alerting (no cue, double cue) and injection condition and orienting (double cue, valid spatial cue) were supported by Bayesian ANOVA showing moderate evidence for the H_0 (Alerting BF_{10} = 0.17;

Orienting BF_{10} = 0.17). Anecdotal evidence was found for an age effect on executive control (BF_{10} = 2.42).

Table 4. Mean Z-score transformed RT attention network scores (SE) shown for each Age and BMI group.

	Alerting	Orienting	Conflicting	Validity
Young Low BMI	0.09 (.24)	0.76 (.21)	2.31 (0.21)	1.63 (0.28)
Young High BMI	0.24 (.24)	1.01 (.22)	2.30 (0.21)	1.39 (0.28)
Old Low BMI	-0.50 (.23)	1.17(.21)	1.54 (0.20)	1.27 (0.27)
Old High BMI	-0.30 (.21)	0.93 (.19)	2.10 (0.18)	1.35 (0.24)

6.6 DISCUSSION

The current study examined the relative contributions of inflammation, body weight and ageing to psychomotor speed and visual attentional processing. As inflammation has been implicated as a putative mechanism for both ageing and BMI associated cognitive decline, the current analyses examined whether these associations, if present, were mediated by inflammation. Although our results confirm earlier findings showing that age and BMI are each associated with psychomotor slowing and inflammation, we show here, for the first time, that inflammation fully mediates BMI-related psychomotor slowing (regardless of age) and that inflammation only partially mediates age-related psychomotor slowing (regardless of body weight). These results partly explain why studies show conflicting results between BMI and psychomotor speed (e.g., Prickett et al., 2018; Walther et al., 2009), since it is not BMI but rather the level of inflammation that is predictive of psychomotor speed. Our results further show that inflammation partially accounts for age-related psychomotor slowing. With regard to visual attention processes, our results show reduced performance benefits of alerting cues ("alerting") in older adults (after adjusting for psychomotor speed), yet intact, efficient use of spatial information ("orienting") (e.g., Fernandez-Duque & Black, 2006; Gamboz et al., 2010; Jennings et

al., 2007; Mahoney, Verghese, Goldin, Lipton, & Holtzer, 2010). No relationships were observed between visual attention measures and BMI or inflammation. Together, these results strongly support the contention that inflammation is a significant biological predictor of psychomotor slowing, especially in individuals with high BMI.

A putative pathway by which inflammation might exert cognitive effects is through modulating dopamine pathways (Felger & Treadway, 2016). The nigrostriatal dopamine pathway is pivotal in the facilitation of movement, signalling stimulus salience, and motivated behaviours, and it has been demonstrated that inflammation modulates this system (Brydon et al., 2008; Felger & Treadway, 2016). Consistent with this speculation, lower levels of striatal dopamine transporter are associated with psychomotor slowing in healthy older adults (van Dyck et al., 2008) and altered function of the dopamine system in individuals with obesity has also been reported (Hanisch & Kettenmann, 2007). Dopamine function may possibly be altered, directly or indirectly, via microglia. Activated microglia, the brain's most prominent immune cells, have the capacity to significantly interfere with synaptic turnover, architecture and function (Hanisch & Kettenmann, 2007). The substania nigra has a very high density of microglia (McCarthy, 2017; Mittelbronn, Dietz, Schluesener, & Meyermann, 2001; Wang, Oyarzabal, Wilson, Qian, & Hong, 2015) and activating nigral microglia, for example via local injection of lipopolysaccharides, results in permanent and selective depletion of nigral dopamine (Flores-Martinez et al., 2018). Further indirect support for a role of activated microglia in inflammation-associated psychomotor slowing in humans is the finding that induced acute inflammation in humans, via injection of typhoid vaccination, altered the functional integrity of the microglial-rich substania nigra and was accompanied by psychomotor slowing (Brydon et al., 2008). Furthermore, in ageing and obesity as well as in certain neurodegenerative conditions, e.g., Alzheimer's and Parkinson's disease, microglia shift from a state of relative quiescence to one of activation (Miller & Streit, 2007; Mosher & Wyss-Coray, 2014). This results in an enhanced inflammatory response that in turn promotes neural damage and leads to cognitive degradation (Cope et al., 2018; Perry, Cunningham, & Holmes, 2007). It is tempting to speculate that disturbance of these functions within brain regions that are involved in psychomotor speed may

represent an etiological factor in the onset or progression of psychomotor slowing in inflammatory states. However, considering that age-related psychomotor slowing is not fully explained by elevated inflammation, other mechanisms that are not directly related to inflammation, such as synaptic alterations, white matter lesions, oxidative damage, hormonal changes, and neurochemical changes (e.g., Hedden & Gabrieli, 2004b; Morrison & Baxter, 2012b) may contribute to psychomotor slowing in advanced age.

The current findings are in support of the proposal that reducing inflammation may be useful for mitigating age-related cognitive impairment (Sartori, Vance, Slater, & Crowe, 2012), and, as suggested by the current data, this benefit may be particularly relevant to individuals with a high BMI. Since psychomotor demands underlie the performance of many routine activities, such as using motorised vehicles, counting out change, and social discourse, means to improve psychomotor speed or delay its age-related degradation are critically. Factors that may partially ameliorate inflammation include modifiable health behaviours such as body weight reduction and improvement of lifestyle (e.g., better diet, more physical activity) (Forsythe, Wallace, & Livingstone, 2008; Nicklas et al., 2004). Lifestyle adjustments that decrease inflammation may improve cognitive and emotional processes that in turn enhance the ability to engage in and adhere to such lifestyle changes. Not only might this further support a positive cycle of improved health behaviours, such lifestyle adjustments are also relevant to healthy ageing, by reducing risk of diabetes, cardiovascular diseases, and depression (Frasca, Blomberg, & Paganelli, 2017; Hawkley & Cacioppo, 2010).

Despite the fact that being overweight or obese is recognised as a major risk factor in the development of health issues and cognitive decline, there is an ongoing debate about possible protective effects of being overweight or obese in old age, referred to as the "obesity paradox". The obesity paradox has mostly been described in terms of advantageous effects of being overweight or obese for chronic diseases and health outcomes such as coronary heart disease, stroke, high blood pressure, and osteoporosis (Gruberg et al., 2002; Oga & Eseyin, 2016), and has more recently been extended to cognitive processes. The literature on the obesity paradox for cognition is less straightforward compared to that for cardiovascular-associated conditions.

Whereas some studies report that higher BMI in midlife is associated with greater cognitive decline in late life (Dahl et al., 2013; Gunstad et al., 2006; Nilsson & Nilsson, 2009), others report that high mid-life BMI is predictive of better cognitive performance in late life (e.g., Gunstad et al., 2010; Van Den Berg, Biessels, De Craen, Gussekloo, & Westendorp, 2007) or is non-predictive (Smith et al., 2011). These contrasting observations expose the need for greater research in this area and for the development of clarity on mechanisms by which high mid-life BMI might affect central nervous system ageing (Hsu et al., 2015). Although not longitudinal in design, the current study points towards negative cognitive effects of high BMI-associated inflammation across adulthood, thus arguing against the possibility of cognitive benefits bestowed via the obesity paradox.

In sum, the present study showed that individual differences in psychomotor speed can be accounted for by elevated inflammation linked to being either excessively overweight or older, with effects of each condition being additive, not interactive. Further research focusing on central pathways may help enhance mechanistic understanding of inflammatory effects on cognition. This and other experimental work could help more firmly establish a causal role of inflammation in determining psychomotor speed and reveal whether periods of chronic inflammation result in temporary versus permanent psychomotor slowing.

6.7 SUPPLEMENTARY MATERIALS

*Table S1. Results from hierarchical regression analysis showing the mediation effect of IL-6 on the relationship between BMI group and response time (RT). Step 1 involved entering BMI group, followed by IL-6 in step 2 and demographic and health variables in step 3. BMI group was coded as low (1) and high (2) BMI, Sex was coded as male (1) and female (2), and Education level was coded as low (1), middle (2), and high (3) education. Statistical significance of the models is indicated as follows; * p < .05, ** p < .01, *** p < .001.*

	Step 1 R2 = .069* ΔF = 5.845*			Step 2 R2 = .184*** ΔF = 10.983**			Step 3 R2 = .346** ΔF = 1.689		
	β	t	*p*	β	t	*p*	β	t	*p*
BMI Group	0.262	2.418	.018	0.006	0.049	.961	-0.018	-0.134	.894
IL-6				0.425	3.314	.001	0.322	2.450	.017
Time of day tested							0.135	1.301	.198
Sex							-0.097	-0.830	.410
Education level							-0.148	-1.220	.227
Perceived health status							-0.005	-0.040	.968
Depression							0.053	0.311	.757
Anxiety							-0.192	-1.348	.182
Stress							-0.198	-1.146	.256
Illness symptoms							0.199	1.744	.086
Smoking status							-0.083	-0.675	.502
Alcohol intake							0.046	0.368	.714

*Table S2. Results from hierarchical regression analysis showing the mediation effect of IL-6 on the relationship between Age group and response time (RT). Step 1 involved entering Age group, followed by IL-6 in step 2 and demographic and health variables in step 3. Age group was coded as young (1) and old (2), Sex was coded as male (1) and female (2), Education level was coded as low (1), middle (2) and high (3) education. Statistical significance of the models is indicated as follows; * p < .05, ** p < .01, *** p < .001*

	Model 1 R^2 = .451*** ΔF = 64.940***			Model 2 R^2 = .529*** ΔF = 12.957**			Model 3 R^2 = .592*** ΔF = 1.043		
	β	t	*p*	β	t	*p*	β	t	*p*
Age Group	0.672	8.059	<.001	0.605	7.568	<.001	0.705	6.401	<.001
IL-6				0.288	3.600	.001	0.264	2.991	.004
Time of day tested							0.135	1.654	.103
Sex							-0.061	-0.658	.513
Education level							-0.133	-1.416	.161
Perceived health status							-0.034	-0.387	.700
Depression							0.109	0.824	.413
Anxiety							0.007	0.058	.954
Stress							-0.115	-0.840	.404
Illness symptoms							-0.082	-0.826	.412
Smoking status							-0.084	-0.871	.387
Alcohol intake							-0.079	-0.783	.437

Table S3. Spearman correlations and ANOVAs for the relationships between IL-6 and age, IL-6 and BMI, and IL-6 and RT, either with (N = 83) or without (N = 74) replacement of IL-6 values for group mean IL-6 values of individuals with missing or extreme IL-6 concentrations.

	N = 83 (with replacement)			N = 74 (without replacement)		
	r_s	*p*	*F*	r_s	*p*	*F*
Age	.320	.003	4.55	.416	<.001	4.85
BMI	.655	<.001	49.25	.638	<.001	33.97
RT	.430	<.001		.489	<.001	

CHAPTER 7

Selective Effects of Acute Low-Grade Inflammation on Human Visual Attention

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Based on: "Balter, L. J. T., Bosch, J. A., Aldred, S., Drayson, M. T., Higgs, S, Veldhuijzen van Zanten, J. J. C. S., Raymond, J. E., & Mazaheri, A. (under review). Selective Effects of Acute Low-Grade Inflammation on Human Visual Attention"

ABSTRACT

Illness is often accompanied by perceived cognitive sluggishness, a symptom that may stem from immune system activation. The current study used electroencephalography (EEG) to assess how inflammation affected three different distinct attentional processes: alerting, orienting, and executive control. In a double-blinded placebo-controlled within-subjects design (20 healthy males, mean age = 24.5, SD = 3.4), Salmonella typhoid vaccination (0.025 mg; Typhim Vi, Sanofi Pasteur) was used to induce transient mild inflammation, while a saline injection served as a placebo-control. Participants completed the Attention Network Test with concurrent EEG recorded six hours post-injection. Analyses focused on behavioral task performance and on modulation of oscillatory EEG activity in the alpha band (9-12 Hz) for alerting as well as orienting attention and frontal theta band (4-8 Hz) for executive control. Vaccination induced mild systemic inflammation, as assessed by interleukin-6 (IL-6) levels. While no behavioral task performance differences between the inflammation and placebo condition were evident, inflammation caused significant alterations to task-related brain activity. Specifically, inflammation produced greater cue-induced suppression of alpha power in the alerting aspect of attention while individual variation in the inflammatory response was significantly correlated with the degree of alpha power suppression. Notably, inflammation did not affect orienting (i.e., alpha lateralization) or executive control (i.e., frontal theta activity). These results reveal a unique neurophysiological vulnerability to acute mild inflammation of the neural network that underpins attentional alerting functions. Observed in the absence of performance decrements, these novel findings suggest that acute inflammation requires individuals to exert greater cognitive effort when preparing for a task in order to maintain adequate behavioral performance.

7.1 INTRODUCTION

Evidence is mounting to support the contention that immune system activation (i.e., inflammation), both chronic and acute, may degrade basic cognitive function (Allison & Ditor, 2014). Common complaints of mild cognitive deficits in conditions associated with chronic inflammation (e.g., aging, obesity, kidney disease, rheumatoid arthritis, virus infection, and neurodegenerative diseases) or acute inflammation (e.g., injury or commonplace infections) include impaired concentration (Vollmer-Conna et al., 2004), cognitive sluggishness (Smith, 2012), as well as depression (Luppino et al., 2010) and anhedonia (Freed et al., 2018). However, it remains unclear how inflammation impacts specific basic brain processes such as attention. The aim of the current study was to investigate the impact of mild acute inflammation on the fundamental cognitive function of visual attention.

Visual attention refers to the capacity to prioritize relevant information from the ever changing visual sensory environment and is a critical brain function that underpins many everyday activities (Broadbent, 1966; Posner & Rothbart, 2007). Three main roles of the attention system have been identified: Preparing the brain for upcoming salient events (alerting); preparing where to look for task relevant information (orienting); and prioritizing task-relevant information (e.g., roads signs) over concurrent, compelling but irrelevant distractions (executive control). These distinct, yet interacting, functions have been previously assessed using the Attention Network Test (ANT) (Fan et al., 2007; Fan, McCandliss, Sommer, Raz, & Posner, 2002; Posner & Rothbart, 2007).

In the current study, we utilized a variant of the ANT (Figure 1) to investigate inflammation-induced changes to the attention system. The ANT requires participants to respond to different types of cue displays that are each quickly followed by a standard target array. The latter contains a target to which a fast, accurate manual response must be made. Different cue types provide different information that can then be used by the brain to prepare for the target array. Effective use of cues is reflected by changes in brain activity that can be measured electrophysiologically during the cue-target interval and by comparing speed and

accuracy of different cue-target conditions (Fan et al., 2007; Fan et al., 2002; Posner & Rothbart, 2007).

Evidence that inflammation may cause degradation of cognitive processes stems from two broad lines of enquiry. First, correlational analyses examining cognitive performance in populations with chronic inflammation (e.g., elderly, overweight, or patients with chronic inflammatory states due to disease or disorder) have generally revealed negative correlations between inflammation and cognitive performance (Lin et al., 2018; Marsland et al., 2006; but Singh-Manoux et al., 2014). However, specific evidence for inflammation-related impairment of visual attention is scant (but see Kurella Tamura et al., 2017). Moreover, these correlational studies only address effects of chronic inflammation and cannot identify inflammation as the cause of cognitive deficits, except perhaps in the rare case where appropriate mediation analyses are used (Bourassa & Sbarra, 2016). Complicating this picture is evidence that depression and poor cardiovascular health, conditions that are more prevalent in inflammation-associated states (Dhar & Barton, 2016), may themselves contribute to reduced cognitive function (e.g., Deckers et al., 2017).

A second line of research linking inflammation and cognitive degradation involves experimental induction of transient inflammation via administration of immune-activating agents, such as bacterial endotoxin, in otherwise healthy participants. However, previous studies using endotoxin induced inflammation reported no effects on cognitive tests presumed to evaluate attention processes (Grigoleit et al., 2010; Krabbe et al., 2005; Reichenberg et al., 2001; van den Boogaard et al., 2010). Similarly, Brydon et al., (2008) reported no effect on putative attention measures using vaccination against *Salmonella typhi* as a low-grade inflammatory stimulus. Interestingly, while performance was not affected, this study showed compared to a placebo condition, greater BOLD activity during task performance, perhaps reflecting that increased effort was needed. Moreover, these aforementioned studies used coarse cognitive tests (e.g., digit span forward, digit symbol test, color-word Stroop test) that more likely index memory, learning, and other high-level executive functions, leaving open the question of whether inflammation disrupts the functions that support visual attention, *per se*. Furthermore,

previous work has focused almost exclusively on behavioral measures of attention, leaving largely unexplored the effects of acute inflammation on the underlying neurophysiological mechanisms. However, knowledge of these effects is of value for at least two reasons. First, absence of behavioral effects does not imply the absence of underlying neurophysiological effects of inflammation. Second, understanding neurophysiological mechanisms that underpin inflammation-associated cognitive changes may open up possibilities for early markers for those at risk to develop cognitive dysfunction. Experimental studies of inflammation have shown that inflammation induced by means of interferon-alpha, typhoid vaccination or endotoxin was associated with increased task-relevant neural responses, while behavioral performance was unaffected (Brydon et al., 2008; Capuron et al., 2005a; Kullmann et al., 2013). This combination of increased neural recruitment and preserved behavioral performance has been interpreted as reflecting increased effort needed to perform the task.

To address effects of inflammation on attention, the current study experimentally induced acute mild inflammation and assessed visual attention using the ANT paradigm. Typhoid vaccination was used to induce mild inflammation without the concurrently inducting fever and flu-like symptoms as typically occurs with endotoxin (e.g., nausea, headache, and extreme fatigue) that could directly degrade cognition (Grigoleit et al., 2011; Lasselin et al., 2016). We used a randomized double-blind crossover design with a saline injection as the placebo condition. The analysis focused on injection condition-dependent changes in oscillatory activity in the electroencephalogram (EEG) alpha band to the onset of visual alerting cues, a measure reflecting mental preparation effort (Fink et al., 2005; Keil et al., 2006; Sawaki, Luck, & Raymond, 2015). Frontal-midline theta-band oscillations were assessed as a measure for executive control (Cavanagh & Frank, 2014). Activity in the theta band has been associated with aspects of task monitoring and error detection, that is linked to executive attention (Fan et al., 2007). According to the inhibition-timing hypothesis (Klimesch, Sauseng, & Hanslmayr, 2007), alpha selectively increases in a region that is task-irrelevant; when attention is being cued to the left or right visual hemifield, higher occipital alpha power is measured contralateral to the unattended side than contralateral to the attended side. The ratio between left and right occipital

alpha, referred to as the Alpha Lateralization Index (ALI), was assessed as an index of the efficiency of orienting attention (Haegens et al., 2011). The primary prediction was that vaccine-induced inflammation would degrade the brain's ability to prepare for upcoming task-relevant events, resulting in either reduced behavioral indices of alerting functions and/or neurophysiological evidence of enhanced preparatory effort allowing behavioral performance to be maintained.

7.2 METHODS & MATERIALS

7.2.1 Participants

Twenty healthy young male students from the University of Birmingham (*M* age = 24.5, SD = 3.4 years) were enrolled via advertisement and participated in exchange for course credits or £40. Individuals were excluded if they reported being a smoker, having a history of or suspected vaccine-related allergy, food allergy or intolerance, inflammatory, cardiovascular, neurological, mental health or immune-related disorder, visual impairment (unless corrected to normal), and those on any medication 7 days prior to the test days. Mean body mass index (BMI) was 24.5 (SD = 3.4), 16.6 – 29.2 kg/m^2. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local Research Ethics Committee of the National Health Service (NHS).

7.2.2 General Procedures

Participants visited the behavioral immunology laboratory on three separate occasions: one familiarization session, followed by two separate test days (i.e., receiving vaccination or saline placebo, randomly assigned) at least one week apart. The study was carried out in a double-blind placebo-controlled crossover fashion. On arrival in the morning, a blood sample was taken before participants received the vaccination or placebo injection. After injection, participants had a 4-hour break followed by a standardized lunch and EEG set-up preparations. A second blood sample was taken about 5h30 post-injection; then, the ANT was completed

while EEG recordings took place (about 6 hours post-injection), followed by a set of other cognitive tests. Other cognitive tests, not reported here, included measures of emotion recognition, memory, learning, and response inhibition. For a detailed description of the complete study procedures, see Balter et al. (2018). The final blood sample was taken about 8 hours post-injection. Mood and sickness symptoms and tympanic body temperature were assessed at several intervals during the test day, including before injection, at 5h30m, and at 8 hours post-injection. Test timings were identical across visits, as were the procedures, except for the type of injection (vaccine or saline placebo).

7.2.3 Randomization

Participants were randomly assigned to receive vaccine or saline placebo on the first day of formal testing. Randomization was performed by supporting staff who had no contact with the study participants. Only the nurse administering the injections was aware of the order. A sealed envelope containing information about the condition was handed to the nurse before administering the injection. The nurse followed identical procedures for placebo and vaccine injection ensuring participant's blindness to the condition. The researchers were not present when the injection was administered. Results indicated that participants were blind to their condition at the first visit: on test day 1, 62.5% of the participants reported the condition correctly at the end of the test day, which is near chance level ($\chi^2(1) = 1$, $p = .317$). At the end of test day 2, 73.3% correctly guessed the condition they were in ($\chi^2(1) = 3.27$, $p = .071$).

7.2.4 Typhoid vaccination

Typhoid vaccine was selected as a low-grade inflammatory stimulus as this vaccine is known to induce increases in circulating pro-inflammatory cytokine levels without inducing significant effects on sickness symptoms such as fever (e.g., Paine, Ring, Bosch, Drayson, & Veldhuijzen van Zanten, 2013). Participants received 0.5 mL Salmonella typhi capsular polysaccharide vaccine (0.025 mg in 0.5 mL, Typhim Vi, Sanofi Pasteur, UK) or a saline placebo

(0.5 mL) via intra-muscular injection in the deltoid muscle of the non-dominant arm by a certified nurse on each test day.

7.2.5 Mood and sickness symptom assessment

Current mood and presence of sickness symptoms was assessed using a modified version of the Profile of Mood States – Short Form (POMS-SF; Curran, Andrykowski, and Studts 1995). The version used here comprised 38 items each beginning with 'How are you feeling right now' followed by a word descriptive of one of eight states (tension–anxiety, anger–hostility, fatigue–inertia, vigor–activity, confusion–bewilderment, depression–dejection, physically well - physically ill, withdrawn-sociable). Ratings were made using a five-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, to 4 = extremely). Scores for POMS subscales were computed by summing ratings on individual items.

7.2.6 Blood sampling

Blood (6 ml) was collected into a vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500 *g* for 10 min at 4 °C and plasma was aliquoted and stored at -80 °C for later cytokine assessment of plasma interleukin-6 (IL-6). Plasma IL-6 was assessed in duplicate using high-sensitivity enzyme-linked immunosorbent assay (ELISA; Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The limit of detection of this assay was 0.11 pg/mL, with an intra-assay coefficient of variation of 4.2%. All samples from the same participant were assayed in the same run. Assessment of IL-6 primarily acted as an inflammation check and no causal assumptions about the role of IL-6 in the observed effects can be made.

7.2.7 Behavioral and Electrophysiological data acquisition

Participants were seated at approximately 80 cm distance in front of a high-resolution 17'' LCD color monitor. E-Prime 2.0 (Psychology Software tools, Inc., Sharpsburg, PA, USA) was

used to present stimuli and record behavioral data. Task responses were recorded via a standard computer keyboard. Electroencephalograms were obtained in a temperature-controlled environment using a 64-channel Ag/AgCl electrode 10-10 WaveGuard cap and eego™ sports amplifier from ANT (https://www.ant-neuro.com). The data was acquired with a sampling rate of 1024 Hz with online Cpz reference. Electrodes impedance was maintained below 20 kΩ as recommended by the manufacturers. Horizontal eye movements were monitored via two bipolar electrodes placed at the external canthi of each eye.

7.2.8 Attention Network Test

In the current study we utilized a lateralized variant of the ANT commonly used in previous studies (Fan et al., 2002). Each trial began with the presentation of a black central fixation cross (1° of visual angle in diameter) that remained on the screen throughout the trial. See Figure 1. After a randomly jittered interval of 400-500 ms, one of three cue conditions occurred. (1) No Cue: A blank screen except for the fixation cross until target array presentation occurred 1200 ms later, providing no information regarding when or where the target would appear; (2) Double Cue: Two asterisks (each 0.6° in diameter) presented 2.3° to the left and right of the fixation cross along the horizontal meridian for 100 ms, providing certainty over when but not where the target would appear; and (3) Spatial Cue: A single asterisk presented either 2.3° to the left or right of the fixation cross for 100 ms, signaled the target location on 100% of the trials (spatial cue) providing both temporal and spatial certainty about the target's appearance. The target array was presented 1200 ms after cue onset and disappeared immediately upon response or within 2000 ms. The target stimulus was a central arrow (0.6° tall X 1.7° wide) presented simultaneously with four similar (flanker) arrows pointing in the same (congruent) or opposite (incongruent) direction as the target. Flankers were positioned directly above and below the target; all arrows were aligned vertically in a column (3.3° total height; arrows separated by 0.1°) and could appear 2.3° to the left or right of fixation. The participant's task was to report the direction of the central target arrow by pressing the "k" key for up or the "m" key for down

using the dominant hand as quickly and accurately as possible. Each trial lasted 2250 ms on average.

Participants performed one practice block of 32 trials followed by six experimental blocks. Within each block, each cue condition was presented 16 times in a pseudorandom order with each combination of target array location and congruency being equally likely to occur within each cue condition.

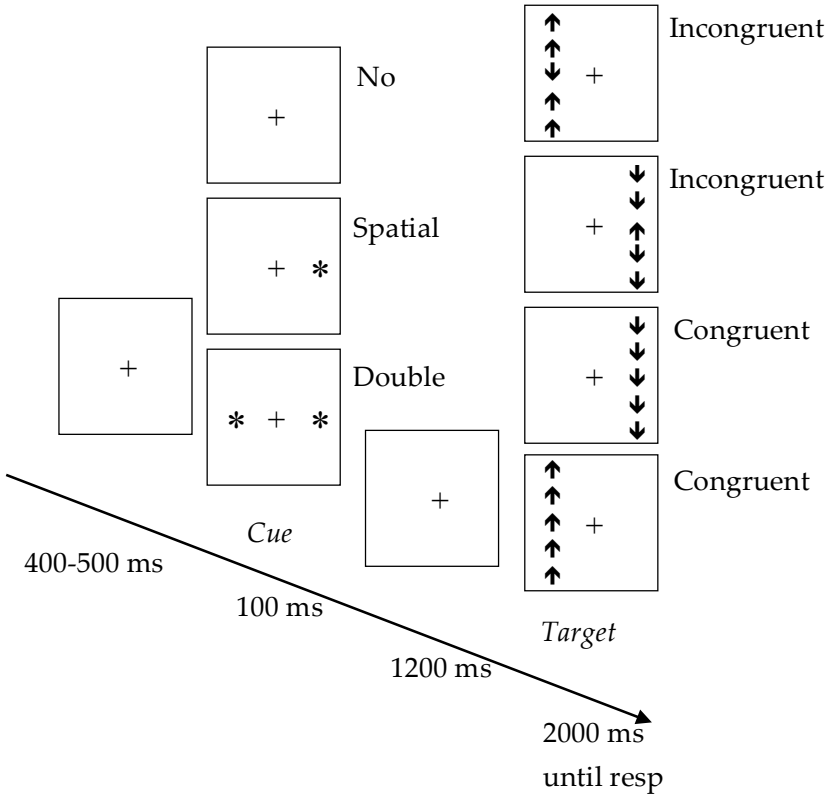


Figure 1. The sequence of displays presented in each trial of the lateralized ANT task. The cue display had three equally likely conditions: No Cue, Double Cue and Spatial Cue (100% valid). The target array comprised a central arrow and four identical flankers that were either congruent or incongruent with the target arrow. The task was to report the direction (up, down) of the center target arrow as quickly and accurately as possible.

In this test, differences in response time (RT) and accuracy for different cue and target flanker conditions were used to index the efficiency of two different cueing effects: (1) alerting, i.e., computing the difference between trials with a double cue and trials with no cue; and (2) orienting, i.e., computing the difference between trials with spatial cues that validly predicted the spatial location of the target and those with double cues, offering no prediction. For EEG, orienting of attention was calculated as the Alpha Lateralization Index (ALI) which is described in the next section. In addition, flanker congruency effect referred to as (3) "executive control" was determined by comparing congruent and incongruent flanker trials regardless of cue condition. Psychomotor speed was computed as the grand average RT across all cue and target-flanker conditions.

7.3 STATISTICAL ANALYSIS

7.3.1 Behavioral data analysis

Trials with incorrect responses or with RTs <150 ms or >1500 ms were excluded from any further analysis, accounting for 1.1% of the data. In addition, RTs >3 SDs from the mean for each combination of cue and target flanker condition for each participant were also excluded, accounting for an additional 1.2% of the data. All data for two participants was excluded from analysis as a result of equipment failure (no behavioral file was saved).

Mixed design repeated measures ANOVAs and paired samples *t*-tests were used where appropriate. We analyzed both RT and accuracy for testing order effects (Day 1 versus Day 2) and found neither main nor interaction effects (all F's < 1). To assess whether the inflammatory response was associated with measures of interest (e.g., behavioral measures, alpha power, frontal theta power, ALI), bivariate correlational analyses were performed with the inflammatory response to the vaccine. The inflammatory response was defined as the IL-6 level at 5h30 post-injection in the vaccine condition minus the IL-6 level at 5h30 post-injection in the placebo condition. In addition to traditional null hypothesis significance testing, Bayes Factors were calculated for non-significant effects via Bayesian ANOVAs and correlation analyses using

default prior probabilities in JASP version 0.9. Bayes factors provide relative evidence of both the null (H_0) and alternative hypothesis (H_A), compared to the conclusions about the null hypothesis proffered by traditional null hypothesis significance testing. To allow for clear interpretation, the approximate classification scheme of Wagenmakers et al., (2017) was used which states that an estimated Bayes Factor (BF_{10}; H_0/ H_A) value <1 supports evidence in favor of H_0. For example, a BF_{10} of 0.25 indicates that the H_0 is 4 times (1:0.25) more likely than the H_A. Values close to 1 are not informative and a BF_{10} between 1 and 3 provides anecdotal evidence for the H_A. A BF_{10} between 1 and 0.33 provides anecdotal evidence for the H_0 (e.g., 1:3 probability in favor of H_0) and a BF_{10} between 0.33 and 0.10 provides moderate evidence for H_0. A $BF_{10} < 0.10$ provides strong evidence for H_0.

7.3.2 Time-Frequency analysis

Offline processing and analyses were performed using the matlab toolbox EEGLAB (Delorme & Makeig, 2004) and Fieldtrip (Oostenveld, Fries, Maris, & Schoffelen, 2011). Continuous EEG data were offline re-referenced to the average of all scalp electrodes, high-pass filtered at 0.1 Hz and low-pass filtered below 40 Hz using a two-way, fourth-order Butterworth filter. To remove ocular and muscle artifacts, independent components analysis (ICA) was performed using the EEGLAB toolbox (on average, 6 components per participant were removed, SD = 2.0). There was no significant difference between the number of components accepted in the vaccine versus placebo condition, $t(19) = 1.24$, $p = .230$. Next, continuous EEG data were epoched from -2000 ms to 2000 ms, time-locked to cue onset. Only correct trials were included in the analyses. Time–frequency representations of power were estimated per trial, using sliding Hanning tapers having an adaptive time window of five cycles for each frequency of interest ($\Delta T = 5/f$). For the 'alerting' component of the ANT we focused on the difference in modulation of alpha power (averaged across 9-12 Hz) in the interval between 200 ms post-cue onset to target onset for double versus no-cues. We chose to analyze activity 200ms after the onset of cues as to avoid spectral contamination from the sensory evoked responses into the alpha band. The difference in alpha modulation (over time and electrodes) between 'placebo vs vaccine'

conditions was assessed by means of cluster-based permutation procedure (Maris & Oostenveld, 2007) implemented in the Fieldtrip toolbox (Oostenveld et al., 2011). This test controls the Type I error rate involving multiple comparisons (e.g. multiple channels or time-frequency tiles). A probability value here is obtained through the Monte Carlo estimate of the permutation *p*-value of the cluster of channels by randomly swapping the labels (i.e., condition) in participants 5000 times and calculating the maximum cluster-level test statistic.

For the 'executive control' aspect of the ANT, we examined the post-target differences in theta power between incongruent target flankers and congruent target flankers in the interval 200-700 ms post-target onset (as to avoid spectral contamination from the sensory evoked responses). The difference in theta modulation related to congruency (over time and electrodes) between 'placebo vs vaccine' conditions was also assessed by means of a cluster-based permutation procedure.

Lastly, for the 'orienting' aspect of the ANT, the alpha lateralization index (ALI) was calculated for the left and right cue for early (0 to 500 ms post-cue) and late (500 to 1000 ms post-cue) time points. The region of interest (ROI) included posterior channels for the left (P3, P5, P7, PO5, PO7) and right hemisphere (P4, P6, P8, PO6, PO8). The ALI was calculated as follows:

$$\mathrm{ALI}_{\mathrm{left}} = \frac{\alpha\ power\ \frac{Left\ ROI}{Right\ Cue} - \alpha\ power\ \frac{Right\ ROI}{Right\ Cue}}{\alpha\ power\ \frac{Leftt\ ROI}{Right\ Cue} + \alpha\ power\ \frac{Right\ ROI}{Right\ Cue}} \qquad \text{Eq 1.1}$$

$$\mathrm{ALI}_{\mathrm{right}} = \frac{\alpha\ power\ \frac{Left\ ROI}{Left\ Cue} - \alpha\ power\ \frac{Right\ ROI}{Leftt\ Cue}}{\alpha\ power\ \frac{Leftt\ ROI}{Leftt\ Cue} + \alpha\ power\ \frac{Right\ ROI}{Left\ Cue}} \qquad \text{Eq 1.2}$$

A repeated measures ANOVA with cue (left, right), time (early, late), and injection condition (placebo, vaccine) was conducted to assess the effect of injection condition on the ALI. Levene's test of Equality of Variances and Mauchly's Test of Sphericity were used to test for assumption violations with adjustments made using the Greenhouse-Geisser corrections;

Bonferroni corrections were applied to post-hoc pairwise comparisons (two-tailed unless stated otherwise) to control for type I error rate; and alpha was set at .05.

7.3.3 RESULTS

7.3.4 Physiological responses

Participants showed a significantly greater peak in plasma IL-6 to typhoid vaccination (+4.0 pg/mL, *SD* = 1.6) as compared to placebo (-0.1 pg/mL, *SD* = .3) (time (0h, 5h30, 8h) x injection condition) ($F(2, 26) = 30.10$, $p < .001$, $\eta_p^2 = .82$). The peak IL-6 response (See Table 1) occurred at 5h30m post-injection for most participants. IL-6 remained elevated at 8 hours post injection (+2.3 pg/mL, SD = 1.9) ($t(16) = -7.03$, $p < .001$). As such from this point on we will refer to the vaccine condition as the inflammation condition for the remainder of the text. Peak body temperature was similar across conditions (inflammation *M* = 36.9°C, SD = 0.3; placebo *M* = 36.8°C, SD = 0.3), confirming absence of fever.

Table 1. Mean (SD) interleukin-6 (IL-6) concentrations (pg/mL) separated by injection condition. Column labels represent time since injection.

	0 hours	5h30m	8 hours
Placebo	0.9 (0.3)	0.8 (0.4)	0.8 (0.4)
Vaccine	1.2 (0.5)	5.1 (1.4)	3.9 (1.3)

7.3.5 Mood and sickness symptoms

No significant time by injection condition interactions were evident for any of the POMS subscales or total mood score (all *F*'s < 1). POMS data are summarized in Table 2.

Table 2. Mean (SD) POMS subscales (mood and physical and behavioral symptoms) separated by injection condition. Column labels represent time since injection.

	0 hours		5h30m		8 hours	
	Placebo	Inflammation	Placebo	Inflammation	Placebo	Inflammation
Anger-Hostility	0.4 (1.0)	0.2 (0.5)	0.6 (1.4)	0.2 (0.4)	0.8 (2.0)	1.0 (2.0)
Confusion-Bewilderment	3.4 (1.6)	3.3 (1.5)	3.3 (1.7)	3.1 (1.0)	4.5 (2.1)	4.5 (1.9)
Depression-Dejection	0.4 (1.0)	0.4 (1.0)	0.3 (0.6)	0.2 (0.6)	0.8 (2.2)	1.5 (2.6)
Fatigue-Inertia	2.6 (2.9)	2.7 (2.3)	2.5 (3.2)	1.8 (2.5)	4.9 (3.2)	5.7 (3.8)
Tension-Anxiety	0.7 (1.1)	0.6 (0.9)	1.1 (2.2)	1.1 (1.6)	1.2 (2.4)	1.0 (1.9)
Vigor-Activity	6.8 (5.5)	6.5 (3.4)	6.6 (3.9)	6.7 (3.5)	3.9 (4.2)	3.4 (3.2)
Withdrawn-Sociable	2.2 (1.2)	2.2 (1.2)	2.1 (1.3)	2.0 (1.0)	2.6 (1.3)	2.4 (1.3)
Physically well-Ill	0.6 (1.2)	0.7 (1.6)	0.8 (2.0)	0.6 (1.5)	0.9 (1.8)	0.7 (1.4)
Total mood	0.7 (8.8)	-0.2 (5.6)	1.3 (9.9)	-0.4 (6.2)	8.2 (12.1)	9.4 (11.4)

7.3.6 Behavioral attention measures

ANOVA of individual condition means revealed no significant main effect of injection condition on RT (placebo M = 480 ms, SE = 11; inflammation M = 486 ms, SE = 11) ($F(1, 16) = 1.00$, $p = .332$, $\eta_p^2 = .06$). However, as expected, main effects of Cue condition ($F(2, 32) = 42$, $p < .001$, $\eta_p^2 = .72$) and Target Flanker condition ($F(1, 16) = 123$, $p < .001$, $\eta_p^2 = .89$) were found. Injection condition did not interact with Cue or Target Flanker condition (F's < 1.1). Mean RTs and accuracy for each cue and target flanker condition for the placebo and inflammation condition are shown in Figure 2 and Figure 3, respectively.

Subsequently, repeated measures ANOVAs were performed for each cueing comparison. Across injection condition, RTs for No Cue trials were 20 ms (SE = 3 ms) slower as compared to Double Cue trials (alerting effect) ($F(1, 16) = 55.73$, $p < .001$, $\eta_p^2 = .78$). RTs for Double Cue trials were 8 ms (SE = 3 ms) slower as compared to Spatial Cue trials (orienting effects) ($F(1, 16) = 9.38$, $p < .001$, $\eta_p^2 = .37$). Lastly, RTs for trials with incongruent target flankers were 38 ms (SE = 3 ms) slower as compared to trials with congruent target flankers (executive control effects) ($F(1, 16) = 124.84$, $p < .001$, $\eta_p^2 = .89$). Injection condition did not significantly affect alerting ($F = 1.71$, $p = .209$), orienting ($F = 1.47$, $p = .243$), or executive control ($F = 0.23$, $p = .883$) performance. None of

the cueing comparisons significantly correlated with the inflammatory response (p's > 0.7). Bayesian repeated measures ANOVA showed moderate (RT alerting BF_{10} = 0.27; RT executive control BF_{10} = 0.14) and anecdotal (RT orienting BF_{10} = 0.41) evidence for the null hypothesis (H_0; no effect of injection condition) against the alternative hypothesis (H_A). Furthermore, Bayesian correlation analysis showed moderate evidence in favor of H_0 for a correlation between IL-6 and cueing effects (alerting: BF_{10} = 0.30; orienting: BF_{10} = 0.30; executive control: BF_{10} = 0.30).

Similar to RT, accuracy was not significantly affected by injection condition (placebo M = 96.8%, SE = 0.5; inflammation M = 97.0%, SE = 0.6) ($F(1, 16) = 0.165$, $p = .690$, $\eta_p^2 = .01$). A main effect of Target Flanker condition ($F(1, 16) = 32$, $p < .001$, $\eta_p^2 = .67$) was found and no effect of Cue condition was evident ($F(2, 32) = 1.42$, $p = .256$, $\eta_p^2 = .08$). Injection condition did not interact with Cue or Target Flanker condition (F's < 1). ANOVAs were performed for each cueing comparison. No significant alerting ($F < 1$) and orienting effects ($F(1, 16) = 3.83$, $p = .068$) of accuracy were found. Across injection condition, a significant executive control effect was found (M = -3.8%, SE = 0.6%) ($F(1, 16) = 35.36$, $p < .001$, $\eta_p^2 = .69$). No effects of injection condition were evident (F's < 1) and correlations between cueing comparisons and the inflammatory response were non-significant (p's > 0.1). Bayesian repeated measures ANOVA and correlation analysis showed moderate (ANOVA alerting BF_{10} = 0.33; ANOVA executive control BF_{10} = 0.33) and anecdotal evidence for H_0 against H_A (ANOVA orienting BF_{10} = 0.45; correlation alerting: BF_{10} = 0.77; correlation orienting: BF_{10} = 0.64; correlation executive control: BF_{10} = 0.37).

These results suggest that information of the cues was used to improve performance to the same degree in the placebo and inflammation condition.

Figure 2. Mean RT for placebo and inflammation condition. Error bars represent standard error of the mean.

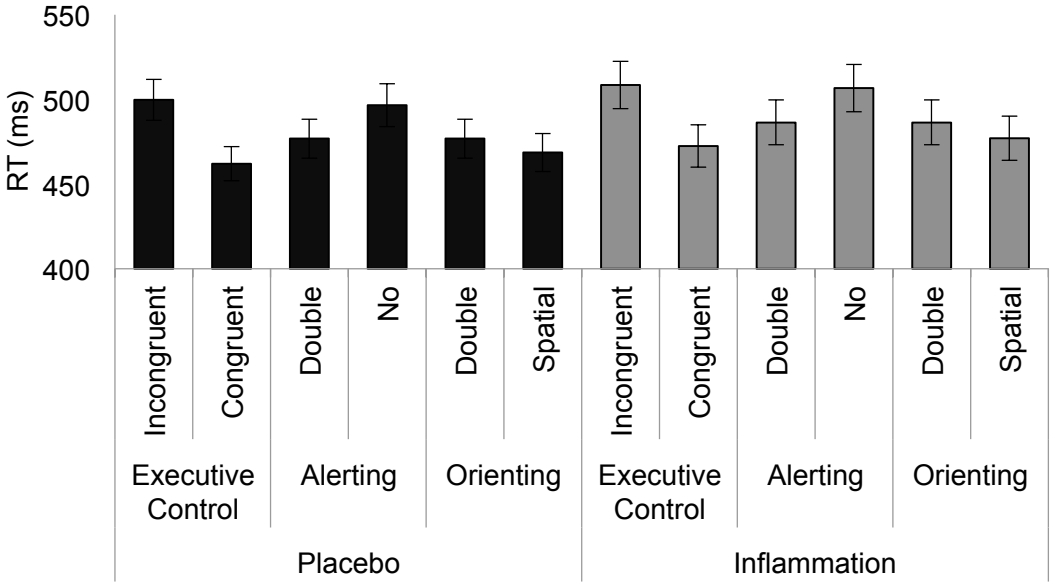
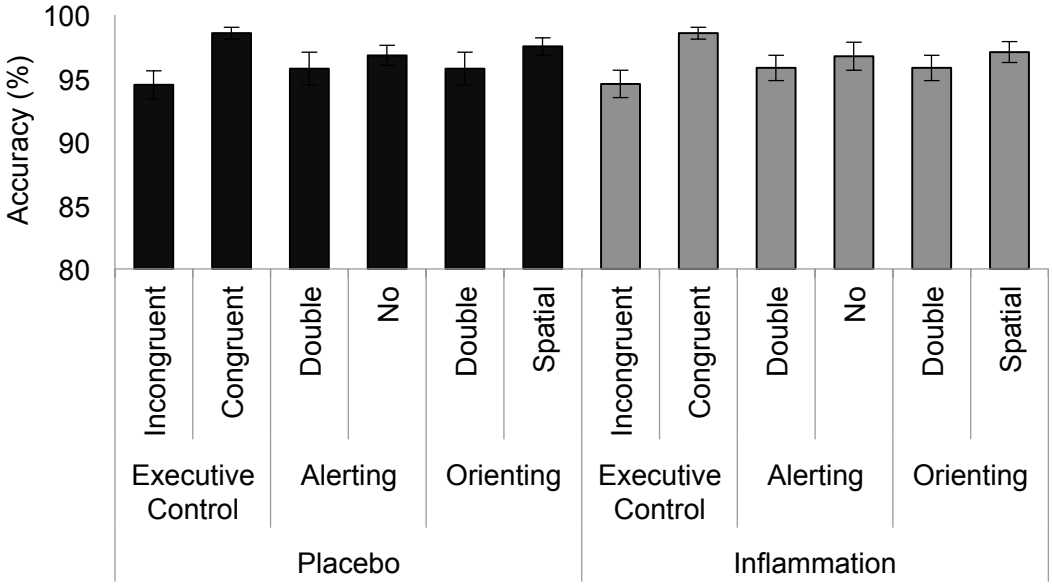


Figure 3. Mean accuracy for placebo and inflammation condition. Error bars represent standard error of the mean.



7.3.7 Inflammation extenuates alpha suppression related to alerting

The cue-induced modulation of alpha power in the alerting dimension of the ANT (i.e. Double Cue - No Cue) can be seen in Figure 4. Cue-induced alpha suppression was significantly greater in the inflammation condition relative to placebo, in the interval 200-300 ms after cue onset ($t(16) = -46.84$, $p = .037$, Monte Carlo p-value, corrected for multiple comparisons). This effect was most pronounced over a central, frontal and frontal-central cluster of electrodes. Individual variation in the log IL-6 response to the vaccine was significantly negatively correlated with alpha power ($r(15) = -.613$, $p = .026$) (Figure 5). Bayesian correlation analysis showed moderate evidence in favor of H_A for a negative correlation between IL-6 and alpha power ($BF_{10} = 3.20$).

Our oscillatory findings are unlikely to be accounted for by differences in cue-evoked potentials. Time-frequency representations that are time-locked to the onset of an experimental event capture activity that is phase locked as well as changes that are non-phase locked. In order to reduce the likelihood that our findings could simply be accounted for by differences in the amplitude of evoked responses, we repeated all our statistical analysis on time-frequency representations after the spectral components of the average ERP were removed from the 'total' spectra measures in each trial (Mazaheri & Picton, 2005; Mazaheri et al., 2018). We found that removal of the ERP spectra had negligible effects on our contrasts. For simplicity, we report here the statistics and scalp topographies for time-frequency representations that were not subjected to this subtraction.

7.3.8 Brain and Performance Interactions

Finally, we assessed the relationship between frontal-central alpha suppression and behavioral performance. Across participants, a correlation analysis was performed between alerting induced (double-cue – no-cue) alpha activity over the frontal-central cluster of electrodes and overall accuracy and overall RT. Interestingly, we found that greater alpha suppression was correlated with lower accuracy both in the inflammation ($r(16) = .699$, $p = .005$)

and placebo ($r(16) = .660$, $p = .010$) condition. This would suggest the more alpha suppression an individual produced in response to the alerting cues, the more mistakes they made.

We did not observe a relationship between alerting induced alpha suppression and RT (inflammation: r (16) = .380, p = .163; placebo: $r(16) = .362$, $p = .185$). We investigated this further by separating data from experimental trials based on whether responses were slow or fast (using a median split). We then determined the power of alpha activity across the significant cluster of electrodes as well as the time interval that was revealed with cluster-based permutation tests for each RT category (fast, slow). We performed a repeated measures ANOVA with Injection condition (placebo, inflammation), RT category (slow, fast), and Cue type (double, no) to assess the relationship of alpha power to RT. As expected (given these were significant clusters obtained from a previous analysis), the Injection condition x Cue condition interaction revealed a greater cue-induced alpha suppression in the inflammation condition ($F(1,17) = 8.19$, $p = .011$, np2 = 0.34. However, the main effect of RT (slow, fast) ($F = 2.24$, $p = .154$) was nonsignificant. Moreover, the Injection condition x RT x Cue type interaction was also nonsignificant (F = 2.37, p = .129), as were all other interaction effects (F's < 1). We conducted an additional trial-by-trial analysis examining a correlation between the power of alpha activity across all electrodes at this time interval and subsequent RT. This resulted in correlation topographies for each participant for each condition. The statistical significance of these topographies at the group level was assessed with a cluster-level randomization test. Again, we did not observe a relationship between post-cue alerting alpha and RTs. The same analysis using alpha lateralization as an index also did not find a relationship between trial-by-trial alpha activity across contra-lateral or ipsi-lateral hemispheres and subsequent RT. These findings suggest that the pre-target alpha modulation (alerting or orienting related) reported here is unlikely to be directly related to trial-by-trial reaction times, or speed of processing.

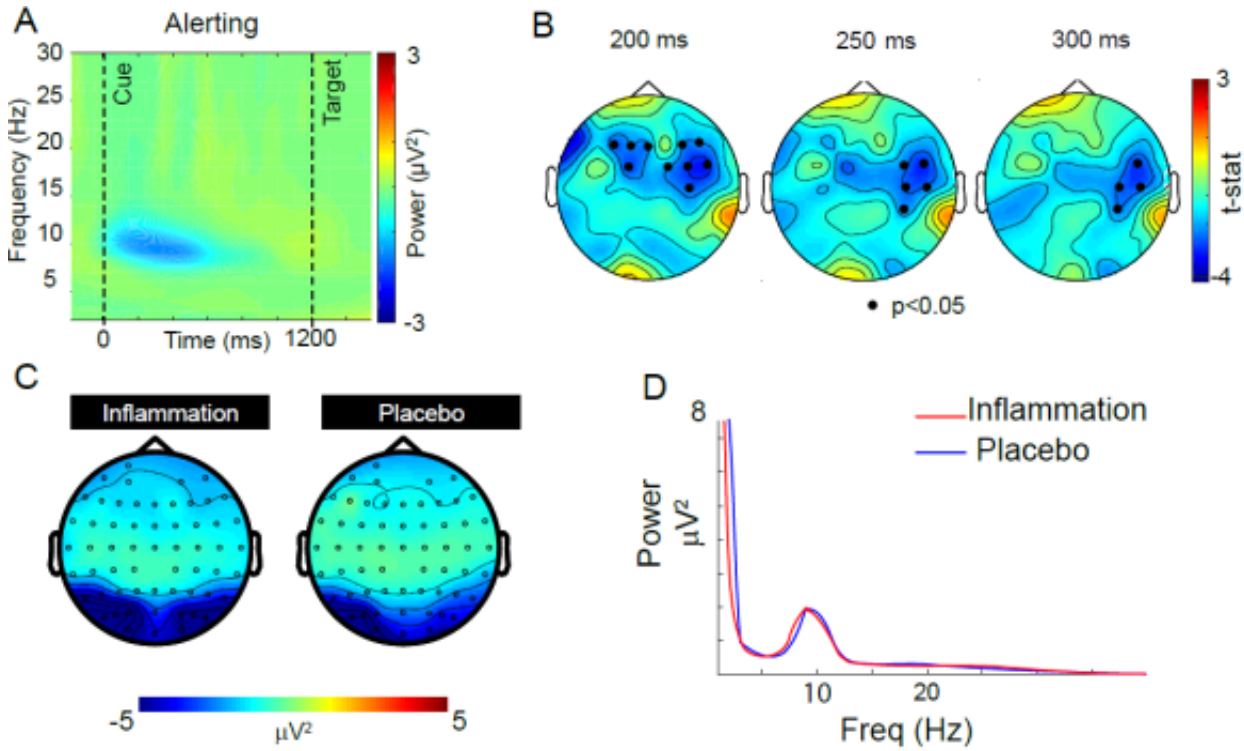


Figure 4. Alpha modulation related to 'alerting' dimension of the ANT (A) Power-locked alpha activity cued to onset of Double versus No Cue across injection conditions averaged over the significant cluster of electrodes and time interval (200-300 ms) that was revealed with random-cluster permutation tests. (B) The random-cluster permutation test revealed significantly greater alpha suppression in the inflammation compared to the placebo condition 200-300 ms after cue onset. The dots illustrate the clusters of electrodes that show the most pronounced difference between placebo and inflammation condition over the time interval in which a significant difference was found using the random-cluster permutation test (200-300 after cue onset). (C) Scalp topography of alerting-related alpha activity for the inflammation and placebo condition over the same interval. (D) Frequency spectra in the baseline period (1 second) for placebo (red) and inflammation (blue) condition averaged over the cluster of electrodes showing a significant difference in alpha power between inflammation and placebo condition.

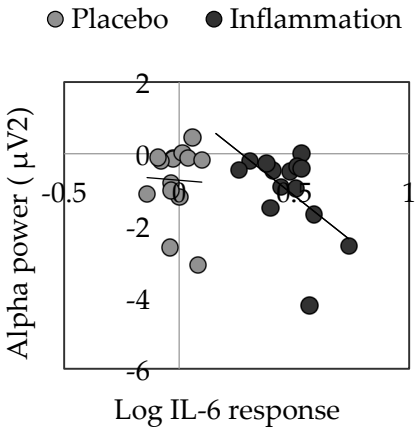


Figure 5. Significant negative correlation between the IL-6 response to the vaccine and alpha power to alerting cues in the inflammation conditions (black dots). Participants with a larger IL-6 response showed greater alpha power suppression. No significant correlation was found between the IL-6 response to the placebo and alpha power to altering cues in the placebo condition (gray dots). Alpha power was averaged across the electrodes (see Figure 4b) and the time period in which placebo and inflammation showed the greatest difference (200-300 ms post-cue).

In addition to investigating the modulation of alpha activity between the baseline no-cues and alerting double cues, we examined if there was a baseline difference in the frequency spectra between the inflammation and placebo condition during the no-cue. The frequency spectra at the baseline period, over the electrodes showing the most pronounced difference between placebo and inflammation can be seen in Figure 4D. Qualitatively, there does not appear to be any difference in the spectra between the placebo and inflammation. Nonetheless, to test for any possible frequency power of 1/f difference between the inflammation and placebo condition, rather than correcting for the broadband or 1/f changes, we chose to look at every frequency in the spectra between 1 and 30 Hz, and apply a cluster-level randomization approach similar to the one used by Segaert et al (2018) to circumvent multiple comparisons. Here, the data was clustered spatially (across electrodes), as well as across the frequency spectra. We did not observe any differences in the frequency spectra of individuals receiving the placebo or vaccine injection during the baseline no-cue interval condition. In addition, we repeated the

analysis this time using pre-defined bands on interest (delta (1-2 Hz), theta (3-7), alpha (9-12 Hz), yet we still failed to observe any statistical differences between the placebo and inflammation condition in the amplitude of these frequency bands at baseline. We believe that these observations suggest that low-grade inflammation does not alter the baseline level of alpha activity, but rather affects its modulation after the onset of alerting cues.

7.3.9 Inflammation did not significantly affect the theta increase related to executive control

While there is greater target-locked frontal theta oscillatory activity in the incongruent versus congruent target flanker condition ($t(16) = 16.7$, $p < .001$), no significant difference in theta increase between placebo and inflammation was found using cluster-based permutation analysis procedure ($t(16) = -18.03$, $p = .433$). See Figure 6. Individual variation in the IL-6 response to the vaccine was not significantly correlated with theta activity (averaged across the electrodes Cz, Cpz) in the executive control domain of attention ($r(16) = .011$, $p = .969$), with anecdotal ($BF_{10} = 0.33$) evidence in favor of H_0.

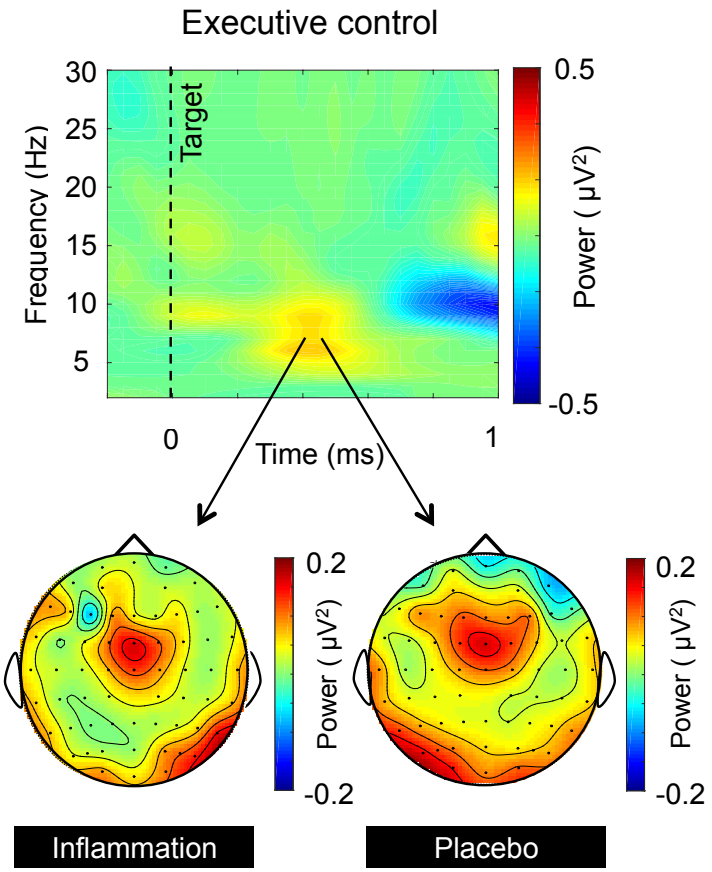


Figure 6. Frontal theta (Cz, Cpz) activity for executive control (A) Time-frequency representations of power-locked activity cued to the onset of targets (Incongruent – Congruent target flankers) of the ANT for placebo and inflammation condition. (B) Scalp topography of target-related theta activity. The distribution of theta power (4-7Hz) from 300 to 500 ms after target onset shown for the placebo and inflammation condition. Theta power is maximal over frontal midline electrodes. Inflammation did not affect the alpha lateralization related to orienting.

7.3.10 Inflammation did not affect the alpha lateralization related to orienting

The cue-induced alpha lateralization index (ALI) left and right for early (200-500 ms post-cue) and late (500-800 post-cue) processing was calculated (see Eq 1.1 and Eq 1.2) and can be seen in Figure 7. No significant effect of injection condition on the ALI (F = .94, p = .347) was found nor interactions between injection condition and cue (left, right) or time (early, late) (F's < 1.3). Interestingly, we found a relationship between the ALI and overall RT (ALI left early = $r(16)$ = -.488, p = .055; ALI right early = $r(16)$ = -.538, p = .032; ALI left late = $r(16)$ = -.554, p = .026; ALI right late = $r(16)$ = -.517, p = .040), suggesting that participants who showed the greatest alpha to the cue also tend to be the ones that were faster in the task. The IL-6 response was not significantly correlated with the early or late ALI_{left} and ALI_{right} (p's > .1). Bayesian correlation analysis showed anecdotal evidence in favor of H_0 for a correlation between IL-6 and alpha power (Early ALI_{left} BF_{10} = 0.51; Late ALI_{left} BF_{10} = 0.36; Early ALI_{right} BF_{10} = 0.88; Late ALI_{right} BF_{10} = 0.34).

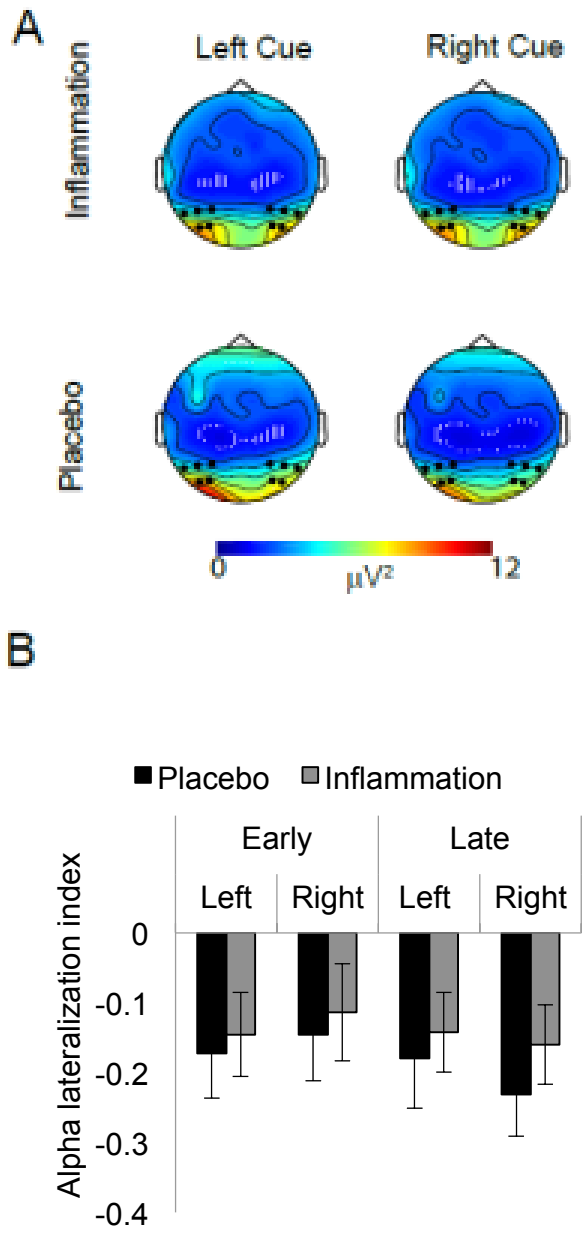


Figure 7 A) The topography of the post-cue (200ms -800 ms) of the alpha power for the inflammation and placebo condition. Mean Alpha Lateralization Index (ALI) 200-500 ms post-cue (early) and 500-800 ms post-cue (late) onset for the left and right cue separately for the placebo and inflammation condition. A more positive value of ALI is indicative of a shift in alpha power to the left hemisphere, a more negative value of ALI means a shift to the right hemisphere.

7.4 DISCUSSION

The current study used the ANT to investigate how acute low-grade inflammation affected the alerting, orienting and executive components of visual attention at a neurophysiological and behavioral level. Typhoid vaccination given to the participants effectively induced mild inflammation, as measured by IL-6, without evoking concurrent fever or flu-like symptoms, a finding in line with results from a larger cohort (Balter et al., 2018). The primary finding of the current study is that transient mild inflammation affected the alerting network, but left the other attention components, orienting and executive relatively unaffected. Specifically, we observed that the alpha suppression response to cues that provide temporal information about imminent targets stimuli was significantly more pronounced in the inflammation compared to the placebo condition. Importantly, this alteration in the brain's preparation for a target display did not result in any overt behavioral change in performance and baseline alpha activity remained unaltered. The results demonstrate for the first time that a sub-process of attention, i.e., alerting, is selectively sensitive to the effects of acute mild inflammation.

The degree to which alpha power is suppressed is thought to reflect the level of cognitive effort required by an upcoming task (reviewed in Van Diepen, Foxe, & Mazaheri, 2019). Greater alpha power suppression is associated with higher task demands (Fink et al., 2005), greater subjective task difficulty (Wostmann, Herrmann, Wilsch, & Obleser, 2015), increased memory load (Stipacek, Grabner, Neuper, Fink, & Neubauer, 2003), and greater discrimination difficulty (Roberts, Fedota, Buzzell, Parasuraman, & McDonald, 2014). Observing greater alpha suppression with no corresponding decrement in performance after inflammation induction in the current study suggests that inflammation may require greater cognitive effort to perform an attentionally demanding task. This explanation seems in apparent contrast with the suggestion that motivational reductions are a characteristic of inflammation (Felger & Treadway, 2016; Draper et al., 2017). However, careful inspection of the literature suggests that inflammation may result in a recalibration of reward-effort trade-offs, i.e., greater effort is invested when the behavior is regarded as especially rewarding (Lasselin, Treadway, et al., 2016; Vichaya et al.,

2014). Although the current task was not designed to assess reward-effort trade-offs, the observed pattern of results extends the notion of a reward-effort recalibration to attentional performance and suggests that increased effort is invested to maintain a high level of attentional performance. Arguing against this idea is the possibility that inflammation might degrade low-level visual sensory processing and thereby demand greater higher level processing that consequently leads to greater alpha power suppression (Roberts et al., 2014). However, such an explanation cannot account for effects based on difference scores between cue conditions. If inflammation degraded visual sensory processes, then greater alpha suppression would have been found for all cue conditions, resulting in no effect on attention difference scores.

In line with previous experimental studies using diverse tasks subsumed under attention, inflammation exhibited no overt behavioral attention effects (Roberts et al., 2014). However, absence of behavioral effects does not imply the absence of an underlying neurophysiological effect of inflammation. It has been shown that EEG can identify early signs of cognitive decline in pathological states, such as mild cognitive impairment (Brydon et al., 2008; Grigoleit et al., 2010; Krabbe et al., 2005; Reichenberg et al., 2001; van den Boogaard et al., 2010) and dementia in Parkinson's disease (Mazaheri et al., 2018). EEG methods, as compared to most behavioral measures, are able to detect subtle aspects of cognitive function, making this method highly suitable for probing neural effects of mild inflammation. Indeed, the current results further underline the need for caution when drawing conclusions from nonsignificant behavioral results. Nevertheless, it remains unclear why behavior is unaffected by inflammation when underlying neurophysiological processes indicate significant alterations. One reason may be that overt behavioral effects of inflammation on attention require persistent (i.e., chronic) or severe inflammation before compensatory mechanisms that maintain performance fail to cope with weakened preparatory attention mechanisms. Typhoid vaccination, as used here, elicited only a 4-fold increase in IL-6 levels, whereas the endotoxin model, often used to experimentally study high inflammation, generally raises IL-6 levels 100-fold up to roughly 1000-fold (e.g., Draper et al., 2017; Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009; Grigoleit et al., 2011; Lasselin et al., 2016; Muscatell et al., 2016). Yet such studies fail to show evidence supporting

the possibility that inflammation affects overt attentional processes (reviewed in Bollen et al., 2017), suggesting that higher inflammation levels alone are not sufficient to induce overt attentional changes. The modest but reliable elevation of IL-6 observed in the current study is akin to the inflammation levels seen in subsets of depressed individuals, as well as in medical conditions such as diabetes and atherosclerosis (e.g., Dowlati et al., 2010; Maes et al., 1995; O'Brien, Scully, Fitzgerald, Scott, & Dinan, 2007). However, a difference between experimental models of inflammation and the aforementioned medical conditions is the duration of inflammation. Here it was up to 8 hours compared to the weeks, months or even years of elevated inflammation in these medical conditions. Considering that patients with chronic inflammation, such as those with cystic fibrosis and inflammatory bowel disease, show reduced attention performance as compared to healthy controls (Piasecki, Stanisławska-Kubiak, Strzelecki, & Mojs, 2017) raises the possibility that overt behavioral effects of inflammation on attention only occur when inflammation is persistent. Perhaps, with chronic inflammation neural compensatory mechanisms eventually fail, allowing behavioral indices dependent on attentional preparation processes to become sensitive to inflammatory states.

A potential pathway by which mild inflammation may modulate alerting attention processes is through modulation of the locus coeruleus (LC). Locus coeruleus-norepinephrinergic (LC-NE) activity has been notoriously involved in alerting of attention (Kane et al., 2017; Rajkowski, Kubiak, & Aston-Jones, 1994). The LC is especially vulnerable to toxins and infection due to a few factors. First, the LC has high energetic demands and may be more susceptible to mitochondrial oxidative stress (Sanchez-Padilla et al., 2014). Second, the LC has a high exposure to blood circulation making LC neurons more likely to take up toxicants (Pamphlett, 2014). Third, the LC's close proximity to the fourth ventricle may also expose it to toxins in cerebrospinal fluid (Mravec, Lejavova, & Cubinkova, 2014). Indirect support for a role of altered LC-NE activity in inflammation-associated cognitive change is the finding that aging is associated with selective reductions in the alerting component of attention (Gamboz et al., 2010; Jennings et al., 2007). Aging is also characterized by elevated inflammatory activity as well as reduced LC integrity (Capuron et al., 2014; Mather & Harley, 2016). While these data are in

support of the hypothesis that altered LC-NE activity may play a role in inflammation-associated changes in alerting, the current study did not directly assess this nor did it allow for localization of the origin of the effect as no structural MRI of the individual was acquired. Future studies, perhaps using MEG or EEG in combination with an individual's structural MRI, could inform the localization of this alpha difference. Unfortunately, we are not able to investigate the role gamma activity plays in our task since we recorded our EEG with a 30 Hz low-pass filter. Our rational for this setting was that it is difficult to disentangle broadband scalp muscle activity from neuronal cortical activity in the high frequency range with EEG (see review: Muthukumaraswamy, 2013). In addition, the data was collected in an unshielded room, a setting with lots of electrical noise. Therefore, we filtered the data to remove these high frequency signals. That being said, the role that inflammation might play in modulating gamma activity would indeed be a worthwhile aim for future studies using MEG, and techniques that could reduce muscle artefacts.

The current findings suggest that EEG correlates of attention may be used to detect subtle neurophysiological changes accompanying inflammation. Future research may assess whether preparatory alpha suppression can be used as a pre-clinical predictive marker to identify those at risk to develop inflammation-associated changes. The current findings are also important in light of the high prevalence of mild cases of flu, colds or minor infections that cause mild, acute inflammation. Previous research has found that high alpha power is a sensitive measure of reduced arousal, sleepiness, and mental fatigue (Käthner, Wriessnegger, Müller-Putz, Kübler, & Halder, 2014). However, we did not observe an overall difference in alpha activity at the baseline period between the placebo and inflammation condition, which suggests that the degree of modulation of alpha from baseline to an alerting state appears to be a key feature. It also provides no support for the notion that fatigue is a contributor to the alpha power effects we observed. As such, mild inflammatory states may enhance feelings of mental fatigue or cognitive stress due to the increase effort needed to perform otherwise effortless tasks.

In sum, the present study showed, for the first time, that transient low-grade inflammation triggers enhanced cognitive efforts to reinstate performance in attention. Future

studies may explore the potential of neurophysiological outcomes as a marker for early detection of inflammation-associated cognitive changes.

CHAPTER 8

Summary and General Discussion

Background

Inflammation is the immune system's response to harmful stimuli in an attempt to remove injurious stimuli and initiate the healing process (Piasecki, Stanisławska-Kubiak, Strzelecki, & Mojs, 2017). The immune system is regarded as a dynamic network that continuously remodels throughout a person's life as a result of the interaction between genes, lifestyles, and environments (Medzhitov, 2008). While the inflammatory response is vital to health, it is an aggressive response that incurs collateral damage to cells and tissues, and can have neurocognitive effects that tend to be unpleasant (i.e., nausea, malaise, fatigue, low mood) and may impair optimal performance in a number of domains (e.g., learning, social behaviour, attention). The latter effects, especially when prolonged, are also thought to contribute to increased vulnerability to psychopathology such as clinical and subclinical depression as well as to other debilitating problems such as fatigue (ter Horst et al., 2016). This thesis attempted to elucidate the relationship between low-grade inflammation and several cognitive processes. In particular, the chapters in the present thesis focussed on three cognitive domains; social cognition (Chapter 2 and 3), reward-motivated behaviour (Chapter 4 and 5), and attention and psychomotor processes (Chapter 6 and 7). The current chapter summarises the main findings and outlines directions for future research.

Summary of main findings and discussion

Social cognition involves mental operations that underlie social interactions (Dantzer et al., 2008). One key aspect of human social interaction is the ability to adequately interpret the mental state of other persons. Earlier research suggested that interpreting the mental state of another person becomes impaired by inflammation (Adolphs, 2001). However, empirical support is based on experiments that induce high levels of inflammatory activity, accompanied by discomfort and sickness; factors that, independent of inflammation, could account for a temporary social impairment. The results in Chapter 2 showed that emotion recognition is also impaired in low-grade inflammation induced using a typhoid vaccination; an inflammatory stimulus that appeared to be subliminal for most subjects, e.g., not detected through sickness

symptoms or other somatic perceptions, thus isolating effects of inflammation and social cognition. Apart from possible implications for psychopathology, which is the main focus of the existing literature, another relevant implication is that understanding the effects of inflammation on the central nervous system do not need be limited to generalisations from sickness, and that the usual term of "sickness behaviour" to describe such effects may be a misnomer because of the absence of physical malaise that more typically denotes sickness (e.g., fever, nausea). Given that in many daily life situations mild immune stressors (e.g., exercise, stress, foods, pollution, subclinical infections) can evoke mild fluctuations in inflammatory activity, healthy individuals may temporarily experience impairments in social emotion recognition. As a further implication, although admittedly still speculative at this point, is that healthy individuals as well as patients experiencing inflammation might be less in tune with their social environment, e.g., less sensitive in picking up social cues that guide essential social behaviours, which in the case of patients involve behaviours such as help-seeking behaviour.

While Chapter 2 showed that activation of the immune system can shape social perceptions, social perceptions may, in turn, also shape immune responsivity (Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015). For example, cognitive states (e.g., stressful experiences, negative mood states) have been shown to regulate responses of peripheral physiological pathways such as the hypothalamic-pituitary-adrenal (HPA) axis and release of cortisol (Dantzer, 2017), that can subsequently modulate activity of immune cells that regulate secretion of inflammatory cytokines (Joseph & Golden, 2017). In Chapter 3 evidence was presented showing that those who are feeling more lonely exhibited an enhanced response to an inflammatory stimulus (i.e., salmonella vaccination). These associations were found independent of negative mood and other factors commonly associated with social behaviour or health (e.g., sleep, anxiety).

In the recent years, loneliness is increasingly recognised for its disruptive effects on health. For example, loneliness has been linked to poor mental health (e.g., depression, substance use) and lonelier individuals show a 30% increased risk of stroke, myocardial infarction, and mortality (Messay, Lim, & Marsland, 2012). The findings from Chapter 3 thus

present a potential biobehavioural pathway linking loneliness to impaired health. More generally the results of Chapter 2 and 3 combined indicate bidirectional links between social cognition and immune system functioning.

Research indicates that activity of the immune system may play a role in responding to appetitive and aversive stimuli (i.e., motivated behaviour) (Holt-Lunstad et al., 2015; Steptoe et al., 2013; Valtorta et al., 2016). However, much is still unclear about which specific aspects of motivated behaviour are sensitive to effects of inflammation. Chapter 4 and 5 tested several independent hypotheses about how low-grade inflammation may affect motivation, focussing on the non-social domain. The results presented in Chapter 4 showed that experimentally induced inflammation had a selective impact on expressions of motivated learning; the rate at which these reward contingencies were acquired was slower. However, once these associations were well-learned, performance was relatively stable suggesting that the motivation to maintain adequate performance was intact once behaviour could be executed with less effort. However, when reward contingencies were suddenly changed, slower adaptation was observed in those with inflammation, suggestive of reduced flexibility. Motor slowing was also observed with inflammation. Sensitivity to the type of reinforcer, i.e. reward, loss, large or small outcome, remained unaffected. As a tentative interpretation is suggested that effortful behaviours are specifically sensitive to the effects of inflammation.

In order to understand whether the effects found with experimentally induced low-grade inflammation replicate to more naturalistic low-grade inflammatory states, in Chapter 5 groups of individuals that generally show basal low-grade inflammatory activity were assessed, i.e., older individuals and individuals with high body mass index (BMI) and these were compared to their young and low BMI counterparts, respectively. Table 1 presents an overview and comparison of these results. Each of these groups indeed showed elevated inflammation in the expected direction. Assessment of these groups allowed a potential conceptual replication of the findings presented in Chapter 2 (emotion recognition) and Chapter 4 (motivated learning). The results of age on motivated learning showed striking similarities with the effects found with vaccine-induced low-grade inflammation; older individuals and transient low-grade

inflammation were similarly associated with a reduced rate of learning, whereby performance remained relatively stable once reward contingencies were acquired, and slower adaptation to the change in reward contingencies as well as motor slowing was observed. Moreover, IL-6 levels were again negatively associated with some of these expressions of motivated learning, which were found independent of age and health and demographic variables. Based on these findings it is tentatively concluded that low-grade inflammation may be associated with transient ageing-like effects on motivated learning.

Replicating the findings presented in Chapter 4 (motivated learning), the results in Chapter 5 further showed that age- and BMI-related chronic low-grade inflammation was negatively associated with emotion recognition, which likewise was independent of health and demographic factors. High BMI was also associated with reduced emotion recognition but in an age-dependent manner: young high BMI individuals performed worse on emotion recognition as compared to young low BMI individuals, while the opposite was found in older individuals. Taken together these results suggest that both acute low-grade inflammation and states associated with protracted low-grade inflammation have an overlapping impact on motivated behaviour and emotion recognition.

Considering the multifactorial nature of cognitive processes, it is a considerable challenge to interpret the relationship between inflammation and cognitive processes. For example, motivated learning may be impaired as a result of reductions of other supportive cognitive processes such as reduced working memory capacity, an inability to focus attention, reduced motor control, other factors, or a combination of these. Chapter 6 focussed on a critical brain function that underpins many higher order cognitive processes which is visual attention. Three main roles of the attention system have been identified: (1) preparing the brain for upcoming events (alerting); (2) preparing where to look for task relevant information (orienting); and (3) prioritising task-relevant information (executive control). In Chapter 6, the relationship between age- and BMI-related inflammation and these distinct, yet interacting, attention functions as well as psychomotor speed were assessed. Results found no evidence for an effect of chronic low-grade inflammation on visual attentional processes. Psychomotor speed,

on the other hand, was negatively and significantly correlated with inflammation, i.e., decreased psychomotor speed was associated with higher inflammation levels. Furthermore, mediation analyses showed that inflammation fully accounted for psychomotor slowing in those with high BMI, and inflammation partly accounted for age-related psychomotor slowing. These results suggest that BMI-related inflammation rather than BMI reduces psychomotor speed. Hence, a possible implication of these findings is that reducing inflammation in those with a high BMI may improve psychomotor speed. A typical example of the importance of psychomotor speed is fast braking in the face of a sudden, unexpected road obstacle. Psychomotor speed is not only necessary for rapidly changing situations like driving, but is also essential for fluent action and decision-making in more mundane tasks such as social discourse, cooking, or using digital media. Indeed, deficits in these functions are associated with poor mental health and lower emotional well-being (Boyle et al., 2019; Draper et al., 2017; Eisenberger et al., 2010; Lasselin, Treadway, et al., 2016).

In Chapter 7, using vaccination as a method to induce acute low-grade inflammation, behavioural data again provided no evidence for an impact of inflammation on attentional functioning. However, electrophysiological data showed a distinguishable neurophysiological vulnerability of the neural network that underpins attentional alerting functions. More specifically, inflammation-induction produced greater alerting-cue induced suppression of oscillatory alpha activity, which was also correlated with the magnitude of the inflammatory response. The amount of oscillatory activity in the alpha band to the onset of visual attention cues is thought to provide neurophysiological information of mental preparatory effort (Fink, Grabner, Neuper, & Neubauer, 2005; Keil, Mussweiler, & Epstude, 2006). Inflammation did not affect the neural network related to orienting of attention (i.e., alpha lateralisation) or executive control (i.e., frontal theta activity). Based on these results, and in conjunction with the results in other chapters, it is proposed that inflammation requires individuals to invest greater cognitive effort in order to maintain adequate behavioural performance. Whether such enhanced investment of cognitive effort reflects a general effect of inflammation or whether it is an alerting-specific effect remains to be determined.

Future directions

The results presented in the current thesis undergird the proposal that activities of the immune system may be a determinant of what people think, feel, and do. Further, these effects may exist even outside the context of illness. That is, the current results may be applicable to clinical conditions characterised by low-grade inflammation but may also be relevant for healthy individuals experiencing fluctuations in inflammatory activity within a normal range, e.g., due to exposure to mild every-day immune-activating stimuli such as physical exercise, stress, pollution, and subclinical infections or more protracted exposures like elevated BMI.

Although all individuals were given a similar inflammatory stimulus not all individuals appeared equally impacted. In Chapter 3 it was shown that individual variation in subjective loneliness before an inflammatory insult predicted the magnitude of the inflammatory response to vaccination. Extrapolating, vaccination may be used as a standardised stimulus to investigate dysregulations in the inflammatory response system. Such dysregulations have for example been proposed for depression, but this proposal is solely based on the finding that basal inflammatory levels are elevated. A further and perhaps stronger test of this idea may thus be provided by studies that investigate if clinical depression predicts an exaggerated response to such standardised stimuli. A similar reasoning may apply to other conditions such as chronic fatigue, for which comparable immune-behavioural models have been proposed.

Taking the above one step further, at present it is also unclear how psychological differences (e.g., social independence, subjective valuation of money) influence down-stream effects of inflammation such as its cognitive effects (e.g., emotion recognition, monetary reward learning). For example, are individuals who heavily rely on close others in daily life, compared to those who regard themselves as more independent, more vulnerable to develop inflammation-induced mood and behavioural changes? Or does lack of money (i.e., poverty), which tends to impose an additional cognitive load, result in an exacerbated response to monetary reward when inflamed? Hence, research aimed at identification of risk factors to develop inflammation-associated cognitive dysfunction is imperative and may, ideally, lead to development of screening measures and early intervention strategies.

It has become clear that inflammation modulates selective aspects of motivation. Most of the existing research focussed on how inflammation changes the reinforcing properties of money. However, other evolutionary relevant reinforcers such as food and sex remain largely unexplored, even though changes in appetite and sex drive are often prevalent in chronic disease populations as well as in depressed patients who exhibit immune dysregulation (Miller & Raison, 2016; Simmons et al., 2018).

The research presented in this thesis may be relevant to research on fatigue. The data, in line with findings by others, propagated the idea that low-grade inflammation affects the comparative assessment of cost and effort. Motivational inputs, such as expected rewards and costs, modulate the exerted effort. A flawed integration of expected costs and benefits has been suggested to underlie perceived fatigue. While fatigue is an adaptive physiological process that signals the body to rest after intense work, sleep loss or illness, pathological fatigue, when the adaptive nature of fatigue has been lost, is experienced as a highly disabling symptom that is commonly present in various medical and psychiatric conditions (Karshikoff et al., 2017). The ubiquitous nature, heterogeneous etiology, and multidimensional manifestation of fatigue impose a challenge to health care professionals in treating and managing this debilitating symptom. Fatigue can be expressed as physical fatigue, lack of motivation, mental fatigue (i.e., reduced concentration) or emotional tiredness. These domains can be measured subjectively through self-report measures, and objectively using motivational, physical, cognitive or neurophysiological measures. Even though fatigue is foremost a subjective experience, there are at least two limitations to solely examining symptom reports. First, subjective fatigue indicates the presence of a problem, whereas early intervention requires availability of markers that precede the problem, and cognitive testing may fill that void. Second, symptom reports present phenotypical endpoints that provide little information on underlying (neurocognitive) processes. For example, a self-reported symptom such as 'low motivation' may reflect a reduction of working memory capacity that makes planning and execution of common daily tasks unusually effortful, or a disruption of central reward systems, causing actions which are normally pleasant (e.g., visiting a friend) now become less rewarding. As such, this research

field would benefit from research aimed to identify predictive markers of fatigue that can help anticipate the development of fatigue and ultimately allow for pre-emptive interventions.

Given that objective measures of fatigue (e.g., behaviours, cognitive responses) do not map in a clear cut manner on subjective reports of fatigue (Karshikoff et al., 2017), it is preferable to combine objective and subjective measures to assess the different dimensions of fatigue. Potentially relevant here is the time course of development of fatigue. For example, objective measures of motivational fatigue perhaps detect early subtle changes in fatigue while subjective fatigue may only occur at later stages. However, at present it is unclear whether objective measures of fatigue may precede development of subjective fatigue. One marker that is worth exploring was presented in Chapter 8, in which was shown that inflammation induced changes in alpha power suppression, a measure reflecting mental preparatory effort (Holtzer et al., 2017; Leavitt & DeLuca, 2010), while behaviour remained unaffected. It has been proposed that greater investment of mental effort can result in increased subjective fatigue (Fink et al., 2005; Keil et al., 2006; Sawaki et al., 2015). Cognitive and neurophysiological markers could potentially be relevant for early detection of subjective fatigue and potentially related cognitive complaints.

Concluding remarks

The studies presented in this dissertation assessed the role of low-grade inflammation in social behaviour, motivated learning, and basic cognitive processes such as attention and psychomotor speed. The notion that inflammation is a regulator of these cognitive functions was corroborated and extended. Analyses in this thesis commenced with the observation that acute low-grade inflammation negatively affected emotion recognition as measured by the Reading the Mind in the Eyes Test. This finding was then replicated in more naturalistic states of chronic low-grade inflammation, i.e., in age- and BMI-related low-grade inflammation. Subsequently it was shown that subjective loneliness before a mild inflammatory insult predicted the magnitude of the inflammatory responses, providing support for a bidirectional link between social behaviour and inflammation. It was further shown that selective aspects of motivated learning

were modulated by low-grade inflammation while leaving others intact. The observations in the chapters on motivated learning lead to two tentative proposals: First, acute low-grade inflammation may transiently induce ageing-like effects on motivated learning, and, second, low-grade inflammation may specifically impair cognitively effortful behaviours. The latter was supported by the observation that although no evidence was found for inflammation-associated changes in overt attentional performance, inflammation induced changes in a neurophysiological marker of mental preparatory effort.

As a closing note, historically, the term sickness behaviour has been borrowed from animal research, based on experiments in which animals receive immunological stressors that evoke potent physiological and behavioural changes, and for which inflammation is thought to be a key biological mediator (Otto, Zijlstra, & Goebel, 2018). However, the use of the term sickness behaviour is perhaps less appropriate when generalised to conditions with low-grade inflammation. E.g., we, and others, have used typhoid vaccination to induce low-grade inflammation, which is associated with (neuro)psychological effects subsumed under sickness behaviour, but without any of the physical malaise that more typically denotes 'sickness' (e.g., fever, nausea). This argument may similarly apply to other human data in which low-grade inflammatory activity is present without overt or detectable sickness (e.g., such as in depression or autoimmune disease in remission), but are still linked with neuropsychological phenomena like fatigue, anhedonia, and motor slowing (e.g., Dantzer & Kelley, 2007). Hence, there seems reasonable ground to revaluate whether the term sickness behaviour remains appropriate, or whether we should consider new terminology (e.g., inflammation-associated cognitive changes) that better capture the non-illness related effects of immune mediators, which we now know also regulate normal 'everyday' cognitive functions.

Table 1. Overview of age and BMI and acute and chronic low-grade inflammation effects on emotion recognition, motivated learning measures and attention and psychomotor slowing; RT = response time; – = not affected by inflammation/age/BMI, 🡻 = lower performance with greater inflammation/older age (vs. young)/high BMI (vs. low BMI) unless otherwise stated, 🡹 = higher performance with greater inflammation/older age (vs. young)/high BMI (vs. low BMI) unless otherwise stated, × = not assessed.

	Acute low-grade inflammation	Chronic low-grade inflammation	Ageing	BMI
Emotion recognition (accuracy) (Chapter 2 & 3)	🡻	🡻	–	🡻 in young, 🡹 in older
Motivated learning (Chapter 4 & 5)				
Ability to learn				
Mean response time (RT)	🡻	–	🡻	–
Mean accuracy	–	🡻	🡻	–
Peak learning	–	🡻	🡻	–
Rate of learning	🡻	🡻	🡻	–
Stable performance	–	–	–	–
Flexibility	🡻	–	🡻	–
Sensitivity to the reinforcer				–
Valence	–	–	–	–
Magnitude	–	–	–	–
Attention (Chapter 6 & 7)				
Behavioural (RT)				
Alerting	–	–	🡻	–
Orienting	–	–	–	–
Executive control	–	–	🡻young vs. older	–
Neurophysiological				
Alerting (alpha suppression)	🡻	×	×	×
Orienting (ALI)	–	×	×	×
Executive control (frontal theta)	–	×	×	×
Psychomotor speed (Chapter 6 & 7)	–	🡻	🡻	🡻

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