Role of neuroinflammation in an amyloid-beta model of Alzheimer's disease and the identification of possible biomarker

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PREFACE

The experimental work described in this thesis was conducted at the University of KwaZulu-Natal (Durban, South Africa), under the supervision of Professor Musa Mabandla.

This work has not been submitted in any form for any degree to any tertiary institution, where use has been made of the work of others, it is duly acknowledged in the text.

---------- 9/12/2020 ----------

Oluwadamilola Faith Shallie **Date**

As the candidate's supervisor, I agree to the submission of this thesis.

---------- 9/12/2020 ----------

Professor Musa Mabandla **Date**

DECLARATION

I, Oluwadamilola Faith Shallie declare that:

(i) The research reported in this thesis, except where otherwise indicated, is my original work.

(ii) This thesis has not been submitted for any degree or examination at any other university.

(iii) This thesis does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.

(iv) That my contribution to the project was as follows:

Identification of research topics, experimental design, execution, data analysis and interpretation, manuscript and thesis write-up.

(v) That the contributions of others to the project were as follows:

Professor Musa Mabandla (supervisor) has reviewed the entire project and the write-up of the manuscripts and thesis. This project was funded by the College of Health Sciences, UKZN (Grant no.: 636801).

9/12/2020

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Oluwadamilola Faith Shallie **Date**

DEDICATION

This thesis is dedicated to God Almighty, my irreplaceable husband and my lovely kids.

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To my supervisor, Professor Musa Mabandla, I deeply appreciate your patience, excellent mentorship and guidance throughout the project.

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
^{o}c	Degree celsius
µ	Micro
µg	Micrograms
µl	Microlitre
Aβ	Amyloid-beta
AD	Alzheimer's disease
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ANOVA	Analysis of variance
BACE1	Beta-site amyloid precursor protein cleaving enzyme 1
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
cm	Centimeter
ATP	Adenosine triphosphate
C	Celsius
Ca^{2+}	Calcium ion
CD33	Cluster of differentiation 33
cDNA	Complementary deoxyribonucleic acid
CNS	Central nervous system
COX-2	Cyclo-oxygenase 2
CS	Conditioned stimulus
CSF	Cerebrospinal fluid
Da	Dalton
DAP12	DNAX-activating protein of 12kDa
DHA	Docosahexaenoic acid
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
EOAD	Early-onset Alzheimer's disease
FAD	Familial Alzheimer's disease
FCT	Fear conditioning test
g	Gram
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein

GSH	Glutathione
h	Hour
hrs	Hours
HRP	Horseradish peroxidise
IBA-1	Ionized calcium-binding adaptor molecule 1
iNOS	Inducible nitric oxide synthase
IP3	Inositol triphosphate
i.p.	Intraperitoneal
IL	Interleukin
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
kDa	Kilodaltons
kg	Kilogram
K^+	Potassium ion
L	Litre
LOAD	Late-onset Alzheimer's disease
MAPK	Mitogen-activated protein kinase
MCI	Mild cognitive impairment
MDA	Malondialdehyde
mg	Milligram
min	Minute
miRNA	MicoRNA
mL	Millilitre
mm	Millimetre
mmol	Millimole
mol	Mole
MWM	Morris water maze
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
Na^+	Sodium ion
NADPH	Nicotinamide adenine dinucleotide phosphate
NBF	Neutral buffered formalin
NF-kB	Nuclear factor kappa-light-chain-enhancer
NFTs	Neurofibrillary tangles
NGF	Nerve growth factor
NO	Nitric oxide

NPY	Neuropeptide Y
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PET	Positron emission tomography
PI3K	Phosphoinositol-3-kinase
PKC	Protein kinase C
PLA2	Phospholipase A2
PLC	Phospholipase C
PS1	Presenilin 1
PS2	Presenilin 2
PSEN	Presenilin
SAD	Sporadic Alzheimer's disease
SD	Sprague-Dawley
SNPs	Single nucleotide polymorphisms
SEM	Standard error of mean
SHP-1	Src homology-2- containing protein tyrosine phosphatase 1
SHP-2	Src homology-2- containing protein tyrosine phosphatase 2
siRNA	Small interfering RNA
SOD	Superoxide dismutase
SPs	Senile plaques
Syk	Tyrosine-protein kinase
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumour necrotic factor
TREM2	Triggering receptor expressed on myeloid cells 2
US	Unconditioned stimulus

PUBLICATIONS AND PRESENTATIONS

Published Manuscripts

Oluwadamilola F. Shallie, Ernest Dalle, Musa V. Mabandla. Memory decline correlates with increased plasma cytokines in amyloid-beta (1-42) rat model of Alzheimer's disease. Neurobiology of Learning and Memory, https://doi.org/10.1016/j.nlm.2020.107187. See chapter 3 and appendix I

Oluwadamilola F. Shallie and Musa V. Mabandla. Amyloid-beta (1-42) lesion of CA1 rat dorsal hippocampus reduces contextual fear memory and increases expression of microglial genes regulating neuroinflammation. Behavioural Brain Research, https://doi.org/10.1016/j.bbr.2020.112795. See chapter 4 and appendix II.

Manuscripts under Peer review

Oluwadamilola F. Shallie and Musa V. Mabandla. Progressive interdependence between neuroinflammation and oxidative stress in an amyloid-beta (1–42) rat model of Alzheimer's disease. *Under Review:* Inflammation (Manuscript number: IFLA-D-20-00599). See chapter 2.

Oluwadamilola F. Shallie and Musa V. Mabandla. MicroRNA-107 as a possible plasma biomarker in an amyloid-beta (1-42) rat model of Alzheimer's disease. *Under Review:* Neurotoxicity Research (Manuscript number: NTRE-D-20-00239). See chapter 5.

Conference Presentations

Oluwadamilola F. Shallie, Ernest Dalle, Musa V. Mabandla. Memory decline correlates with increased plasma cytokines in amyloid-beta (1-42) rat model of Alzheimer's disease. Annual Laboratory Medicine and Medical Sciences (LMMS) Research Symposium 2019, 6th September 2019, Westville, Durban, South Africa. See appendix VII.

Oluwadamilola F. Shallie, Musa V. Mabandla. Progressive interdependence between neuroinflammation and oxidative stress in an amyloid-beta (1-42) rat model of Alzheimer's disease. Annual College of Health Sciences (CHS) Research Symposium 2019. K-RITH Tower Building, Nelson R Mandela School of Medicine Campus, University of KwaZulu-Natal, Durban, South Africa.

THESIS OUTLINE

The principal findings of this PhD research study have been compiled into an article format and presented as a thesis by manuscript.

Chapter 1: Provides background information with a brief review of selected topics relevant to the study. Study aims and objectives, hypotheses and potential benefits of this research are also highlighted.

Chapter 2: This chapter is the first manuscript which provides information on the progressive interdependence between neuroinflammation and oxidative stress in an amyloid-beta (1–42) rat model of Alzheimer's disease. *Under Review:* Inflammation (Manuscript number: IFLA-D-20-00599).

Chapter 3: This chapter is the second manuscript which provides information on how Memory decline correlates with increased plasma cytokines in amyloid-beta (1-42) rat model of Alzheimer's disease. Neurobiology of Learning and Memory, https://doi.org/10.1016/j.nlm.2020.107187. See appendix I.

Chapter 4: This chapter is the third manuscript which provides information on how amyloid-beta (1-42) lesion of CA1 rat dorsal hippocampus reduces contextual fear memory and increases expression of microglial genes regulating neuroinflammation. Behavioural Brain and Research, https://doi.org/10.1016/j.bbr.2020.112795. See appendix II.

Chapter 5: This chapter is the fourth manuscript which provides information on the identification of MicroRNA-107 as a possible plasma biomarker in an amyloid-beta (1-42) rat model of Alzheimer's disease. *Under Review:* Neurotoxicity Research (Manuscript number: NTRE-D-20-00239).

Chapter 6: This chapter provides synthesis of the research findings and general conclusions. It also highlights limitations and makes recommendations for future studies.

ABSTRACT

Introduction

Alzheimer's disease (AD) is the most common form of neurodegenerative disorder that results in dementia. It currently affects 75 million people worldwide and is predicted to affect as many as 135 million people by 2050. Despite considerable research, current medication provides only modest relief to symptoms and does not cure the underlying disease. The delay in identifying a definitive cure is probably due to the scant knowledge of the cellular and molecular mechanisms implicated in its pathogenesis. However, the role of neuroinflammation has been acknowledged. Neuroinflammation is generally due to sustained activation of the brain's resident immune cells, including microglia and astrocytes. Although the importance of amyloid-beta (Aβ) in the aetiology of AD has recently come into question, there is still consensus that Aβ is closely related to AD. Therefore, a reappraisal of the amyloid hypothesis focusing on the role of neuroinflammation will open new avenues for identifying novel targets for AD treatment. In this study, we sought to assess neuroinflammation and identify possible biomarkers in an Aβ(1-42) rat model of AD over a progressive period of time.

Materials and Methods

Male Sprague-Dawley rats were used in all experiments. The animals were randomly divided into a vehicle group of rats that were infused with phosphate-buffered saline (PBS) and an Aβ(1–42) group that was lesioned with the Aβ(1–42) peptide. Each group was further sub-divided into four groups (Day 3 group: animals euthanised 3 days after infusion; Day 7 group: animals euthanised 7 days after infusion; Day 10 group: animals euthanised 10 days after infusion, and Day 14 group: animals euthanised 14 days after infusion). Animals were subjected to neurobehavioral tests pre and post-infusion. The Morris water maze test was used to assess spatial learning and memory and the fear conditioning test was used to assess associative fear learning and memory. After euthanisation, whole blood sample acquired aseptically from both the vehicle and Aβ(1–42) lesioned group of rats was collected into ethylenediaminetetraacetic acid (EDTA) coated tubes for cytokine, oxidative stress markers and microRNA assays using multiplex immunoassay, spectrophotometric and real-time polymerase chain reaction analysis respectively. The excised whole brain was post-fixed in 10% neutral buffered formalin (NBF) for immunofluorescence and immunohistochemical analysis. Other brain tissue was placed in frozen 0.9% saline slush before the hippocampus was carefully dissected out and placed in a bio-freezer at -80 ºC post dissection. The tissue was later used for messenger RNA analysis using the real-time polymerase chain reaction technique.

Results

We observed impaired spatial and reduced contextual fear memory, which was exacerbated as the post-lesion days increased. Our results also showed increased expression of ionized calcium-binding adaptor molecule 1 (IBA-1), glial fibrillary acidic protein (GFAP), and beta-site amyloid precursor protein cleaving enzyme1(BACE1) antibodies and upregulated mRNA expression levels of cluster of differentiation 33 (CD33) and triggering receptor expressed on myeloid cells 2 (TREM2) genes in the

hippocampus, as well as downregulated expression of miRNA107 in the plasma. In addition, our results showed a positive relationship between the activated glial cell markers and lipid peroxidation. Furthermore, elevated plasma concentration of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) with a concomitantly lowered levels of the anti-inflammatory cytokine (IL-10) in the Aβ(1–42) lesioned rats was observed when compared to the vehicle groups. A negative correlation between the decline in spatial memory and plasma levels of the pro-inflammatory cytokines and a positive correlation between the decline in spatial memory and plasma concentration of the anti-inflammatory cytokine was observed.

Conclusion

Our findings implicate cellular and molecular mechanisms, as shown by prolonged and progressive activation of the glial cells, resulting in a bidirectional interplay between neuroinflammation and oxidative stress. These interconnections result in the concomitant release of brain cytokines as a secondary response to the hallmarks of AD, which impacts both neural circuit activity and expression of microglial genes regulating neuroinflammation, indicating dynamic crosstalk between the immune and nervous systems. These interactions facilitate the understanding of AD's pathogenesis and provide the basis for an integrative approach to validate the role of neuroinflammation in memory processes and, importantly, identify a potential biomarker for the early diagnosis of Alzheimer's diseases. As a contribution to knowledge, this study unveils the connection between memory decline and plasma cytokine concentration, as well as the relationship between genes regulating neuroinflammation in AD. Therefore, it is incontrovertible that neuroinflammation holds a pivotal role in AD pathology.

Keywords: Alzheimer’s disease; Neuroinflammation; Glia; Cytokines; Microglial genes; Memory.

CHAPTER 1

Introduction and Literature Review

1.1 Background

Alzheimer's disease (AD) which is the most common form of neurodegenerative disorder leading to dementia, currently affects 75 million people worldwide with a predicted increase to 135 million people by 2050 (Shi et al., 2018, Leidinger et al., 2013, Brookmeyer et al., 2007). Due to the high prevalence and the generation of a substantial socioeconomic burden, Alzheimer's disease is one of the significant unmet health concerns in the 21st century (Hurd et al., 2013). Alzheimer's disease is perceived as a biological and clinical continuum. It starts with the preclinical stage, manifesting with molecular alterations in the absence of clinical dementia (Anand et al., 2014, Cummings et al., 2016), progresses through mild cognitive impairment (MCI), prodromal Alzheimer's disease towards mild, moderate and severe dementia stages (Aisen et al., 2017, Jack Jr et al., 2018).

Currently, there are several forms of Alzheimer's disease with early-onset (before age 65) accounting for up to 5% of all cases (Bettens et al., 2013). Most of the early-onset cases are familial, a rare form of Alzheimer's disease caused by mutations in the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) or *PSEN2* genes (Bettens et al., 2013). Patients with non-familial (sporadic) early-onset Alzheimer's disease have no reliable family history. Usually, they have an older age of onset than patients with familial early-onset (Joshi et al., 2012). Late-onset sporadic Alzheimer's disease (from 65 years of age) is the most common form, accounting for about 95% of all cases. The pattern of molecular alterations and associated neurodegeneration in the brain changes during the disease progression, with the hippocampus being the earliest and most severely affected (Dhikav and Anand, 2007, Frisoni et al., 2010). The main pathological hallmarks of Alzheimer's disease are senile plaques (SPs) consisting of accumulated β-amyloid peptides (Aβ) and neurofibrillary tangles (NFTs) primarily containing highly phosphorylated tau protein (Baranello et al., 2015). The two most commonly accepted hypotheses, the Aβ and the tau hypotheses, are also based on these two pathological characteristics.

Despite considerable amount of research, current medication provide only modest relief to symptoms and does not cure the underlying disease (Yiannopoulou and Papageorgiou, 2013). This failure is probably due to the scant knowledge of the cellular and molecular mechanisms implicated in AD pathogenesis. However, it is now well recognized that AD is a multifactorial disorder impacted by other factors in its pathogenesis and progression. These include prominent activation of inflammatory and innate immune responses (Heppner et al., 2015, Zhao and Lukiw, 2018) as well as calcium dyshomeostasis, oxidative stress, mitochondrial damage, and alterations in the cell cycle regulatory mechanisms (Zhao and Lukiw, 2018, Pchitskaya et al., 2018, Wojsiat et al., 2018). Among these, the role of neuroinflammation has been confirmed (Ferreira et al., 2014, Prokop et al., 2013, Heneka et al., 2014, Kim et al., 2018). This role is not exclusively attributable to innate immunity which is constituted

by microglia in the brain. It is also caused by other brain resident cells like the astrocytes which are found throughout the central nervous system and diverse and critical roles in both homeostasis and pathophysiology of the central nervous system (Verkhratsky et al., 2014, Lecuyer et al., 2016, Steardo Jr et al., 2015).

Although the primacy of Aβ in the aetiology of AD has been questioned (Morris et al., 2018), there is still consensus that Aβ is linked to AD pathology (Murpy and LeVine III, 2010). Therefore, a reappraisal of the amyloid hypothesis focusing on the role of neuroinflammation in a beta-amyloid model of AD will open new avenues for identifying biomarkers for early diagnosis and therapeutic targets.

1.2 Epidemiology

Alzheimer's disease accounts for 60 to 80% of dementia cases (Barker et al., 2002). Other common causes of dementia include Parkinson's disease, Lewy body dementia, vascular dementia, and frontotemporal lobar degeneration, with each of these accounting for between 5 and 10% of cases (Barker et al., 2002). Alzheimer's disease is a financially devastating and debilitating disease expected to increase into the middle of the century, and it is estimated that more than 135 million individuals will be affected by 2050 (Shi et al., 2018, Leidinger et al., 2013, Brookmeyer et al., 2007). Aging is the most substantial risk factor for Alzheimer's disease; every 6.3 years, the incidence for all dementias doubles from 3.9 per 1000 for ages 60–90 to 104.8 per 1000 above age 90 (report, 2015, Nelson et al., 2011). The prevalence is estimated at 40% for those over the age of 80 and 10% for individuals over 65 years, while life expectancy varies from 7 to 10 years for patients whose conditions are diagnosed in their 60s and early 70s to about 3 years or less for patients whose conditions are diagnosed in their 90s (Zanetti et al., 2009).

1.3 Aetiology and Pathogenesis of Alzheimer's Disease

The rare familial form of Alzheimer's disease accounts for less than 1% of cases and is due to mutations in the amyloid precursor protein (APP), presenilin 1 (PSEN1), or PSEN2 genes (Ryman et al., 2014). Dominantly inherited FAD can present as early as age 20, with the average age of onset being 46.2 years (Ryman et al., 2014). Early-onset Alzheimer's disease (EOAD) presents atypically before age 65 in cases (Mendez, 2017). This form of Alzheimer's disease is slightly more common than FAD cases and accounts for fewer than 5% of the pathologically diagnosed Alzheimer's disease cases (Bettens et al., 2013). The late-onset Alzheimer's disease (LOAD) is sporadic, although genetic risk factors have been identified, most notably the apolipoprotein E gene (APOE) (Hardy and Higgins, 1992). Age, APOE4 genotype, and family history in a first-degree relative, present the most significant risks of developing Alzheimer's disease (Verghese et al., 2011). The pathophysiology of Alzheimer's disease is linked to several factors, such as cholinergic dysfunction, amyloid/tau toxicity, neuroinflammation, and oxidative

stress/mitochondrial dysfunction (Mohamed et al., 2016). Among these factors, the critical role of neuroinflammation has been confirmed (Ferreira et al., 2014, Prokop et al., 2013, Heneka et al., 2014, Kim et al., 2018).

1.4 The role of neuroinflammation in the pathogenesis of Alzheimer's Disease

The activation of different brain cells drives the neuroinflammatory pathophysiology of Alzheimer's disease (Verkhratsky et al., 2015). Evidence suggests that glial cells are primarily involved in this phenomenon, as they immediately respond to brain injuries, restoring brain physiology by activating a series of repair mechanisms (Verkhratsky et al., 2015). These cells are highly heterogeneous and are responsible for many essential brain functions (Verkhratsky et al., 2015). Microglia are the first responders for immune defence in the brain, while astrocytes are an essential neuro-supportive cell type (Fakhoury, 2018). Astrocytes control the environment by regulating pH, ion homeostasis, oxidative stress, and blood flow (Deitmer and Rose, 1996, Iadecola and Nedergaard, 2007). Also, astrocytes modulate information processing and signal transmission, synaptogenesis, regulate neural plasticity, provide trophic and metabolic support to neurons (Perea et al., 2009, Khakh and Sofroniew, 2015). Interestingly, human autopsy and data from animal models showed that senile plaques and neurofibrillary tangles cause an immune response in the brain and co-localize around the activated glial cells (Verkhratsky et al., 2014).

Astrocyte and microglia acquire a reactive phenotype (Verkhratsky et al., 2014) and rapidly act in response to pathology undergoing essential changes in their morphology, molecular presentation (and functioning (Sofroniew and Vinters, 2010, Scuderi et al., 2013). Such activation is fundamentally a protective response aimed at removing injurious stimuli (Sofroniew and Vinters, 2010, Scuderi et al., 2013). Astrocytes create a protective barrier around plaques by modulating neurotoxicity, degrading, internalising, and removing Aβ (Paradisi et al., 2004, Thal, 2012, Mathur et al., 2015). However, uncontrolled activation beyond physiological control causes detrimental effects and override the beneficial ones (Mrak and Griffinb, 2001, Tuppo and Arias, 2005). At this stage, the synthesis of different cytokines and mediators by glial cells promotes neuroinflammation (Mrak and Griffinb, 2001, Tuppo and Arias, 2005) (see Figure 1). This is a characteristic event of AD brains and is called reactive gliosis (Fakhoury, 2018). Studies have demonstrated relevant action of glial cells from an early stage of the pathogenic process, with indications that the cycle becomes independent on Aβ presence, neural dysfunction, cell death, and disease progression (Gandy and Heppner, 2013, Sudduth et al., 2013, Holmes et al., 2009). Chronic inflammation caused by the release of proinflammatory molecules acts both in an autocrine and paracrine way (Fakhoury, 2018). This action is by fostering the perpetuation of reactive gliosis and neuronal death (Scuderi et al., 2014, Block and Hong, 2005). The release of inflammatory mediators and reactive oxygen species result in neuronal death (Christov et al., 2004).

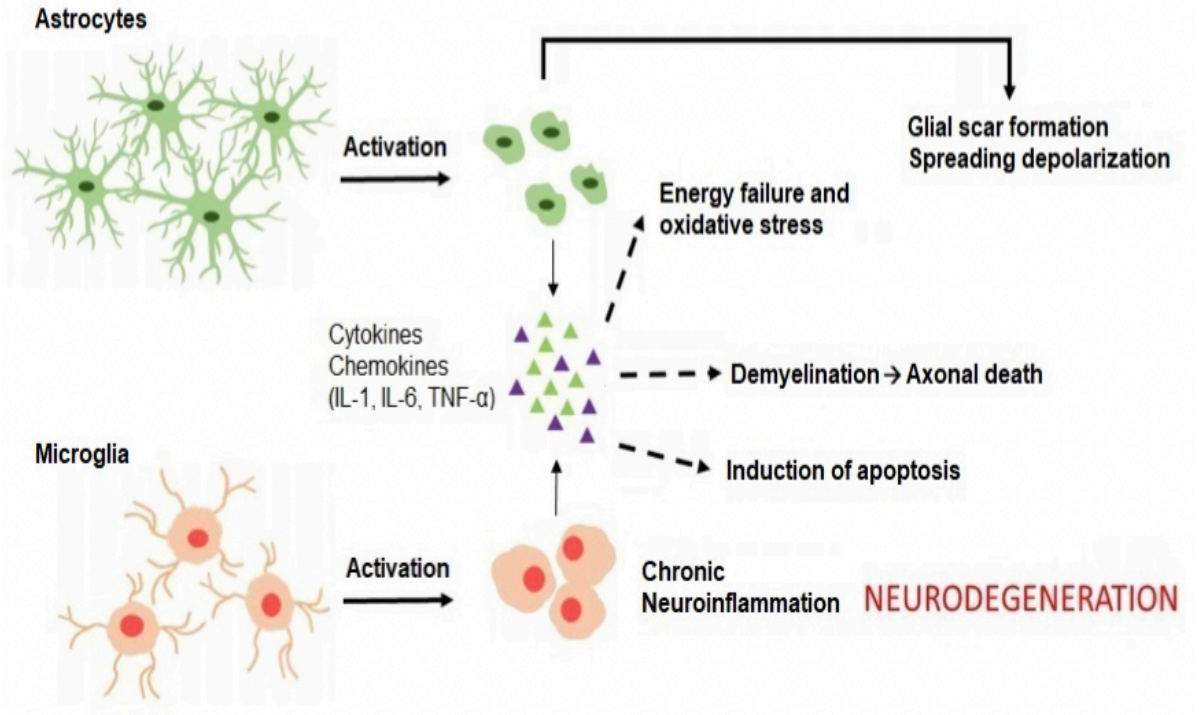

Figure 1: Detrimental effects of glial-mediated inflammation. Activation of microglia and astrocytes by Aβ or following a signal of damage leads to the secretion and release of inflammatory chemokines and cytokines, including IL-1, IL-6, and TNF-α. These pro-inflammatory elements trigger a cascade of events, such as oxidative stress, demyelination and apoptosis, which eventually lead to neurodegeneration and cognitive decline. Reactive astrocytes also contribute to scar formation around injured tissue by accumulating around amyloid plaques. Adopted from (Fakhoury, 2018).

1.4.1 Cell mediators of neuroinflammation

Microglia and astrocytes are the major glial cell types that respond to disease stressors by innate immune responses such as the production and release of inflammatory mediators (Verkhratsky et al., 2015). Initially regarded merely as structural support for neurons, evidence indicates that glial cells are active and responsive to environmental changes (Fakhoury, 2018). Their processes become hypertrophied when activated and produce multiple inflammatory factors, including cytokines (Heneka and O'Banion, 2007). Reactive glial cells are associated with plaques and tangles in AD (Serrano-Pozo et al., 2011). Generally, activation refers to an enhanced ability of a cell to perform a function beyond that present in the basal state (Kettenmann and Verkhratsky, 2008). The activation of microglia and astrocytes is multi-dimensional; that is, they proliferate, phagocytose, and release proinflammatory cytokines or growth factors (Kettenmann and Verkhratsky, 2008). The cross-talk between activated glial cells and neurons has also become an area of focus. The neuron can activate glia via various neurotransmitters or modulators, such as glutamate, fractalkine and nitric oxide (Liu et al., 2006, Verge et al., 2004).

Conversely, the activated glial cells affect neuronal function and contribute to the development of various diseases such as Alzheimer's disease (Liu et al., 2006, Verge et al., 2004).

1.4.1.1 Microglia

Microglia which constitute around 10–20% of all glial cells, are derived from bone marrow precursors, and represent the brain's internal immune system (Czeh et al., 2011). They are the resident macrophages and are thus considered the first line of defence (Czeh et al., 2011). In physiological conditions, microglia are ramified and have highly motile processes, surveying the microenvironment in the CNS (Nimmerjahn et al., 2005). Furthermore, microglia exist at all stages of brain development (Ginhoux et al., 2013). Destruction of invading pathogens, debris elimination, tissue repair, and homeostasis are their main functions in maintaining host defence. (Glass et al., 2010). Swift release of cytokines on activation of microglia is due to their role as inflammatory regulators in the CNS (Block et al., 2007). Their morphology changes from small cell bodies with fine processes to large cell bodies with amoeboid processes upon activation (Fakhoury, 2018). They also undergo rapid proliferation in order to increase their number for the upcoming battle, demonstrated by the upregulation of complement receptor type 3 (OX42) immunostaining (Kim and de Vellis, 2005). In vitro studies have shown that cytotoxic substances such as cytokines, reactive oxygen intermediates, neurotrophic factors and various arachidonic acid derivatives are released from microglia (Harry and Kraft, 2012).

Significantly increased activated microglia were observed in a triple-transgenic model of AD compared to non-transgenic controls (Rodriguez et al., 2010). The activated microglia were closely associated with Aβ plaque formation, smaller Aβ deposits, and Aβ plaques (Rodriguez et al., 2010). A study by Griciuc *et al.* revealed an increased CD33 level and CD33-positive microglia in AD brains, while mice lacking the gene had less AD pathology, revealing the role of microglia in Aβ clearance (Griciuc et al., 2013). Microglia are also strongly activated early in the emergence of senile plaques, to limit their growth and reduce inflammatory damage to brain components (Scheffler et al., 2011). Besides, microglia also secrete proteolytic enzymes such as insulin-degrading enzyme, matrix metalloproteinase, and plasminogen–plasmin complex that degrade Aβ, and promote phagocytosis by the expression of receptors for advanced-glycosylation end products (Leissring et al., 2003, Li et al., 2011, Du Yan et al., 1996, El Khoury et al., 1998). Moreover, microglia can secrete several soluble factors such as glia-derived neurotrophic factor which plays a role in promoting neuronal survival (Liu and Hong, 2003).

Although there is evidence to support a role for microglia in neuroprotection and Aβ clearance, continuous Aβ accumulation and progression of AD pathology despite continued microglia activation and recruitment remain unanswered. Possible explanations would be the overload that microglia become subjected to by a large amount of Aβ production, uncontrolled generation of Aβ (Hickman et al., 2008) and the diminished activation of these cells in the later stages of plaque formation (Scheffler et al., 2011). Interaction between microglia and Aβ is age-dependent, and a decrease in this interaction

leads to loss of phagocytotic ability (Floden and Combs, 2011); thus, the uptake and degradation of Aβ are reduced. Primary microglial functions progressively decline with the appearance of Aβ plaques in AD, and lowering Aβ burden could reverse this functional impairment (Krabbe et al., 2013b). Proliferation and activation of microglia and neuronal damage result in a vicious cycle in AD (Hanisch and Kettenmann, 2007). Most importantly, the microglia role in the neuropathophysiology of AD could be viewed as a double-edged sword. Therefore, to be able to estimate the effect of potential therapies, it is crucial to understand the activation of microglia at different stages of AD.

1.4.1.2 Astrocytes

Astrocytes account for approximately 35% of CNS cell population and are found all over the CNS (Sherwood et al., 2006). They are derived from the neuroectoderm and are morphologically heterogeneous (Chan et al., 2007). These star-shaped cells have a central cell body, approximately 15–17 μm in diameter, and long processes extending in all directions (Sherwood et al., 2006). A network of coupled astrocytes is formed from contacts of processes from different cells via gap junctions (Blomstrand et al., 1999, Cornell-Bell et al., 1990, Guthrie et al., 1999, Nedergaard et al., 2003). The extension of these processes envelope synapses of neurons and makes contact with capillary vessels (Sherwood et al., 2006). Therefore astrocytes are the bridge for all cells in CNS, including neurons, oligodendrocytes, microglia, endothelia, and astrocytes themselves (Sherwood et al., 2006).

Removal of toxins from the cerebrospinal fluid, maintenance of redox potential, production of trophic factors, regulation of neurotransmitter, and ion concentrations are some of the functional roles of astrocytes (Blomstrand et al., 1999, Cornell-Bell et al., 1990, Guthrie et al., 1999, Nedergaard et al., 2003). Functional impairment associated with the injury of astrocytes during physiological reactions can trigger or exacerbate neuronal dysfunction (Sidoryk-Wegrzynowicz et al., 2011). When there is injury or trauma, astrocytes often withdraw their arms and slack off on their stabilizing chores resulting in weakening or disappearance of their role as neuronal partners (Sidoryk-Wegrzynowicz et al., 2011). However, they also release neurotrophic factors such as transforming growth factor-beta (TGF-β) and nerve growth factor (NGF) (Escartin and Bonvento, 2008). These neurotrophic factors are beneficial for the repair, proliferation, and filling up the space to form glial scar replacing the cells that cannot regenerate (Escartin and Bonvento, 2008). Another notable characteristic of activated astrocytes is elevated intracellular calcium (Ca^{2+}) (Haydon, 2001). Through the diffusion of inositol triphosphate (IP3) by gap junctions and extracellular adenosine triphosphate (ATP) signaling, astrocytes signal each other in the form of a calcium wave, resulting in elevation of Ca^{2+} concentration in adjacent cells (Haydon, 2001). Increased Ca^{2+} binds to various molecular targets, trigger or contribute to intracellular signal transduction pathways including dependent phospholipases such as phospholipase C and phospholipase A2 as well as downstream calcium-dependent elements (phosphatases and protein kinases), some of which contribute to the rapid motility and morphological changes of astrocytes

(Scemes, 2000). Additionally, the calcium wave can also propagate to neighbouring microglia (Schipke et al., 2002).

Interferon-γ can induce Aβ production by human astrocytes or astrocytoma cells in combination with IL-1β or TNF-α (Blasko et al., 2000). Proinflammatory factors secreted by astrocytes contribute to the level of expression of secretases and increase the conversion of APP to neurotoxic Aβ (Yu et al., 2009, Tang, 2009). Reactive astrocytes express BACE, the enzyme responsible for the generation of Aβ, suggesting that they may promote Aβ accumulation in aged transgenic AD mice model (Hartlage-Rübsamen et al., 2003, Robner et al., 2005). Sporadic AD patients have increased levels of presenilin-1 astrocytes, the catalytic component of the γ-secretase complex that is involved in the formation of Aβ (Weggen et al., 1998). As the largest number of brain cells, activated astrocytes may represent a significant source of Aβ during neuroinflammation in AD (Zhao et al., 2011).

On the other hand, by providing trophic support to neurons and forming a protective barrier between neurons and Aβ deposits, astrocytes are known to promote the Aβ clearance and degradation (Roßner et al., 2005). Thus, astrocytes undergoing modifications and chronic inflammation might suffer a deleterious transformation, acquire the capacity to generate Aβ, and lose the ability to remove and degrade them (Rossi and Volterra, 2009). Astrocytes are intimately involved in inflammatory and immunological events occurring in the CNS. This involvement is due to their ability to secrete and respond to a vast number of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-8, IL-10, transforming growth factor (TGF)-β, interferon-γ, and interferon-β (Qin and Benveniste, 2012). The mechanisms of astrocyte activation in response to Aβ may include NF-κB–mediated and inflammatory gene expression (Carrero et al., 2012). In vitro, Aβ42 triggered senescence, driving the expression of positive senescent astrocytes. Senescent astrocytes produce several inflammatory cytokines, including IL-6, and an accumulation of senescent astrocytes may be associated with increased risk of sporadic AD with advancing age (Bhat et al., 2012).

1.4.2 Cytokines and neuroinflammation

Cytokines are small, non-structural proteins produced by a broad range of cells (McGeer and McGeer, 1997).Their molecular weights range from 8,000 Da to 40,000 Da (McGeer and McGeer, 1997). Cytokines can stimulate the secretion of several proteins found in senile plaques (McGeer and McGeer, 1997). The biological effects induced by cytokines include the stimulation or inhibition of cell proliferation, cytotoxicity/apoptosis, antiviral activity, cell growth and differentiation, inflammatory responses, and upregulation of expression of surface membrane proteins (Meager, 2006). Chronic neuroinflammation by cytokines released from activated microglia and astrocytes is recognised as one of the significant mechanisms of AD neuropathology (Meager, 2006). Cytokines are extremely pleiotropic (Guzmán et al., 2010) and exhibit extensive redundancy, with many distinct proteins

exerting overlapping effects (Liu et al., 2013). Some cytokines promote inflammation and are called proinflammatory cytokines, whereas other cytokines suppress the activity of proinflammatory cytokines and are called anti-inflammatory cytokines (Liu et al., 2013). The downstream effects of cytokines differ depending on the presence of other cytokines and specific cell types (Lund et al., 2006, Norden et al., 2014).

1.4.2.1 Cytokines modulate memory

Both the anti- and pro-inflammatory responses mediated by cytokines modulate memory (Donzis and Tronson, 2014). The most commonly studied cytokines in the brain are Interleukin 1β (IL-1β), Interleukin 6 (IL-6), and tumour necrosis factor-α (TNF-α) (Capuron and Miller, 2011, Goehler, 2008). They increase in circulation after systemic inflammatory events such as surgery (Terrando et al., 2011), peripheral injuries and LPS injection (Skelly et al., 2013). Besides, these cytokines are appropriately suited to modulate memory as they are highly expressed in the hippocampus after manipulations in the periphery (Burton et al., 2011) or brain (Belarbi et al., 2012). Memory processes, such as acquisition (the process of copying the contents of physical memory for preservation), consolidation (the narrowing down process through which short term memory is converted into the long term) or retrieval (the process of remembering information stored in long-term memory), involve specific cytokines (Donzis and Tronson, 2014). Peripheral IL-6 levels correlate with memory retrieval (Elderkin-Thompson et al., 2012), while IL-1 disrupts consolidation of context fear conditioning after post-training injection of LPS (Pugh et al., 1998). However, most studies demonstrating the roles of cytokines in the modulation of learning and memory use an acute or chronic injection of cytokine, inflammatory stimulus models, or transgenic models injection before training (Pugh et al., 1998).

The feed-forward nature of cytokine expression indicates that many of the effects on learning and memory attributed to any individual cytokine are more likely due to the cumulative effect of all active cytokines (See Figure 2). For instance, IL-1β is not increased in isolation; it leads to increases in TNF-α, IL- 6, IL-1 family proteins, and cytokine receptors (Anisman et al., 2008, Moore et al., 2009) across multiple brain regions (Anisman et al., 2008, Moore et al., 2009). Similarly, targeting either TNF-α or IL-6 leads to changes in the expression of other inflammatory cytokines (Balschun et al., 2004, del Rey et al., 2013). This dependent nature among cytokines provide answers to discrepancies that arise in the action of some cytokines because activation of these cytokines results in altered expression of a variety of additional cytokines including IL-10 (Platzer et al., 1995, Steensberg et al., 2003) and IL-4 (Nolan et al., 2005). Rather than direct effects on memory, cytokines indirectly exert their effects via network properties of inflammatory signaling (Donzis and Tronson, 2014). For example, IL-1β has been shown to both enhance (Goshen et al., 2007) and impair (Gonzalez et al., 2013, Gonzalez et al., 2009) context fear conditioning.

The modulation of learning and memory by commonly studied inflammatory markers is likely due to indirect effects via a network of inflammation-related signals (Donzis and Tronson, 2014). Consistent with a regulatory role, IL-4 and IL-10 attenuates the deleterious impact of inflammatory processes on memory and plasticity (Lynch et al., 2004, Richwine et al., 2009). Both IL-4 and IL-10 can abrogate learning and memory deficits observed in inflammatory models of Alzheimer's disease (Kawahara et al., 2012, Kiyota et al., 2012a, Kiyota et al., 2010). Therefore, cytokine network interactions are not limited to regulation of, and between IL-1β, TNF-α, and IL-6. Rather, activation of these cytokines results in altered expression of a variety of additional cytokines (Donzis and Tronson, 2014).

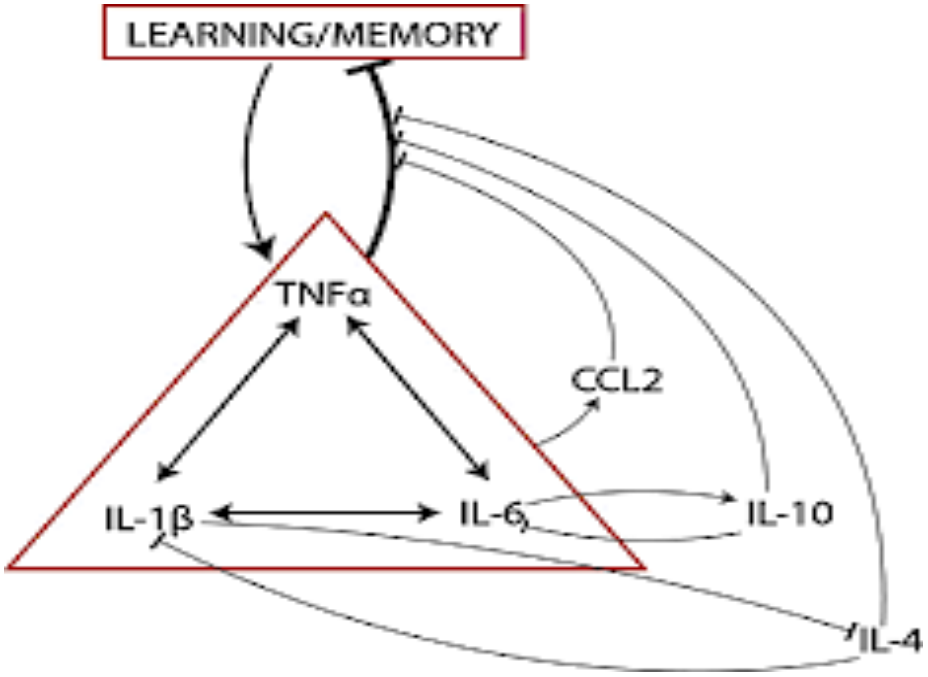

Figure 2: Modulation of learning and memory by networked activation of cytokines. IL-1b, TNF-α, and IL-6 indirectly modify memory processes via interactions and regulation of cytokines and chemokines with similar and opposing effects. The state of the cytokine network is therefore more predictive of the effect on memory than the level of any single cytokine. Arrows represent excitatory connections, bars represent inhibitory connections. IL-1β Interleukin 1 β; TNF-α Tumour Necrosis Factor α; IL-6 Interleukin 6; IL-4 Interleukin 4; IL-10 Interleukin 10; CCL2 C-C-motif ligand 2. Arrows represent positive influence. Blocked head represents negative regulation. Adopted from (Donzis and Tronson, 2014).

1.5 Microglial genes regulating neuroinflammation

Genome-wide association studies have identified a set of single nucleotide polymorphisms (SNPs) associated with AD risk (Lambert et al., 2013, Hollingworth et al., 2011). Several of the genes

underlying these SNPs such as TREM2 and CD33 encode proteins relevant to microglial function and inflammation (Malik et al., 2015). Before these studies, inflammation was recognized to occur in AD with an agreement that anti-inflammatory agents may be helpful. However, the specific aspects of inflammation that were beneficial or detrimental remained unidentified (Akiyama et al., 2000, Wyss-Coray and Rogers, 2012). These recent genetic studies pinpoint the contributions of specific proteins and associated pathways in AD risk. Consideration of APOE4, the prototypical AD genetic risk factor, along its pro-inflammatory, indicates that AD risk is reduced by processes that suppress inflammatory cytokine signaling and enhance debris clearance, including amyloid.

1.5.1 Cluster of Differentiation 33 (CD33)

Cluster of differentiation 33 is a type 1 transmembrane protein expressed mainly in microglial cells of the brain (Varki and Angata, 2006). CD33 is a member of the sialic acid-binding immunoglobulin-like lectin (SIGLEC) family of receptors (Griciuc et al., 2013, Malik et al., 2013). CD33 ligands include sialylated cell surface proteins acting in cis as well as other sialylated agents (Malik et al., 2015). CD33 just like other inhibitor SIGLECs limits immune activation in response to "self" macromolecules that bear a "self-associated molecular pattern" of sialylation (Schwarz et al., 2015). Hence plaque vicinity rich in sialylated agents, including gangliosides and apoE stimulates CD33 thus concealing amyloid plaques from microglia (Salminen and Kaarniranta, 2009). Sialic acid-binding activates CD33 and results in phosphorylation of the CD33 immunoreceptor tyrosine-based inhibitory motif (ITIM) domains and activation of the SHP-1 and SHP-2 tyrosine phosphatases (Ulyanova et al., 1999, Walter et al., 2008) (see Figure 3). The action of these phosphatases on diverse substrates, including spleen tyrosine kinase (Syk), inhibits immune activation (Reth and Brummer, 2004). Consequently, CD33 activation yields increased SHP-1 and SHP-2 activity that antagonizes Syk, inhibiting ITAM-signaling proteins, possibly TREM2/DAP12 (Linnartz and Neumann, 2013). Following this possibility, long-term CD33 inhibition (by antibodies or siRNA) induces the production of cytokines such as IL1β, TNFα, and IL-8 (Lajaunias et al., 2005). CD33 as a possible target for AD prevention or therapy is currently an active research area. Studies with CD33 deficient mice develop normally and yet have reduced amyloid accumulation (Griciuc et al., 2013, Brinkman-Van der Linden et al., 2003). This suggests that more robust CD33 inhibition may reduce AD risk further.

1.5.2 Triggering receptor expressed on myeloid cells 2 (TREM2)

Triggering receptor expressed on myeloid cells 2 is a type 1 transmembrane receptor protein expressed on myeloid cells, including microglia and osteoclasts, bone-marrow-derived macrophages and monocyte-derived dendritic cells (Bouchon et al., 2001). TREM2 is expressed by microglia in the brain, found abundant in the vicinity of plaques in APP mice (Bouchon et al., 2001). It controls two signaling pathways: regulation of phagocytosis and suppressing inflammatory reactivity (Neumann and Takahashi, 2007, Frank et al., 2008, Melchior et al., 2010). TREM2 expression increased during the

alternative activation of microglia on exposure to IL-4 (Turnbull et al., 2006). TREM2 ligands include anionic lipids and perhaps other unknown elements from apoptotic neurons (Hsieh et al., 2009, Wang et al., 2015c). TREM2 possesses an immunoglobulin-like extracellular domain, a transmembrane region, and a short cytoplasmatic tail lacking an extended cytosolic domain (Malik et al., 2015). It signals through the immunoreceptor tyrosine-based activating motif (ITAM) of its co-receptor, DAP12 (Bouchon et al., 2001) (Figure 3). Activated TREM2 stimulates DAP12 through an intramembrane lysine residue, resulting in phosphorylation of the DAP12 ITAM, and activation of the kinase Syk (Bakker et al., 1999). This leads to the activation of PI3K, resulting in actin rearrangement and phagocytic cup formation for target engulfment (Rougerie et al., 2013). TREM2-activated phagocytosis occurs without a commensurate activation of cytokine production (Takahashi et al., 2005). Indeed, TREM2 activation decreases cytokine production in response to Toll-like receptor (TLR) activation (Takahashi et al., 2005). Altogether, TREM2 stimulation via apoptotic neuronal fragments or TREM2 antibodies appears to result in the activation of microglial phagocytosis with minimal changes in cytokine levels.

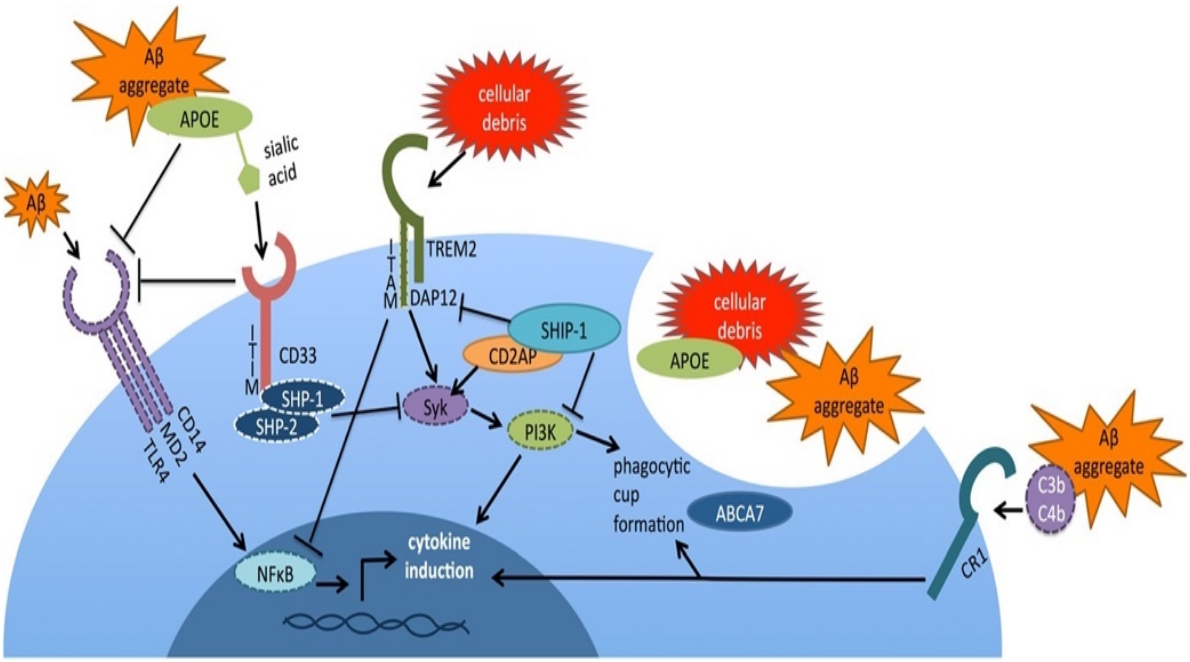

Figure 3: Interactions between the AD risk genes involved in inflammation. TREM2 signals through the ITAM of DAP12 to activate microglial phagocytosis; however, TREM2 expression has also been shown to dampen pro-inflammatory cytokine production activated by TLRs. Activated CD33 recruits SHP-1 and SHP-2 to inhibit Syk signaling; CD33 has also been shown to antagonize CD14/TLR4 signaling. Sialylated apoE, which complexes with Aβ, may serve as a CD33 ligand. Adopted from (Malik et al., 2015).

1.6 Animal models of neuroinflammation in Alzheimer's Disease

Several experimental animal models have been developed that mimic the pathophysiology of AD in humans, most of which share many features with AD in humans (Orta-Salazar et al., 2016). These models have suitable features to understand the aetiology and progression of AD better, as well as to pharmacologically screen potential drugs for better treatment options. The aetiological factors that contribute to human AD include activation of inflammatory and innate immune responses (Heppner et al., 2015, Zhao and Lukiw, 2018), as well as calcium dyshomeostasis, oxidative stress, mitochondrial damage, and alterations in the cell cycle regulatory mechanisms (Zhao and Lukiw, 2018, Pchitskaya et al., 2018, Wojsiat et al., 2018).

Studies have shown that a single animal model may not have all the answers to the pathogenesis and treatment of AD, as its aetiopathology is multifactorial (Gong et al., 2018, Iqbal and Grundke-Iqbal, 2010). However, each model explains a particular pathway for the progression of AD, and the studied model is therefore associated with the aetiology and possible identification of biomarkers. The current animal models for neuroinflammation in AD are categorised based on the mechanism of their creation, such as immune challenge-based, neurotoxin-induced, and transgenic models (Nazem et al., 2015). Examples of the neurotoxin-induced neuroinflammatory model of AD include the amyloid-beta (Aβ), streptozotocin, okadaic acid, and colchicine induced models.

1.6.1 The amyloid-beta induced neuroinflammatory model of AD

The discovery that amyloid-beta (Aβ) is the main constituent of the amyloid plaques in the brains of AD patients (Glenner and Wong, 1984, Masters et al., 1985) and is toxic to neurons (Yankner et al., 1989, Pike et al., 1991) led to in vivo studies on the effects of Aβ in the brain. The acute neurodegenerative effect of Aβ and amyloid cores from the brains of AD patients was demonstrated in vivo when these substances were injected into the brain of two different rat models (Kowall et al., 1991, Frautschy et al., 1991). In both cases a significant induction of abnormal tau phosphorylation was observed in the immediate vicinity of the Aβ immunoreactive sites. Subsequently, several laboratories reported contradictory results from acute injections or continuous infusion of Aβ directly into the rat brain.

Whereas many groups demonstrated neurotoxicity, AD-like astrogliosis, tau hyperphosphorylation (Nitta et al., 1994, Weldon et al., 1998, Nakamura et al., 2001) and/or memory decline in the experimental models (McDonald et al., 1994, Sweeney et al., 1997, Oka et al., 1999), others showed no significant effect of the peptides (Winkler et al., 1994, Clemens and Stephenson, 1992). Much of the variance in the results obtained depended on the nature of the peptide (fibrillated or soluble Aβ) or solvent used, the concentration of the solution and manner of introduction (single injections or continuous infusions over different periods into rat ventricles, hippocampus or septum), age of the treated animals (young versus old) and the time frame when the effects were assessed (immediate or

long-term). More models demonstrating a deteriorating effect of Aβ in vivo followed in the 2000s, proving that this is still a viable approach for modelling different aspects of AD pathology. These models have been used for testing the protective effects of ginkgo biloba extracts, docosahexaenoic acid (DHA), ginseng, oestradiol, green tea, synthetic cognitive enhancers or antioxidants (Yamada et al., 1999, Hruska and Dohanich, 2007) and the deteriorating effects of chronic stress (Srivareerat et al., 2009) on memory in Aβ infused rats. (Takata et al., 2007), showed that exogenous microglia transplanted into the brains of rats microinjected with Aβ, participate in Aβ clearance.

1.6.2 Amyloid-beta as a cause and consequence of neuroinflammation in AD

Amyloid-beta (Aβ) peptides, the main constituents of amyloid plaques, are derived from the proteolytic cleavage of APP (Citron, 2010, De Strooper et al., 2010). Diverse lines of evidence suggest that Aβ and APP contribute to the pathogenesis of AD causally. The conversion from preclinical AD to clinical AD is associated with widespread Aβ plaque deposition and NFT pathology and the appearance of various soluble/dispersible Aβ aggregates in the neuropil (Thal et al., 2013). Aβ regulates synaptic and neuronal activities and Aβ accumulation in the brain leads to an exciting combination of synaptic depression and aberrant network activity (Palop and Mucke, 2010). For neuroinflammation, Aβ plaques are frequently associated with the activation of microglia and astrocytes (Thal et al., 2013). Aβ induces the expression of inflammatory cytokines and inflammatory enzymes such as COX-2 and iNOS (Chen et al., 2012, Chami et al., 2012). Inflammatory cytokines (such as TNF-α and IL-1β), in turn, enhance APP production and the process of APP proteolytic cleavage to increase the production of Aβ42 peptide (Citron, 2010, De Strooper et al., 2010). Aβ can stimulate NF-κB and MAPK signaling pathways (Chen et al., 2012, Chami et al., 2012). Both these signaling pathways are associated with the transcription of inflammatory mediators. Also, NF-κB participates in Aβ production through regulation of APP and BACE and γ-secretases; NF-κB/p65 is closely related to BACE1 expression, cleavage and Aβ production (Chen et al., 2012, Chami et al., 2012). Aβ can disrupt gliotransmitter release and astrocytic calcium signaling and alter synaptic plasticity which are vital processes for astrocyte-neuron communication (Vincent et al., 2010). Thus, Aβ can be viewed both as a cause and consequence of neuroinflammation in AD.

1.6.3 The amyloid-beta hypothesis

The amyloid hypothesis considers Aβ deposition to be the causative event of AD pathology; neurofibrillary tangles, cell loss, vascular damage, and dementia occur as a consequence of this event (Hardy and Allsop, 1991). It suggests that the imbalance between the production and clearance of Aβ is the key trigger of a cascade of events that leads to AD (Baranello et al., 2015). Many observations supported the evolution of this hypothesis. One of the most persuasive arguments for the amyloid hypothesis came from genetic studies which brought to light many FAD cases that resulted from inherited APP or PS1 mutations leading to increased deposition of Aβ in plaques (Bertram et al., 2010).

Moreover, transgenic models of AD harbouring APP mutations develop age-dependent pathology similar to that seen in AD (Philipson et al., 2010). Also, the fourth allelic variant (ε4) of the apolipoprotein (APOE) polymorphism, which represents the most important genetic risk factor for the sporadic form of AD, is associated with Aβ accumulation and reduced clearance (Castellano et al., 2011).

On the other hand, mutations in tau lead to tauopathies and not AD, thereby suggesting that NFTs occur after Aβ aggregation(Castellano et al., 2011). Soluble oligomers and intermediate amyloids are the most toxic forms of Aβ (Walsh and Selkoe, 2007), which can cause neurotoxicity, neuron apoptosis, inflammation and mitochondrion dysfunction (Walsh et al., 2005, Hauptmann et al., 2006, Klyubin et al., 2008). Although this hypothesis has been questioned, there is still consensus that Aβ is closely related to AD. Therefore, a reappraisal of the amyloid hypothesis focusing on the role of neuroinflammation in a beta-amyloid model of AD will open new avenues for identifying targets for AD treatment.

1.7 MicroRNAs as biomarkers for early diagnosis of AD

MicroRNAs are a class of non-coding RNAs acknowledged as essential regulators for post-transcriptional gene expression by either repressing translation or degrading the target messenger RNAs (Maoz et al., 2017). MicroRNAs have been identified as the most frequently implicated regulators in many critical biological events such as neurodegenerative processes, development, growth, and differentiation (Huang et al., 2011, Hammond, 2015). A single microRNA can target several genes, and a single gene can be regulated by numerous microRNAs, granting microRNAs the power as potential tools to investigate multifactorial diseases like AD (Iqbal and Grundke-Iqbal, 2010). Depending on the algorithm used, bioinformatics predicts that miRNAs regulate 30% of human genes (Lewis et al., 2003, Lewis et al., 2005) to as much as 92% (Miranda et al., 2006). Expression profiling has provided essential insight into miRNA biology. Microarray studies have helped describe which miRNAs are expressed during various normal and abnormal brain states, including in brain diseases (Babak et al., 2004, Ciafre et al., 2005, Nelson et al., 2006, Perkins et al., 2007).

MicroRNA expression analyses can provide ground-breaking data for this new research field, however, profiling it benefits from the parallel use of more than a single technique. Previous work confirmed that understanding cellular level expression patterns is vital for brain microRNA studies (Nelson et al., 2006). In situ hybridization pinpoints exactly where specific microRNAs are expressed, whether in neurons, astrocytes, oligodendrocytes, microglia, or vasculature. Neurodegenerative diseases tend to affect distinct cell populations (Nelson et al., 2006). Hence, knowing which microRNAs are expressed in which cell populations can facilitate the identification of disease-relevant microRNA genes and their messenger RNA targets (Zhang et al., 2018). MicroRNAs, unlike messenger RNAs, are stable enough in biological fluids including serum, plasma, and CSF (Zhang et al., 2018). Besides, many of them

target genes directly involved in AD pathophysiology such as presenilin, beta-site amyloid precursor protein cleaving enzyme1 (BACE-1) and amyloid precursor protein (Liu et al., 2014).

MicroRNA107 is implicated in AD-related phenotypes in early phases of the disease (Wang et al., 2010, Nelson and Wang, 2010, Wang et al., 2008) and is especially dysregulated in the brain as well as in the blood of patients with AD, making it an ideal candidate biomarker (Wang et al., 2008, Wang et al., 2015a, Leidinger et al., 2013). MicroRNA107 targets BACE1, an enzyme that cleaves the amyloid precursor protein, creating the neurotoxic β-amyloid peptide, $A\beta_{(1–42)}$ (Stratman et al., Haniu et al., 2000, Vassar et al., 1999). BACE1 is the initiating and putatively rate-limiting enzyme in Aβ generation (Fukumoto et al., 2002, Li and Südhof, 2004). While its inhibition blocks the production of Aβ and prevent the development of Aβ-associated pathologies, overexpression of this enzyme has been shown to initiate or accelerate AD pathogenesis (Fukumoto et al., 2002, Li and Südhof, 2004). Studies have shown that the dysregulation of BACE1 directly contributes to the pathogenesis of AD (Dominguez et al., 2004, Durham and Shepherd, 2006, Guo and Hobbs, 2006, John, 2006).

1.8 Motivation and rationale of the study

At present, AD is incurable. Despite the considerable amount of research, current medications provide only modest relief to symptoms and do not cure the underlying disease. This failure is probably due to the scant knowledge of the cellular and molecular mechanisms implicated in AD pathogenesis. However, it is now well recognized that AD is a multifactorial disorder impacted by other factors in its pathogenesis and progression. Among these, the role of inflammation has been affirmed. Although the inflammatory process is aimed at controlling injuries through several mechanisms to repair tissues, the sustained immune response in the brain accelerates other core pathologies. Hence, it is clear that there are many characters involved in this inflammatory process. Therefore, a better understanding of the fundamental role of neuroinflammation in AD can be an excellent jump-off point for the development of viable therapeutic targets.

1.9 Aim of the study

The aim of this study is to assess the role of neuroinflammation and to identify possible biomarker in a progressive $A\beta_{(1-42)}$ rat model of AD.

1.10 Study objectives

The objectives of this study are to assess the effects of $A\beta_{(1-42)}$ lesion progressively on:

- learning and memory using Morris water maze and fear conditioning test.
- the activity of microglia and astrocytes as neuroinflammatory markers using immunofluorescence technique.

- plasma lipid peroxidation and glutathione levels as biomarkers of oxidative stress using spectrophotometric analysis.
- cytokine levels (interleukin 1-beta, interleukin 6, interleukin10 and Tumour necrosis factor -alpha) in the plasma with the aid of multi-plex immunoassay.
- the expression some microglial genes (TREM2 & CD33) regulating neuroinflammation using real-time polymerase chain reaction.
- the feasibility of plasma based miRNA-107 as a possible biomarker of AD.

1.11 Brief overview of methodology and study design

To accomplish the objectives, standardized laboratory methods and protocols were strictly adhered to, as illustrated in Chapters 2, 3, 4 and 5. Animal experimental work was conducted as per the approval of the Animal Ethics Committee of UKZN (Ref: AREC/015/018D) according to the guidelines of National Institutes of Health for the Care and Use of Laboratory Animals, South Africa.

1.12 Potential benefit of this research

This study is the first to showcase the role of neuroinflammation in a beta-amyloid model of AD, hence reappraising the amyloid hypothesis. It unravels the significance of neuroinflammation as an early entity in the pathogenesis of AD, offers new avenues in the search for improved therapeutic approaches for the disease. Therefore, the outcomes from this study are novel and also contribute to the global understanding of the pathophysiology of AD, which will serve as a precursor for the development of possible targets for AD treatment in relation to neuroinflammation.

REFERENCES

AHMAD, M. H., FATIMA, M. & MONDAL, A. C. 2018. Influence of microglia and astrocyte activation in the neuroinflammatory pathogenesis of Alzheimer's disease: rational insights for the therapeutic approaches. *Journal of Clinical Neuroscience*.

AISEN, P. S., CUMMINGS, J., JACK, C. R., MORRIS, J. C., SPERLING, R., FRÖLICH, L., JONES, R. W., DOWSETT, S. A., MATTHEWS, B. R. & RASKIN, J. 2017. On the path to 2025: understanding the Alzheimer's disease continuum. *Alzheimer's research & therapy,* 9**,** 60.

AKIYAMA, H., BARGER, S., BARNUM, S., BRADT, B., BAUER, J., COLE, G., COOPER, N., EIKELENBOOM, P., EMMERLING, M. & FIEBICH, B. 2000. Griffi n WS. *Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Stromeyer R, Tooyoma I, Van Muiswonkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T***,** 383-421.

ALAM, Q., ZUBAIR ALAM, M., MUSHTAQ, G., A DAMANHOURI, G., RASOOL, M., AMJAD KAMAL, M. & HAQUE, A. 2016. Inflammatory process in Alzheimer's and Parkinson's diseases: central role of cytokines. *Current pharmaceutical design,* 22**,** 541-548.

ALBERT, M. S., DEKOSKY, S. T., DICKSON, D., DUBOIS, B., FELDMAN, H. H., FOX, N. C., GAMST, A., HOLTZMAN, D. M., JAGUST, W. J. & PETERSEN, R. C. 2013. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Focus,* 11**,** 96-106.

AMARAL, D. & LAVENEX, P. 2007. Hippocampal neuroanatomy.

ANAND, R., GILL, K. D. & MAHDI, A. A. 2014. Therapeutics of Alzheimer's disease: Past, present and future. *Neuropharmacology,* 76**,** 27-50.

ANISMAN, H., GIBB, J. & HAYLEY, S. 2008. Influence of continuous infusion of interleukin-1β on depression-related processes in mice: corticosterone, circulating cytokines, brain monoamines, and cytokine mRNA expression. *Psychopharmacology,* 199**,** 231.

BABAK, T., ZHANG, W., MORRIS, Q., BLENCOWE, B. J. & HUGHES, T. R. 2004. Probing microRNAs with microarrays: tissue specificity and functional inference. *Rna,* 10**,** 1813-1819.

BAĐDATOĐLU, Ö. T., POLAT, G., BAĐDATOĐLU, C. & UĐUR, A. Effects of Peripheral Nerve Ischemia-Reperfusion Model on Serum Cytokine Levels. *Turkish Neurosurgery,* 18.

BAGHERI, M., JOGHATAEI, M.-T., MOHSENI, S. & ROGHANI, M. 2011. Genistein ameliorates learning and memory deficits in amyloid β (1–40) rat model of Alzheimer's disease. *Neurobiology of learning and memory,* 95**,** 270-276.

BAIK, S. H., KANG, S., SON, S. M. & MOOK-JUNG, I. 2016. Microglia contributes to plaque growth by cell death due to uptake of amyloid β in the brain of Alzheimer's disease mouse model. *Glia,* 64**,** 2274-2290.

BAKKER, A. B., BAKER, E., SUTHERLAND, G. R., PHILLIPS, J. H. & LANIER, L. L. 1999. Myeloid DAP12-associating lectin (MDL)-1 is a cell surface receptor involved in the activation of myeloid cells. *Proceedings of the National Academy of Sciences,* 96**,** 9792-9796.

BALSCHUN, D., WETZEL, W., DEL REY, A., PITOSSI, F., SCHNEIDER, H., ZUSCHRATTER, W. & BESEDOVSKY, H. 2004. Interleukin-6: A cytokine to forget. *The FASEB journal,* 18**,** 1788-1790.

BARKER, W. W., LUIS, C. A., KASHUBA, A., LUIS, M., HARWOOD, D. G., LOEWENSTEIN, D., WATERS, C., JIMISON, P., SHEPHERD, E. & SEVUSH, S. 2002. Relative frequencies of

Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank. *Alzheimer Disease & Associated Disorders,* 16**,** 203-212.

BARRIENTOS, S. A. & TIZNADO, V. 2016. Hippocampal CA1 Subregion as a Context Decoder. *Journal of Neuroscience,* 36**,** 6602-6604.

BARTSCH, T., DÖHRING, J., ROHR, A., JANSEN, O. & DEUSCHL, G. 2011. CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness. *Proceedings of the National Academy of Sciences,* 108**,** 17562-17567.

BATTISTA, D., FERRARI, C. C., GAGE, F. H. & PITOSSI, F. J. 2006. Neurogenic niche modulation by activated microglia: transforming growth factor β increases neurogenesis in the adult dentate gyrus. *European Journal of Neuroscience,* 23**,** 83-93.

BELARBI, K., JOPSON, T., TWEEDIE, D., ARELLANO, C., LUO, W., GREIG, N. H. & ROSI, S. 2012. TNF-α protein synthesis inhibitor restores neuronal function and reverses cognitive deficits induced by chronic neuroinflammation. *Journal of neuroinflammation,* 9**,** 23.

BERTRAM, L., LILL, C. M. & TANZI, R. E. 2010. The genetics of Alzheimer disease: back to the future. *Neuron,* 68**,** 270-281.

BERTRAM, L. & TANZI, R. E. 2008. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nature Reviews Neuroscience,* 9**,** 768.

BETTENS, K., SLEEGERS, K. & VAN BROECKHOVEN, C. 2013. Genetic insights in Alzheimer's disease. *The Lancet Neurology,* 12**,** 92-104.

BHAT, R., CROWE, E. P., BITTO, A., MOH, M., KATSETOS, C. D., GARCIA, F. U., JOHNSON, F. B., TROJANOWSKI, J. Q., SELL, C. & TORRES, C. 2012. Astrocyte senescence as a component of Alzheimer's disease. *PloS one,* 7.

BINDERUP, H. G., HOULIND, K., MADSEN, J. S. & BRASEN, C. L. 2016. Pre-storage centrifugation conditions have significant impact on measured microRNA levels in biobanked EDTA plasma samples. *Biochemistry and biophysics reports,* 7**,** 195-200.

BITTNER, K. C., GRIENBERGER, C., VAIDYA, S. P., MILSTEIN, A. D., MACKLIN, J. J., SUH, J., TONEGAWA, S. & MAGEE, J. C. 2015. Conjunctive input processing drives feature selectivity in hippocampal CA1 neurons. *Nature neuroscience,* 18**,** 1133.

BLASKO, I., VEERHUIS, R., STAMPFER-KOUNTCHEV, M., SAURWEIN-TEISSL, M., EIKELENBOOM, P. & GRUBECK-LOEBENSTEIN, B. 2000. Costimulatory effects of interferon-γ and interleukin-1β or tumor necrosis factor α on the synthesis of Aβ1-40 and Aβ1-42 by human astrocytes. *Neurobiology of disease,* 7**,** 682-689.

BLOCK, M. L. & HONG, J.-S. 2005. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Progress in neurobiology,* 76**,** 77-98.

BLOCK, M. L., ZECCA, L. & HONG, J.-S. 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nature Reviews Neuroscience,* 8**,** 57-69.

BLOMSTRAND, F., ÅBERG, N., ERIKSSON, P., HANSSON, E. & RÖNNBÄCK, L. 1999. Extent of intercellular calcium wave propagation is related to gap junction permeability and level of connexin-43 expression in astrocytes in primary cultures from four brain regions. *Neuroscience,* 92**,** 255-265.

BONDI, M. W., JAK, A. J., DELANO-WOOD, L., JACOBSON, M. W., DELIS, D. C. & SALMON, D. P. 2008. Neuropsychological contributions to the early identification of Alzheimer's disease. *Neuropsychology review,* 18**,** 73-90.

BOUCHON, A., HERNÁNDEZ-MUNAIN, C., CELLA, M. & COLONNA, M. 2001. A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells. *The Journal of experimental medicine,* 194**,** 1111-1122.

BRADSHAW, E. M., CHIBNIK, L. B., KEENAN, B. T., OTTOBONI, L., RAJ, T., TANG, A., ROSENKRANTZ, L. L., IMBOYWA, S., LEE, M. & VON KORFF, A. 2013a. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nature neuroscience,* 16**,** 848.

BRADSHAW, E. M., CHIBNIK, L. B., KEENAN, B. T., OTTOBONI, L., RAJ, T., TANG, A., ROSENKRANTZ, L. L., IMBOYWA, S., LEE, M. & VON KORFF, A. 2013b. CD33

Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nature neuroscience,* 16**,** 848-850.

BRAIDA, D., SACERDOTE, P., PANERAI, A. E., BIANCHI, M., ALOISI, A. M., IOSUÈ, S. & SALA, M. 2004. Cognitive function in young and adult IL (interleukin)-6 deficient mice. *Behavioural brain research,* 153**,** 423-429.

BRINKMAN-VAN DER LINDEN, E. C., ANGATA, T., REYNOLDS, S. A., POWELL, L. D., HEDRICK, S. M. & VARKI, A. 2003. CD33/Siglec-3 binding specificity, expression pattern, and consequences of gene deletion in mice. *Molecular and cellular biology,* 23**,** 4199-4206.

BROOKMEYER, R., JOHNSON, E., ZIEGLER-GRAHAM, K. & ARRIGHI, H. M. 2007. Forecasting the global burden of Alzheimer's disease. *Alzheimer's & dementia,* 3**,** 186-191.

BURTON, M. D., SPARKMAN, N. L. & JOHNSON, R. W. 2011. Inhibition of interleukin-6 trans-signaling in the brain facilitates recovery from lipopolysaccharide-induced sickness behavior. *Journal of neuroinflammation,* 8**,** 54.

BUSTIN, S. A., BENES, V., GARSON, J. A., HELLEMANS, J., HUGGETT, J., KUBISTA, M., MUELLER, R., NOLAN, T., PFAFFL, M. W. & SHIPLEY, G. L. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical chemistry,* 55**,** 611-622.

BUTLER, M., O'CONNOR, J. & MOYNAGH, P. N. 2004. Dissection of tumor-necrosis factor-α inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early—but not late—phase LTP. *Neuroscience,* 124**,** 319-326.

CAPLAN, B., BOGNER, J., BRENNER, L., KUMAR, R. G., BOLES, J. A. & WAGNER, A. K. 2015. Chronic inflammation after severe traumatic brain injury: characterization and associations with outcome at 6 and 12 months postinjury. *Journal of Head Trauma Rehabilitation,* 30**,** 369-381.

CAPURON, L. & MILLER, A. H. 2011. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacology & therapeutics,* 130**,** 226-238.

CARRERO, I., GONZALO, M., MARTIN, B., SANZ-ANQUELA, J., AREVALO-SERRANO, J. & GONZALO-RUIZ, A. 2012. Oligomers of beta-amyloid protein (Aβ1-42) induce the activation of cyclooxygenase-2 in astrocytes via an interaction with interleukin-1beta, tumour necrosis factor-alpha, and a nuclear factor kappa-B mechanism in the rat brain. *Experimental neurology,* 236**,** 215-227.

CASSIM, S., QULU, L. & MABANDLA, M. V. 2015. Prenatal stress and early life febrile convulsions compromise hippocampal genes MeCP2/REST function in mid-adolescent life of Sprague-Dawley rats. *Neurobiology of learning and memory,* 125**,** 195-201.

CASTELLANO, J. M., KIM, J., STEWART, F. R., JIANG, H., DEMATTOS, R. B., PATTERSON, B. W., FAGAN, A. M., MORRIS, J. C., MAWUENYEGA, K. G. & CRUCHAGA, C. 2011. Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. *Science translational medicine,* 3**,** 89ra57-89ra57.

CHAMI, L., BUGGIA-PRÉVOT, V., DUPLAN, E., DELPRETE, D., CHAMI, M., PEYRON, J.-F. & CHECLER, F. 2012. Nuclear factor-κB regulates βAPP and β-and γ-secretases differently at physiological and supraphysiological Aβ concentrations. *Journal of Biological Chemistry,* 287**,** 24573-24584.

CHAN, W., KOHSAKA, S. & REZAIE, P. 2007. The origin and cell lineage of microglia—new concepts. *Brain research reviews,* 53**,** 344-354.

CHAVAN, S. S., PAVLOV, V. A. & TRACEY, K. J. 2017. Mechanisms and therapeutic relevance of neuro-immune communication. *Immunity,* 46**,** 927-942.

CHEN, C.-H., ZHOU, W., LIU, S., DENG, Y., CAI, F., TONE, M., TONE, Y., TONG, Y. & SONG, W. 2012. Increased NF-κB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease. *International Journal of Neuropsychopharmacology,* 15**,** 77-90.

CHOI, S. S., LEE, H. J., LIM, I., SATOH, J.-I. & KIM, S. U. 2014. Human astrocytes: secretome profiles of cytokines and chemokines. *PloS one,* 9**,** e92325.

CHOWDHURY, P. & SOULSBY, M. 2002. Lipid peroxidation in rat brain is increased by simulated weightlessness and decreased by a soy-protein diet. *Annals of Clinical & Laboratory Science,* 32**,** 188-192.

CHRISTENSEN, R., MARCUSSEN, A. B., WÖRTWEIN, G., KNUDSEN, G. & AZNAR, S. 2008. Aβ (1–42) injection causes memory impairment, lowered cortical and serum BDNF levels, and decreased hippocampal 5-HT2A levels. *Experimental neurology,* 210**,** 164-171.

CHRISTOV, A., OTTMAN, J. T. & GRAMMAS, P. 2004. Vascular inflammatory, oxidative and protease-based processes: implications for neuronal cell death in Alzheimer's disease. *Neurological research,* 26**,** 540-546.

CIAFRE, S., GALARDI, S., MANGIOLA, A., FERRACIN, M., LIU, C.-G., SABATINO, G., NEGRINI, M., MAIRA, G., CROCE, C. M. & FARACE, M. 2005. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochemical and biophysical research communications,* 334**,** 1351-1358.

CIOANCA, O., HRITCU, L., MIHASAN, M., TRIFAN, A. & HANCIANU, M. 2014. Inhalation of coriander volatile oil increased anxiolytic–antidepressant-like behaviors and decreased oxidative status in beta-amyloid (1–42) rat model of Alzheimer's disease. *Physiology & behavior,* 131**,** 68-74.

CITRON, M. 2010. Alzheimer's disease: strategies for disease modification. *Nature reviews Drug discovery,* 9**,** 387.

CLEMENS, J. A. & STEPHENSON, D. T. 1992. Implants containing β-amyloid protein are not neurotoxic to young and old rat brain. *Neurobiology of aging,* 13**,** 581-586.

COGSWELL, J. P., WARD, J., TAYLOR, I. A., WATERS, M., SHI, Y., CANNON, B., KELNAR, K., KEMPPAINEN, J., BROWN, D. & CHEN, C. 2008. Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. *Journal of Alzheimer's disease,* 14**,** 27-41.

COLONNA, M. 2003. TREMs in the immune system and beyond. *Nature Reviews Immunology,* 3**,** 445-453.

CORNELL-BELL, A. H., FINKBEINER, S. M., COOPER, M. S. & SMITH, S. J. 1990. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science,* 247**,** 470-473.

COTRAN, R. S., KUMAR, V. & STANLEY, R. 2004. *Robbins pathologic basis of disease*, WB Saunders CompHny, Philadelphia, USA.

CRAWLEY, J. N. 2007. *What's wrong with my mouse?: behavioral phenotyping of transgenic and knockout mice*, John Wiley & Sons.

CROCE, N., GELFO, F., CIOTTI, M. T., FEDERICI, G., CALTAGIRONE, C., BERNARDINI, S. & ANGELUCCI, F. 2013. NPY modulates miR-30a-5p and BDNF in opposite direction in an in vitro model of Alzheimer disease: a possible role in neuroprotection? *Molecular and cellular biochemistry,* 376**,** 189-195.

CROCKER, P. R., PAULSON, J. C. & VARKI, A. 2007. Siglecs and their roles in the immune system. *Nature Reviews Immunology,* 7**,** 255.

CSUKA, E., MORGANTI-KOSSMANN, M. C., LENZLINGER, P. M., JOLLER, H., TRENTZ, O. & KOSSMANN, T. 1999. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-α, TGF-β1 and blood–brain barrier function. *Journal of neuroimmunology,* 101**,** 211-221.

CUMMINGS, J., AISEN, P. S., DUBOIS, B., FRÖLICH, L., JACK, C. R., JONES, R. W., MORRIS, J. C., RASKIN, J., DOWSETT, S. A. & SCHELTENS, P. 2016. Drug development in Alzheimer's disease: the path to 2025. *Alzheimer's research & therapy,* 8**,** 39.

CZEH, M., GRESSENS, P. & KAINDL, A. M. 2011. The yin and yang of microglia. *Developmental neuroscience,* 33**,** 199-209.

CZERNIAWSKI, J. & GUZOWSKI, J. F. 2014. Acute neuroinflammation impairs context discrimination memory and disrupts pattern separation processes in hippocampus. *Journal of Neuroscience,* 34**,** 12470-12480.

DALLÉ, E., MABANDLA, M. V. & DANIELS, W. M. 2020. Dielectric Constant and Conductivity of Blood Plasma: Possible Novel Biomarkers for Alzheimer's Disease. *Oxidative Medicine and Cellular Longevity,* 2020.

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

DE STROOPER, B., VASSAR, R. & GOLDE, T. 2010. The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nature Reviews Neurology,* 6**,** 99.

DEITMER, J. W. & ROSE, C. R. 1996. pH regulation and proton signalling by glial cells. *Progress in neurobiology,* 48**,** 73-103.

DEL REY, A., BALSCHUN, D., WETZEL, W., RANDOLF, A. & BESEDOVSKY, H. O. 2013. A cytokine network involving brain-borne IL-1β, IL-1ra, IL-18, IL-6, and TNFα operates during long-term potentiation and learning. *Brain, behavior, and immunity,* 33**,** 15-23.

DHIKAV, V. & ANAND, K. 2007. Hippocampal atrophy may be a predictor of seizures in Alzheimer's disease. *Medical hypotheses,* 1**,** 234-235.

DOMINGUES, C., AB DA CRUZ E SILVA, O. & HENRIQUES, A. 2017. Impact of cytokines and chemokines on Alzheimer's disease neuropathological hallmarks. *Current Alzheimer research,* 14**,** 870-882.

DOMINGUEZ, D.-I., HARTMANN, D. & DE STROOPER, B. 2004. BACE1 and presenilin: two unusual aspartyl proteases involved in Alzheimer's disease. *Neurodegenerative Diseases,* 1**,** 168-174.

DONZIS, E. J. & TRONSON, N. C. 2014. Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences. *Neurobiology of learning and memory,* 115**,** 68-77.

DU YAN, S., CHEN, X., FU, J., CHEN, M., ZHU, H., ROHER, A., SLATTERY, T., ZHAO, L., NAGASHIMA, M. & MORSER, J. 1996. RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. *Nature,* 382**,** 685-691.

DURHAM, T. B. & SHEPHERD, T. A. 2006. Progress toward the discovery and development of efficacious BACE inhibitors. *Current opinion in drug discovery & development,* 9**,** 776-791.

ECKMAN, C. B. & ECKMAN, E. A. 2007. An update on the amyloid hypothesis. *Neurologic clinics,* 25**,** 669-682.

EKDAHL, C., KOKAIA, Z. & LINDVALL, O. 2009. Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience,* 158**,** 1021-1029.

EL KHOURY, J., HICKMAN, S., THOMAS, C., LOIKE, J. & SILVERSTEIN, S. 1998. Microglia, scavenger receptors, and the pathogenesis of Alzheimer's disease. *Neurobiology of aging,* 19**,** S81-S84.

ELDERKIN-THOMPSON, V., IRWIN, M. R., HELLEMANN, G. & KUMAR, A. 2012. Interleukin-6 and memory functions of encoding and recall in healthy and depressed elderly adults. *The American Journal of Geriatric Psychiatry,* 20**,** 753-763.

ELLMAN, G. L. 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics,* 82**,** 70-77.

ESCARTIN, C. & BONVENTO, G. 2008. Targeted activation of astrocytes: a potential neuroprotective strategy. *Molecular neurobiology,* 38**,** 231-241.

FACCHINETTI, R., BRONZUOLI, M. R. & SCUDERI, C. 2018. An animal model of Alzheimer disease based on the intrahippocampal injection of amyloid β-peptide (1–42). *Neurotrophic Factors.* Springer.

FAKHOURY, M. 2018. Microglia and astrocytes in Alzheimer's disease: Implications for therapy. *Current neuropharmacology,* 16**,** 508-518.

FANSELOW, M. S. 2000. Contextual fear, gestalt memories, and the hippocampus. *Behavioural brain research,* 110**,** 73-81.

FANSELOW, M. S. & POULOS, A. M. 2005. The neuroscience of mammalian associative learning. *Annu. Rev. Psychol.,* 56**,** 207-234.

FAUCHER, P., MONS, N., MICHEAU, J., LOUIS, C. & BERACOCHEA, D. J. 2016. Hippocampal injections of oligomeric amyloid β-peptide (1–42) induce selective working memory deficits and long-lasting alterations of ERK signaling pathway. *Frontiers in Aging Neuroscience,* 7**,** 245.

FERREIRA, S. T., CLARKE, J. R., BOMFIM, T. R. & DE FELICE, F. G. 2014. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. *Alzheimer's & dementia,* 10**,** S76-S83.

FERREIRA, S. T., LOURENCO, M. V., OLIVEIRA, M. M. & DE FELICE, F. G. 2015. Soluble amyloid-b oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. *Frontiers in cellular neuroscience,* 9**,** 191.

FIALKOW, L., WANG, Y. & DOWNEY, G. P. 2007. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. *Free Radical Biology and Medicine,* 42**,** 153-164.

FLODEN, A. M. & COMBS, C. K. 2011. Microglia demonstrate age-dependent interaction with amyloid-β fibrils. *Journal of Alzheimer's Disease,* 25**,** 279-293.

FRANK, S., BURBACH, G. J., BONIN, M., WALTER, M., STREIT, W., BECHMANN, I. & DELLER, T. 2008. TREM2 is upregulated in amyloid plaque-associated microglia in aged APP23 transgenic mice. *Glia,* 56**,** 1438-1447.

FRAUTSCHY, S. A., BAIRD, A. & COLE, G. M. 1991. Effects of injected Alzheimer beta-amyloid cores in rat brain. *Proceedings of the National Academy of Sciences,* 88**,** 8362-8366.

FRAUTSCHY, S. A., YANG, F., CALDERÓN, L. & COLE, G. M. 1996. Rodent models of Alzheimer's disease: rat Aβ infusion approaches to amyloid deposits. *Neurobiology of aging,* 17**,** 311-321.

FRISONI, G. B., FOX, N. C., JACK, C. R., SCHELTENS, P. & THOMPSON, P. M. 2010. The clinical use of structural MRI in Alzheimer disease. *Nature Reviews Neurology,* 6**,** 67-77.

FUKUMOTO, H., CHEUNG, B. S., HYMAN, B. T. & IRIZARRY, M. C. 2002. β-Secretase protein and activity are increased in the neocortex in Alzheimer disease. *Archives of neurology,* 59**,** 1381-1389.

GANDY, S. & HEPPNER, F. L. 2013. Microglia as dynamic and essential components of the amyloid hypothesis. *Neuron,* 78**,** 575-577.

GARCIA, J. M., STILLINGS, S. A., LECLERC, J. L., PHILLIPS, H., EDWARDS, N. J., ROBICSEK, S. A., HOH, B. L., BLACKBURN, S. & DORÉ, S. 2017. Role of interleukin-10 in acute brain injuries. *Frontiers in neurology,* 8**,** 244.

GARTHE, A. & KEMPERMANN, G. 2013. An old test for new neurons: refining the Morris water maze to study the functional relevance of adult hippocampal neurogenesis. *Frontiers in neuroscience,* 7**,** 63.

GEEKIYANAGE, H., JICHA, G. A., NELSON, P. T. & CHAN, C. 2012. Blood serum miRNA: non-invasive biomarkers for Alzheimer's disease. *Experimental neurology,* 235**,** 491-496.

GEORGE, P. & CHARLES, W. 2007. The rat brain in stereotaxic coordinates. *Qingchuan Zhuge translate,* 32.

GIBERTINI, M., NEWTON, C., FRIEDMAN, H. & KLEIN, T. W. 1995. Spatial learning impairment in mice infected with Legionella pneumophila or administered exogenous interleukin-1-β. *Brain, behavior, and immunity,* 9**,** 113-128.

GINHOUX, F., LIM, S., HOEFFEL, G., LOW, D. & HUBER, T. 2013. Origin and differentiation of microglia. *Frontiers in cellular neuroscience,* 7**,** 45.

GINSBERG, S. D., CRINO, P. B., LEE, V. M. Y., EBERWINE, J. H. & TROJANOWSKI, J. Q. 1997. Sequestration of RNA in Alzheimer's disease neurofibrillary tangles and senile plaques. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society,* 41**,** 200-209.

GIULIAN, D., LI, J., LI, X., GEORGE, J. & RUTECKI, P. A. 1994. The impact of microglia-derived cytokines upon gliosis in the CNS. *Developmental neuroscience,* 16**,** 128-136.

GLASS, C. K., SAIJO, K., WINNER, B., MARCHETTO, M. C. & GAGE, F. H. 2010. Mechanisms underlying inflammation in neurodegeneration. *Cell,* 140**,** 918-934.

GLENNER, G. G. & WONG, C. W. 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and biophysical research communications,* 120**,** 885-890.

GOEDERT, M. 2015. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein. *Science,* 349**,** 1255555.

GOEHLER, L. E. 2008. Cytokines in neural signaling to the brain. *Neuroimmune Biology,* 6**,** 337-352.

GÓMEZ-ISLA, T., HOLLISTER, R., WEST, H., MUI, S., GROWDON, J. H., PETERSEN, R. C., PARISI, J. E. & HYMAN, B. T. 1997. Neuronal loss correlates with but exceeds neurofibrillary

tangles in Alzheimer's disease. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society,* 41**,** 17-24.
GONG, C.-X., LIU, F. & IQBAL, K. 2018. Multifactorial hypothesis and multi-targets for Alzheimer's disease. *Journal of Alzheimer's Disease,* 64**,** S107-S117.
GONZALEZ, P., MACHADO, I., VILCAES, A., CARUSO, C., ROTH, G. A., SCHIÖTH, H., LASAGA, M. & SCIMONELLI, T. 2013. Molecular mechanisms involved in interleukin 1-beta (IL-1β)-induced memory impairment. Modulation by alpha-melanocyte-stimulating hormone (α-MSH). *Brain, behavior, and immunity,* 34**,** 141-150.
GONZALEZ, P. V., SCHIÖTH, H. B., LASAGA, M. & SCIMONELLI, T. N. 2009. Memory impairment induced by IL-1β is reversed by α-MSH through central melanocortin-4 receptors. *Brain, behavior, and immunity,* 23**,** 817-822.
GOODALL, E. F., HEATH, P. R., BANDMANN, O., KIRBY, J. & SHAW, P. J. 2013. Neuronal dark matter: the emerging role of microRNAs in neurodegeneration. *Frontiers in cellular neuroscience,* 7**,** 178.
GOSHEN, I., BRODSKY, M., PRAKASH, R., WALLACE, J., GRADINARU, V., RAMAKRISHNAN, C. & DEISSEROTH, K. 2011. Dynamics of retrieval strategies for remote memories. *Cell,* 147**,** 678-689.
GOSHEN, I., KREISEL, T., OUNALLAH-SAAD, H., RENBAUM, P., ZALZSTEIN, Y., BEN-HUR, T., LEVY-LAHAD, E. & YIRMIYA, R. 2007. A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology,* 32**,** 1106-1115.
GRASSO, M., PISCOPO, P., CONFALONI, A. & DENTI, M. A. 2014. Circulating miRNAs as biomarkers for neurodegenerative disorders. *Molecules,* 19**,** 6891-6910.
GRICIUC, A., SERRANO-POZO, A., PARRADO, A. R., LESINSKI, A. N., ASSELIN, C. N., MULLIN, K., HOOLI, B., CHOI, S. H., HYMAN, B. T. & TANZI, R. E. 2013. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron,* 78**,** 631-643.
GUERREIRO, R., WOJTAS, A., BRAS, J., CARRASQUILLO, M., ROGAEVA, E., MAJOUNIE, E., CRUCHAGA, C., SASSI, C., KAUWE, J. S. & YOUNKIN, S. 2013. TREM2 variants in Alzheimer's disease. *New England Journal of Medicine,* 368**,** 117-127.
GUICHET, P.-O., GUELFI, S., RIPOLL, C., TEIGELL, M., SABOURIN, J.-C., BAUCHET, L., RIGAU, V., ROTHHUT, B. & HUGNOT, J.-P. 2016. Asymmetric distribution of GFAP in glioma multipotent cells. *PloS one,* 11**,** e0151274.
GUO, C., LIU, S. & SUN, M.-Z. 2013. Novel insight into the role of GAPDH playing in tumor. *Clinical and Translational Oncology,* 15**,** 167-172.
GUO, T. & HOBBS, D. W. 2006. Development of BACE1 inhibitors for Alzheimer's disease. *Current medicinal chemistry,* 13**,** 1811-1829.
GUO, Y., XIANG, Y. & YANG, Z.-W. 2016. Drying paraffin sections on hotplate unadvisable. *Journal of Histology & Histopathology,* 3**,** 4.
GUTHRIE, P. B., KNAPPENBERGER, J., SEGAL, M., BENNETT, M. V., CHARLES, A. C. & KATER, S. B. 1999. ATP released from astrocytes mediates glial calcium waves. *Journal of Neuroscience,* 19**,** 520-528.
GUZMÁN, C., HALLAL-CALLEROS, C., LÓPEZ-GRIEGO, L. & MORALES-MONTOR, J. 2010. Interleukin-6: a cytokine with a pleiotropic role in the neuroimmunoendocrine network. *The Open Neuroendocrinology Journal,* 3.
HA, M. & KIM, V. N. 2014. Regulation of microRNA biogenesis. *Nature reviews Molecular cell biology,* 15**,** 509-524.
HAMMOND, S. M. 2015. An overview of microRNAs. *Advanced drug delivery reviews,* 87**,** 3-14.
HANISCH, U.-K. & KETTENMANN, H. 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nature neuroscience,* 10**,** 1387-1394.
HANIU, M., DENIS, P., YOUNG, Y., MENDIAZ, E. A., FULLER, J., HUI, J. O., BENNETT, B. D., KAHN, S., ROSS, S. & BURGESS, T. 2000. Characterization of Alzheimer's β-secretase protein BACE a pepsin family member with unusual properties. *Journal of Biological Chemistry,* 275**,** 21099-21106.
HARDY, J. & ALLSOP, D. 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in pharmacological sciences,* 12**,** 383-388.

HARDY, J. & SELKOE, D. J. 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *science,* 297**,** 353-356.
HARDY, J. A. & HIGGINS, G. A. 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science,* 256**,** 184-186.
HARRY, G. J. & KRAFT, A. D. 2012. Microglia in the developing brain: a potential target with lifetime effects. *Neurotoxicology,* 33**,** 191-206.
HARTLAGE-RÜBSAMEN, M., ZEITSCHEL, U., APELT, J., GÄRTNER, U., FRANKE, H., STAHL, T., GÜNTHER, A., SCHLIEBS, R., PENKOWA, M. & BIGL, V. 2003. Astrocytic expression of the Alzheimer's disease β-secretase (BACE1) is stimulus-dependent. *Glia,* 41**,** 169-179.
HAUPTMANN, S., KEIL, U., SCHERPING, I., BONERT, A., ECKERT, A. & MÜLLER, W. E. 2006. Mitochondrial dysfunction in sporadic and genetic Alzheimer's disease. *Experimental gerontology,* 41**,** 668-673.
HAYDON, P. G. 2001. GLIA: listening and talking to the synapse. *Nature Reviews Neuroscience,* 2**,** 185-193.
HELMY, A., ANTONIADES, C. A., GUILFOYLE, M. R., CARPENTER, K. L. & HUTCHINSON, P. J. 2012. Principal component analysis of the cytokine and chemokine response to human traumatic brain injury. *PloS one,* 7**,** e39677.
HENEKA, M. T., CARSON, M. J., EL KHOURY, J., LANDRETH, G. E., BROSSERON, F., FEINSTEIN, D. L., JACOBS, A. H., WYSS-CORAY, T., VITORICA, J. & RANSOHOFF, R. M. 2015. Neuroinflammation in Alzheimer's disease. *The Lancet Neurology,* 14**,** 388-405.
HENEKA, M. T., KUMMER, M. P. & LATZ, E. 2014. Innate immune activation in neurodegenerative disease. *Nature Reviews Immunology,* 14**,** 463-477.
HENEKA, M. T. & O'BANION, M. K. 2007. Inflammatory processes in Alzheimer's disease. *Journal of neuroimmunology,* 184**,** 69-91.
HENSLER, T., SAUERLAND, S., BOUILLON, B., RAUM, M., RIXEN, D., HELLING, H.-J., ANDERMAHR, J. & NEUGEBAUER, E. A. 2002. Association between injury pattern of patients with multiple injuries and circulating levels of soluble tumor necrosis factor receptors, interleukin-6 and interleukin-10, and polymorphonuclear neutrophil elastase. *Journal of Trauma and Acute Care Surgery,* 52**,** 962-970.
HENSLER, T., SAUERLAND, S., RIESS, P., HESS, S., HELLING, H., ANDERMAHR, J., BOUILLON, B. & NEUGEBAUER, E. 2000. The effect of additional brain injury on systemic interleukin (IL)-10 and IL-13 levels in trauma patients. *Inflammation Research,* 49**,** 524-528.
HEPPNER, F. L., RANSOHOFF, R. M. & BECHER, B. 2015. Immune attack: the role of inflammation in Alzheimer disease. *Nature Reviews Neuroscience,* 16**,** 358-372.
HEYSER, C. J., MASLIAH, E., SAMIMI, A., CAMPBELL, I. L. & GOLD, L. H. 1997. Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin 6 in the brain. *Proceedings of the National Academy of Sciences,* 94**,** 1500-1505.
HICKMAN, S. E., ALLISON, E. K. & EL KHOURY, J. 2008. Microglial dysfunction and defective β-amyloid clearance pathways in aging Alzheimer's disease mice. *Journal of Neuroscience,* 28**,** 8354-8360.
HOLLINGWORTH, P., HAROLD, D., SIMS, R., GERRISH, A., LAMBERT, J.-C., CARRASQUILLO, M. M., ABRAHAM, R., HAMSHERE, M. L., PAHWA, J. S. & MOSKVINA, V. 2011. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nature genetics,* 43**,** 429.
HOLMES, C., CUNNINGHAM, C., ZOTOVA, E., WOOLFORD, J., DEAN, C., KERR, S. U., CULLIFORD, D. & PERRY, V. 2009. Systemic inflammation and disease progression in Alzheimer disease. *Neurology,* 73**,** 768-774.
HOLSINGER, R. D., MCLEAN, C. A., BEYREUTHER, K., MASTERS, C. L. & EVIN, G. 2002. Increased expression of the amyloid precursor β-secretase in Alzheimer's disease. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society,* 51**,** 783-786.
HOPPERTON, K., MOHAMMAD, D., TREPANIER, M., GIULIANO, V. & BAZINET, R. 2018. Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: a systematic review. *Molecular psychiatry,* 23**,** 177.

HRUSKA, Z. & DOHANICH, G. P. 2007. The effects of chronic estradiol treatment on working memory deficits induced by combined infusion of β-amyloid (1–42) and ibotenic acid. *Hormones and behavior,* 52**,** 297-306.

HSIEH, C. L., KOIKE, M., SPUSTA, S. C., NIEMI, E. C., YENARI, M., NAKAMURA, M. C. & SEAMAN, W. E. 2009. A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. *Journal of neurochemistry,* 109**,** 1144-1156.

HUANG, Y., SHEN, X. J., ZOU, Q., WANG, S. P., TANG, S. M. & ZHANG, G. Z. 2011. Biological functions of microRNAs: a review. *Journal of physiology and biochemistry,* 67**,** 129-139.

HUNSAKER, M. R. & KESNER, R. P. 2008. Dissociations across the dorsal–ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear. *Neurobiology of learning and memory,* 89**,** 61-69.

HURD, M. D., MARTORELL, P., DELAVANDE, A., MULLEN, K. J. & LANGA, K. M. 2013. Monetary costs of dementia in the United States. *New England Journal of Medicine,* 368**,** 1326-1334.

HYMAN, B. T., PHELPS, C. H., BEACH, T. G., BIGIO, E. H., CAIRNS, N. J., CARRILLO, M. C., DICKSON, D. W., DUYCKAERTS, C., FROSCH, M. P. & MASLIAH, E. 2012. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's & dementia,* 8**,** 1-13.

IADECOLA, C. & NEDERGAARD, M. 2007. Glial regulation of the cerebral microvasculature. *Nature neuroscience,* 10**,** 1369-1376.

IJOMONE, O. K., SHALLIE, P. D. & NAICKER, T. 2019. Nω-nitro-l-arginine methyl model of pre-eclampsia elicits differential IBA1 and EAAT1 expressions in brain. *Journal of chemical neuroanatomy,* 100**,** 101660.

INTERNATIONAL, A. S. D. 2010. *World Alzheimer report 2010: the global economic impact of dementia*, Alzheimer's Disease International.

IQBAL, K. & GRUNDKE-IQBAL, I. 2010. Alzheimer's disease, a multifactorial disorder seeking multitherapies. Elsevier.

JACK JR, C. R., BENNETT, D. A., BLENNOW, K., CARRILLO, M. C., DUNN, B., HAEBERLEIN, S. B., HOLTZMAN, D. M., JAGUST, W., JESSEN, F. & KARLAWISH, J. 2018. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimer's & Dementia,* 14**,** 535-562.

JANERO, D. R. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine,* 9**,** 515-540.

JARSKY, T., ROXIN, A., KATH, W. L. & SPRUSTON, N. 2005. Conditional dendritic spike propagation following distal synaptic activation of hippocampal CA1 pyramidal neurons. *Nature neuroscience,* 8**,** 1667.

JEAN, Y. Y., BALERIOLA, J., FÀ, M., HENGST, U. & TROY, C. M. 2015. Stereotaxic infusion of oligomeric amyloid-beta into the mouse hippocampus. *JoVE (Journal of Visualized Experiments)***,** e52805.

JIANG, T. & CADENAS, E. 2014. Astrocytic metabolic and inflammatory changes as a function of age. *Aging cell,* 13**,** 1059-1067.

JIANG, T., TAN, L., ZHU, X.-C., ZHANG, Q.-Q., CAO, L., TAN, M.-S., GU, L.-Z., WANG, H.-F., DING, Z.-Z. & ZHANG, Y.-D. 2014. Upregulation of TREM2 ameliorates neuropathology and rescues spatial cognitive impairment in a transgenic mouse model of Alzheimer's disease. *Neuropsychopharmacology,* 39**,** 2949-2962.

JIANG, T., ZHANG, Y.-D., CHEN, Q., GAO, Q., ZHU, X.-C., ZHOU, J.-S., SHI, J.-Q., LU, H., TAN, L. & YU, J.-T. 2016. TREM2 modifies microglial phenotype and provides neuroprotection in P301S tau transgenic mice. *Neuropharmacology,* 105**,** 196-206.

JOHN, V. 2006. Human β-secretase (BACE) and BACE inhibitors: progress report. *Current topics in medicinal chemistry,* 6**,** 569-578.

JONSSON, T., STEFANSSON, H., STEINBERG, S., JONSDOTTIR, I., JONSSON, P. V., SNAEDAL, J., BJORNSSON, S., HUTTENLOCHER, J., LEVEY, A. I. & LAH, J. J. 2013. Variant of TREM2 associated with the risk of Alzheimer's disease. *New England Journal of Medicine,* 368**,** 107-116.

JOSHI, A., RINGMAN, J. M., LEE, A. S., JUAREZ, K. O. & MENDEZ, M. F. 2012. Comparison of clinical characteristics between familial and non-familial early onset Alzheimer's disease. *Journal of neurology,* 259**,** 2182-2188.

JUNG, M. W., WIENER, S. I. & MCNAUGHTON, B. L. 1994. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *Journal of Neuroscience,* 14**,** 7347-7356.

KABBA, J. A., XU, Y., CHRISTIAN, H., RUAN, W., CHENAI, K., XIANG, Y., ZHANG, L., SAAVEDRA, J. M. & PANG, T. 2018. Microglia: housekeeper of the central nervous system. *Cellular and molecular neurobiology,* 38**,** 53-71.

KALOGIANNI, D. P., KALLIGOSFYRI, P. M., KYRIAKOU, I. K. & CHRISTOPOULOS, T. K. 2018. Advances in microRNA analysis. *Analytical and bioanalytical chemistry,* 410**,** 695-713.

KAMETANI, F. & HASEGAWA, M. 2018. Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Frontiers in neuroscience,* 12**,** 25.

KAMM, K., VANDERKOLK, W., LAWRENCE, C., JONKER, M. & DAVIS, A. T. 2006. The effect of traumatic brain injury upon the concentration and expression of interleukin-1β and interleukin-10 in the rat. *Journal of Trauma and Acute Care Surgery,* 60**,** 152-157.

KARTHICK, C., NITHIYANANDAN, S., ESSA, M. M., GUILLEMIN, G. J., JAYACHANDRAN, S. K. & ANUSUYADEVI, M. 2019. Time-dependent effect of oligomeric amyloid-β (1–42)-induced hippocampal neurodegeneration in rat model of Alzheimer's disease. *Neurological research,* 41**,** 139-150.

KAWAHARA, K., SUENOBU, M., YOSHIDA, A., KOGA, K., HYODO, A., OHTSUKA, H., KUNIYASU, A., TAMAMAKI, N., SUGIMOTO, Y. & NAKAYAMA, H. 2012. Intracerebral microinjection of interleukin-4/interleukin-13 reduces β-amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice. *Neuroscience,* 207**,** 243-260.

KAYANO, M., HIGAKI, S., SATOH, J.-I., MATSUMOTO, K., MATSUBARA, E., TAKIKAWA, O. & NIIDA, S. 2016. Plasma microRNA biomarker detection for mild cognitive impairment using differential correlation analysis. *Biomarker research,* 4**,** 22.

KEIFER, J., ZHENG, Z. & AMBIGAPATHY, G. 2015. A microRNA-BDNF negative feedback signaling loop in brain: implications for Alzheimer's disease. *MicroRNA,* 4**,** 101-108.

KETTENMANN, H. & VERKHRATSKY, A. 2008. Neuroglia: the 150 years after. *Trends in neurosciences,* 31**,** 653-659.

KHAKH, B. S. & SOFRONIEW, M. V. 2015. Diversity of astrocyte functions and phenotypes in neural circuits. *Nature neuroscience,* 18**,** 942.

KHEMKA, V. K., GANGULY, A., BAGCHI, D., GHOSH, A., BIR, A., BISWAS, A., CHATTOPADHYAY, S. & CHAKRABARTI, S. 2014. Raised serum proinflammatory cytokines in Alzheimer's disease with depression. *Aging and disease,* 5**,** 170.

KIM, S. U. & DE VELLIS, J. 2005. Microglia in health and disease. *Journal of neuroscience research,* 81**,** 302-313.

KIM, Y. S., JUNG, H. M. & YOON, B.-E. 2018. Exploring glia to better understand Alzheimer's disease. *Animal cells and systems,* 22**,** 213-218.

KINNEY, J. W., BEMILLER, S. M., MURTISHAW, A. S., LEISGANG, A. M., SALAZAR, A. M. & LAMB, B. T. 2018. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions,* 4**,** 575-590.

KIYOTA, T., INGRAHAM, K. L., SWAN, R. J., JACOBSEN, M. T., ANDREWS, S. J. & IKEZU, T. 2012a. AAV serotype 2/1-mediated gene delivery of anti-inflammatory interleukin-10 enhances neurogenesis and cognitive function in APP+ PS1 mice. *Gene therapy,* 19**,** 724.

KIYOTA, T., INGRAHAM, K. L., SWAN, R. J., JACOBSEN, M. T., ANDREWS, S. J. & IKEZU, T. 2012b. AAV serotype 2/1-mediated gene delivery of anti-inflammatory interleukin-10 enhances neurogenesis and cognitive function in APP+ PS1 mice. *Gene therapy,* 19**,** 724-733.

KIYOTA, T., OKUYAMA, S., SWAN, R. J., JACOBSEN, M. T., GENDELMAN, H. E. & IKEZU, T. 2010. CNS expression of anti-inflammatory cytokine interleukin-4 attenuates Alzheimer's disease-like pathogenesis in APP+ PS1 bigenic mice. *The FASEB Journal,* 24**,** 3093-3102.

KLYUBIN, I., BETTS, V., WELZEL, A. T., BLENNOW, K., ZETTERBERG, H., WALLIN, A., LEMERE, C. A., CULLEN, W. K., PENG, Y. & WISNIEWSKI, T. 2008. Amyloid β protein

dimer-containing human CSF disrupts synaptic plasticity: prevention by systemic passive immunization. *Journal of Neuroscience,* 28**,** 4231-4237.

KORZHEVSKII, D. & KIRIK, O. 2016. Brain microglia and microglial markers. *Neuroscience and Behavioral Physiology,* 46**,** 284-290.

KOWALL, N. W., BEAL, M. F., BUSCIGLIO, J., DUFFY, L. K. & YANKNER, B. A. 1991. An in vivo model for the neurodegenerative effects of beta amyloid and protection by substance P. *Proceedings of the National Academy of Sciences,* 88**,** 7247-7251.

KRABBE, G., HALLE, A., MATYASH, V., RINNENTHAL, J. L., EOM, G. D., BERNHARDT, U., MILLER, K. R., PROKOP, S., KETTENMANN, H. & HEPPNER, F. L. 2013a. Functional impairment of microglia coincides with Beta-amyloid deposition in mice with Alzheimer-like pathology. *PloS one,* 8.

KRABBE, G., HALLE, A., MATYASH, V., RINNENTHAL, J. L., EOM, G. D., BERNHARDT, U., MILLER, K. R., PROKOP, S., KETTENMANN, H. & HEPPNER, F. L. 2013b. Functional impairment of microglia coincides with Beta-amyloid deposition in mice with Alzheimer-like pathology. *PloS one,* 8**,** e60921.

KRISHNAN, S. & RANI, P. 2014. Evaluation of selenium, redox status and their association with plasma amyloid/tau in Alzheimer's disease. *Biological trace element research,* 158**,** 158-165.

KUMAR, S., VIJAYAN, M., BHATTI, J. & REDDY, P. H. 2017. MicroRNAs as peripheral biomarkers in aging and age-related diseases. *Progress in molecular biology and translational science.* Elsevier.

LAJAUNIAS, F., DAYER, J. M. & CHIZZOLINI, C. 2005. Constitutive repressor activity of CD33 on human monocytes requires sialic acid recognition and phosphoinositide 3-kinase-mediated intracellular signaling. *European journal of immunology,* 35**,** 243-251.

LAMBERT, J.-C., IBRAHIM-VERBAAS, C. A., HAROLD, D., NAJ, A. C., SIMS, R., BELLENGUEZ, C., JUN, G., DESTEFANO, A. L., BIS, J. C. & BEECHAM, G. W. 2013. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nature genetics,* 45**,** 1452.

LE MOINE, O., STORDEUR, P., SCHANDENÉ, L., MARCHANT, A., DE GROOTE, D., GOLDMAN, M. & DEVIÈRE, J. 1996. Adenosine enhances IL-10 secretion by human monocytes. *The Journal of immunology,* 156**,** 4408-4414.

LECUYER, M.-A., KEBIR, H. & PRAT, A. 2016. Glial influences on BBB functions and molecular players in immune cell trafficking. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 1862**,** 472-482.

LEDOUX, J. E. 2000. Emotion circuits in the brain. *Annual review of neuroscience,* 23**,** 155-184.

LEE, C. D., DAGGETT, A., GU, X., JIANG, L.-L., LANGFELDER, P., LI, X., WANG, N., ZHAO, Y., PARK, C. S. & COOPER, Y. 2018. Elevated TREM2 gene dosage reprograms microglia responsivity and ameliorates pathological phenotypes in Alzheimer's disease models. *Neuron,* 97**,** 1032-1048. e5.

LEIDINGER, P., BACKES, C., DEUTSCHER, S., SCHMITT, K., MUELLER, S. C., FRESE, K., HAAS, J., RUPRECHT, K., PAUL, F. & STÄHLER, C. 2013. A blood based 12-miRNA signature of Alzheimer disease patients. *Genome biology,* 14**,** R78.

LEISSRING, M. A., FARRIS, W., CHANG, A. Y., WALSH, D. M., WU, X., SUN, X., FROSCH, M. P. & SELKOE, D. J. 2003. Enhanced proteolysis of β-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron,* 40**,** 1087-1093.

LEWIS, B. P., BURGE, C. B. & BARTEL, D. P. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *cell,* 120**,** 15-20.

LEWIS, B. P., SHIH, I.-H., JONES-RHOADES, M. W., BARTEL, D. P. & BURGE, C. B. 2003. Prediction of mammalian microRNA targets. *Cell,* 115**,** 787-798.

LI, F., WANG, Y., YANG, H., XU, Y., ZHOU, X., ZHANG, X., XIE, Z. & BI, J. 2019. The effect of BACE1-AS on β-amyloid generation by regulating BACE1 mRNA expression. *BMC molecular biology,* 20**,** 23.

LI, J., LAN, T., ZHANG, C., ZENG, C., HOU, J., YANG, Z., ZHANG, M., LIU, J. & LIU, B. 2015. Reciprocal activation between IL-6/STAT3 and NOX4/Akt signalings promotes proliferation and survival of non-small cell lung cancer cells. *Oncotarget,* 6**,** 1031.

LI, Q. & SÜDHOF, T. C. 2004. Cleavage of amyloid-β precursor protein and amyloid-β precursor-like protein by BACE 1. *Journal of Biological Chemistry,* 279**,** 10542-10550.
LI, W., POTEET, E., XIE, L., LIU, R., WEN, Y. & YANG, S.-H. 2011. Regulation of matrix metalloproteinase 2 by oligomeric amyloid β protein. *Brain research,* 1387**,** 141-148.
LIANG, H., GONG, F., ZHANG, S., ZHANG, C. Y., ZEN, K. & CHEN, X. 2014. The origin, function, and diagnostic potential of extracellular microRNAs in human body fluids. *Wiley Interdisciplinary Reviews: RNA,* 5**,** 285-300.
LIM, D., IYER, A., RONCO, V., GROLLA, A. A., CANONICO, P. L., ARONICA, E. & GENAZZANI, A. A. 2013. Amyloid beta deregulates astroglial mGluR5-mediated calcium signaling via calcineurin and Nf-kB. *Glia,* 61**,** 1134-1145.
LINNARTZ, B. & NEUMANN, H. 2013. Microglial activatory (immunoreceptor tyrosine-based activation motif)-and inhibitory (immunoreceptor tyrosine-based inhibition motif)-signaling receptors for recognition of the neuronal glycocalyx. *Glia,* 61**,** 37-46.
LIU, B. & HONG, J.-S. 2003. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *Journal of Pharmacology and Experimental Therapeutics,* 304**,** 1-7.
LIU, C.-G., WANG, J.-L., LI, L. & WANG, P.-C. 2014. MicroRNA-384 regulates both amyloid precursor protein and β-secretase expression and is a potential biomarker for Alzheimer's disease. *International journal of molecular medicine,* 34**,** 160-166.
LIU, W., CAI, H., LIN, M., ZHU, L., GAO, L., ZHONG, R., BI, S., XUE, Y. & SHANG, X. 2016. MicroRNA-107 prevents amyloid-beta induced blood-brain barrier disruption and endothelial cell dysfunction by targeting Endophilin-1. *Experimental cell research,* 343**,** 248-257.
LIU, W., TANG, Y. & FENG, J. 2011. Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system. *Life sciences,* 89**,** 141-146.
LIU, W., WANG, C.-H., CUI, Y., MO, L.-Q., ZHI, J.-L., SUN, S.-N., WANG, Y.-L., YU, H.-M., ZHAO, C.-M. & FENG, J.-Q. 2006. Inhibition of neuronal nitric oxide synthase antagonizes morphine antinociceptive tolerance by decreasing activation of p38 MAPK in the spinal microglia. *Neuroscience letters,* 410**,** 174-177.
LIU, X., FANG, L., GUO, T. B., MEI, H. & ZHANG, J. Z. 2013. Drug targets in the cytokine universe for autoimmune disease. *Trends in immunology,* 34**,** 120-128.
LIVAK, K. J. & SCHMITTGEN, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods,* 25**,** 402-408.
LLORENS-MARTÍN, M., BLAZQUEZ-LLORCA, L., BENAVIDES-PICCIONE, R., RABANO, A., HERNANDEZ, F., AVILA, J. & DEFELIPE, J. 2014. Selective alterations of neurons and circuits related to early memory loss in Alzheimer's disease. *Frontiers in neuroanatomy,* 8**,** 38.
LOANE, D. J. & KUMAR, A. 2016. Microglia in the TBI brain: the good, the bad, and the dysregulated. *Experimental neurology,* 275**,** 316-327.
LONAPPAN, A., DALLÉ, E. R. H. E. A., AFULLO, T. J. O. & DANIELS, W. M. U. 2017. Analysis of Behaviour Pattern in Sprague-Dawley Rats Using Microwave Techniques. *Journal of Electromagnetic Analysis and Applications,* 9**,** 155.
LUGLI, G., COHEN, A. M., BENNETT, D. A., SHAH, R. C., FIELDS, C. J., HERNANDEZ, A. G. & SMALHEISER, N. R. 2015. Plasma exosomal miRNAs in persons with and without Alzheimer disease: altered expression and prospects for biomarkers. *PloS one,* 10.
LUND, S., CHRISTENSEN, K. V., HEDTJÄRN, M., MORTENSEN, A. L., HAGBERG, H., FALSIG, J., HASSELDAM, H., SCHRATTENHOLZ, A., PÖRZGEN, P. & LEIST, M. 2006. The dynamics of the LPS triggered inflammatory response of murine microglia under different culture and in vivo conditions. *Journal of neuroimmunology,* 180**,** 71-87.
LYNCH, A. M., WALSH, C., DELANEY, A., NOLAN, Y., CAMPBELL, V. A. & LYNCH, M. A. 2004. Lipopolysaccharide-induced increase in signalling in hippocampus is abrogated by IL-10–a role for IL-1β? *Journal of neurochemistry,* 88**,** 635-646.
MAIER, B., SCHWERDTFEGER, K., MAUTES, A., HOLANDA, M., MÜLLER, M., STEUDEL, W. I. & MARZI, I. 2001. Differential release of interleukines 6, 8, and 10 in cerebrospinal fluid and plasma after traumatic brain injury. *Shock (Augusta, Ga.),* 15**,** 421-426.
MAIER, T., GÜELL, M. & SERRANO, L. 2009. Correlation of mRNA and protein in complex biological samples. *FEBS letters,* 583**,** 3966-3973.

MALARKEY, E. B. & PARPURA, V. 2008. Mechanisms of glutamate release from astrocytes. *Neurochemistry international,* 52**,** 142-154.
MALIK, M., PARIKH, I., VASQUEZ, J. B., SMITH, C., TAI, L., BU, G., LADU, M. J., FARDO, D. W., REBECK, G. W. & ESTUS, S. 2015. Genetics ignite focus on microglial inflammation in Alzheimer's disease. *Molecular neurodegeneration,* 10**,** 1-12.
MALIK, M., SIMPSON, J. F., PARIKH, I., WILFRED, B. R., FARDO, D. W., NELSON, P. T. & ESTUS, S. 2013. CD33 Alzheimer's risk-altering polymorphism, CD33 expression, and exon 2 splicing. *Journal of Neuroscience,* 33**,** 13320-13325.
MALPASS, K. 2013. Alzheimer disease: functional dissection of CD33 locus implicates innate immune response in Alzheimer disease pathology. *Nature Reviews Neurology,* 9**,** 360.
MAOZ, R., GARFINKEL, B. P. & SOREQ, H. 2017. Alzheimer's disease and ncRNAs. *Neuroepigenomics in Aging and Disease.* Springer.
MAREN, S. 2001. Neurobiology of Pavlovian fear conditioning. *Annual review of neuroscience,* 24**,** 897-931.
MARTUCCI, M., SARRIA, S., TOLEDO, M., COSCOJUELA, P., VERT, C., SIURANA, S., AUGER, C. & ROVIRA, A. 2014. Cerebral amyloid angiopathy-related inflammation: imaging findings and clinical outcome. *Neuroradiology,* 56**,** 283-289.
MASTERS, C. L., SIMMS, G., WEINMAN, N. A., MULTHAUP, G., MCDONALD, B. L. & BEYREUTHER, K. 1985. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proceedings of the National Academy of Sciences,* 82**,** 4245-4249.
MATHUR, R., INCE, P. G., MINETT, T., GARWOOD, C. J., SHAW, P. J., MATTHEWS, F. E., BRAYNE, C., SIMPSON, J. E., WHARTON, S. B., FUNCTION, M. C. & GROUP, A. N. S. 2015. A reduced astrocyte response to β-amyloid plaques in the ageing brain associates with cognitive impairment. *PLoS One,* 10**,** e0118463.
MATSUOKA, Y., PICCIANO, M., MALESTER, B., LAFRANCOIS, J., ZEHR, C., DAESCHNER, J. M., OLSCHOWKA, J. A., FONSECA, M. I., O'BANION, M. K. & TENNER, A. J. 2001. Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer's disease. *The American journal of pathology,* 158**,** 1345-1354.
MAWUENYEGA, K. G., SIGURDSON, W., OVOD, V., MUNSELL, L., KASTEN, T., MORRIS, J. C., YARASHESKI, K. E. & BATEMAN, R. J. 2010. Decreased clearance of CNS β-amyloid in Alzheimer's disease. *Science,* 330**,** 1774-1774.
MCDONALD, M. P., DAHL, E. E., OVERMIER, J. B., MANTYH, P. & CLEARY, J. 1994. Effects of an exogenous β-amyloid peptide on retention for spatial learning. *Behavioral and neural biology,* 62**,** 60-67.
MCGEER, E. G. & MCGEER, P. L. 1997. Inflammatory cytokines in the CNS. *CNS drugs,* 7**,** 214-228.
MCKENZIE, J. A., SPIELMAN, L. J., POINTER, C. B., LOWRY, J. R., BAJWA, E., LEE, C. W. & KLEGERIS, A. 2017. Neuroinflammation as a common mechanism associated with the modifiable risk factors for Alzheimer's and Parkinson's diseases. *Current aging science,* 10**,** 158-176.
MCLARNON, J. G. & RYU, J. K. 2008. Relevance of Aβ 1-42 intrahippocampal injection as an animal model of inflamed Alzheimer's disease brain. *Current Alzheimer Research,* 5**,** 475-480.
MEAGER, A. 2006. Cytokines: interleukins. *Reviews in Cell Biology and Molecular Medicine*.
MELCHIOR, B., GARCIA, A. E., HSIUNG, B.-K., LO, K. M., DOOSE, J. M., THRASH, J. C., STALDER, A. K., STAUFENBIEL, M., NEUMANN, H. & CARSON, M. J. 2010. Dual induction of TREM2 and tolerance-related transcript, Tmem176b, in amyloid transgenic mice: implications for vaccine-based therapies for Alzheimer's disease. *ASN neuro,* 2**,** AN20100010.
MENDEZ, M. F. 2017. Early-onset Alzheimer disease. *Neurologic clinics,* 35**,** 263-281.
MESSING, A. & BRENNER, M. 2003. GFAP: functional implications gleaned from studies of genetically engineered mice. *Glia,* 43**,** 87-90.
MIRANDA, K. C., HUYNH, T., TAY, Y., ANG, Y.-S., TAM, W.-L., THOMSON, A. M., LIM, B. & RIGOUTSOS, I. 2006. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell,* 126**,** 1203-1217.
MITTAL, M., SIDDIQUI, M. R., TRAN, K., REDDY, S. P. & MALIK, A. B. 2014. Reactive oxygen species in inflammation and tissue injury. *Antioxidants & redox signaling,* 20**,** 1126-1167.

MOHAJERI, M. H., TROESCH, B. & WEBER, P. 2015. Inadequate supply of vitamins and DHA in the elderly: implications for brain aging and Alzheimer-type dementia. *Nutrition,* 31**,** 261-275.
MOHAMED, T., SHAKERI, A. & RAO, P. P. 2016. Amyloid cascade in Alzheimer's disease: recent advances in medicinal chemistry. *European journal of medicinal chemistry,* 113**,** 258-272.
MOORE, A. H., WU, M., SHAFTEL, S. S., GRAHAM, K. A. & O'BANION, M. K. 2009. Sustained expression of interleukin-1β in mouse hippocampus impairs spatial memory. *Neuroscience,* 164**,** 1484-1495.
MORRIS, R. 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of neuroscience methods,* 11**,** 47-60.
MORRIS, R. G. & BADDELEY, A. D. 1988. Primary and working memory functioning in Alzheimer-type dementia. *Journal of clinical and experimental neuropsychology,* 10**,** 279-296.
MORRIS, G. P., CLARK, I. A. & VISSEL, B. 2018. Questions concerning the role of amyloid-β in the definition, aetiology and diagnosis of Alzheimer's disease. *Acta neuropathologica,* 136**,** 663-689.
MOSER, E. I., KROPFF, E. & MOSER, M.-B. 2008. Place cells, grid cells, and the brain's spatial representation system. *Annu. Rev. Neurosci.,* 31**,** 69-89.
MRAK, R. E. & GRIFFINB, W. S. T. 2001. The role of activated astrocytes and of the neurotrophic cytokine S100B in the pathogenesis of Alzheimer's disease. *Neurobiology of aging,* 22**,** 915-922.
MULLER, J., CORODIMAS, K. P., FRIDEL, Z. & LEDOUX, J. E. 1997. Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral neuroscience,* 111**,** 683.
MURGAS, P., GODOY, B. & VON BERNHARDI, R. 2012. Aβ potentiates inflammatory activation of glial cells induced by scavenger receptor ligands and inflammatory mediators in culture. *Neurotoxicity research,* 22**,** 69-78.
MURPY, M. & LEVINE III, H. 2010. Alzheimer's disease and the β-amyloid peptide. *J Alzheimers Dis,* 19**,** 311-323.
NAGARAJ, S., LASKOWSKA-KASZUB, K., DĘBSKI, K. J., WOJSIAT, J., DĄBROWSKI, M., GABRYELEWICZ, T., KUŹNICKI, J. & WOJDA, U. 2017. Profile of 6 microRNA in blood plasma distinguish early stage Alzheimer's disease patients from non-demented subjects. *Oncotarget,* 8**,** 16122.
NAJ, A. C., JUN, G., BEECHAM, G. W., WANG, L.-S., VARDARAJAN, B. N., BUROS, J., GALLINS, P. J., BUXBAUM, J. D., JARVIK, G. P. & CRANE, P. K. 2011. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nature genetics,* 43**,** 436.
NAKAMURA, S., MURAYAMA, N., NOSHITA, T., ANNOURA, H. & OHNO, T. 2001. Progressive brain dysfunction following intracerebroventricular infusion of beta1–42-amyloid peptide. *Brain research,* 912**,** 128-136.
NAZEM, A., SANKOWSKI, R., BACHER, M. & AL-ABED, Y. 2015. Rodent models of neuroinflammation for Alzheimer's disease. *Journal of neuroinflammation,* 12**,** 74.
NAZIROĞLU, M. 2009. Role of selenium on calcium signaling and oxidative stress-induced molecular pathways in epilepsy. *Neurochemical research,* 34**,** 2181-2191.
NEDERGAARD, M., RANSOM, B. & GOLDMAN, S. A. 2003. New roles for astrocytes: redefining the functional architecture of the brain. *Trends in neurosciences,* 26**,** 523-530.
NELSON, P. T., BALDWIN, D. A., KLOOSTERMAN, W. P., KAUPPINEN, S., PLASTERK, R. H. & MOURELATOS, Z. 2006. RAKE and LNA-ISH reveal microRNA expression and localization in archival human brain. *Rna,* 12**,** 187-191.
NELSON, P. T., HEAD, E., SCHMITT, F. A., DAVIS, P. R., NELTNER, J. H., JICHA, G. A., ABNER, E. L., SMITH, C. D., VAN ELDIK, L. J. & KRYSCIO, R. J. 2011. Alzheimer's disease is not "brain aging": neuropathological, genetic, and epidemiological human studies. *Acta neuropathologica,* 121**,** 571-587.
NELSON, P. T. & KELLER, J. N. 2007. RNA in brain disease: no longer just" the messenger in the middle". *Journal of Neuropathology & Experimental Neurology,* 66**,** 461-468.

NELSON, P. T. & WANG, W.-X. 2010. MiR-107 is reduced in Alzheimer's disease brain neocortex: validation study. *Journal of Alzheimer's Disease,* 21**,** 75-79.
NEUMANN, H. & TAKAHASHI, K. 2007. Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. *Journal of neuroimmunology,* 184**,** 92-99.
NIMMERJAHN, A., KIRCHHOFF, F. & HELMCHEN, F. 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science,* 308**,** 1314-1318.
NITTA, A., ITOH, A., HASEGAWA, T. & NABESHIMA, T. 1994. β-Amyloid protein-induced Alzheimer's disease animal model. *Neuroscience letters,* 170**,** 63-66.
NOLAN, Y., MAHER, F. O., MARTIN, D. S., CLARKE, R. M., BRADY, M. T., BOLTON, A. E., MILLS, K. H. & LYNCH, M. A. 2005. Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus. *Journal of Biological Chemistry,* 280**,** 9354-9362.
NORDEN, D. M., FENN, A. M., DUGAN, A. & GODBOUT, J. P. 2014. TGFβ produced by IL-10 redirected astrocytes attenuates microglial activation. *Glia,* 62**,** 881-895.
NORRIS, G. T. & KIPNIS, J. 2019. Immune cells and CNS physiology: microglia and beyond. *Journal of Experimental Medicine,* 216**,** 60-70.
OKA, J.-I., SUZUKI, E., GOTO, N. & KAMEYAMA, T. 1999. Endogenous GLP-1 modulates hippocampal activity in β-amyloid protein-treated rats. *Neuroreport,* 10**,** 2961-2964.
OLABARRIA, M., NORISTANI, H. N., VERKHRATSKY, A. & RODRÍGUEZ, J. J. 2010. Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. *Glia,* 58**,** 831-838.
ORGANIZATION, W. H. 2018. Dementia fact sheet. 2017.
ORTA-SALAZAR, E., VARGAS-RODRÍGUEZ, I., CASTRO-CHAVIRA, S. A., FERIA-VELASCO, A. I. & DÍAZ-CINTRA, S. 2016. Alzheimer's Disease: From Animal Models to the Human Syndrome. *Update on Dementia*.
PALOP, J. J. & MUCKE, L. 2010. Amyloid-β–induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nature neuroscience,* 13**,** 812-818.
PARADISI, S., SACCHETTI, B., BALDUZZI, M., GAUDI, S. & MALCHIODI-ALBEDI, F. 2004. Astrocyte modulation of in vitro β-amyloid neurotoxicity. *Glia,* 46**,** 252-260.
PAXINOS, G. & WATSON, C. 2006. *The rat brain in stereotaxic coordinates: hard cover edition*, Elsevier.
PCHITSKAYA, E., POPUGAEVA, E. & BEZPROZVANNY, I. 2018. Calcium signaling and molecular mechanisms underlying neurodegenerative diseases. *Cell calcium,* 70**,** 87-94.
PEREA, G., NAVARRETE, M. & ARAQUE, A. 2009. Tripartite synapses: astrocytes process and control synaptic information. *Trends in neurosciences,* 32**,** 421-431.
PERKINS, D. O., JEFFRIES, C. D., JARSKOG, L. F., THOMSON, J. M., WOODS, K., NEWMAN, M. A., PARKER, J. S., JIN, J. & HAMMOND, S. M. 2007. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. *Genome biology,* 8**,** R27.
PERRY, R. J., WATSON, P. & HODGES, J. R. 2000. The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: relationship to episodic and semantic memory impairment. *Neuropsychologia,* 38**,** 252-271.
PHILIPSON, O., LORD, A., GUMUCIO, A., O'CALLAGHAN, P., LANNFELT, L. & NILSSON, L. N. 2010. Animal models of amyloid-β-related pathologies in Alzheimer's disease. *The FEBS journal,* 277**,** 1389-1409.
PHILLIPS, R. & LEDOUX, J. 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral neuroscience,* 106**,** 274.
PIKE, C. J., WALENCEWICZ, A. J., GLABE, C. G. & COTMAN, C. W. 1991. In vitro aging of ß-amyloid protein causes peptide aggregation and neurotoxicity. *Brain research,* 563**,** 311-314.
PLATZER, C., MEISEL, C., VOGT, K., PLATZER, M. & VOLK, H.-D. 1995. Up-regulation of monocytic IL-10 by tumor necrosis factor-α and cAMP elevating drugs. *International immunology,* 7**,** 517-523.
PROKOP, S., MILLER, K. R. & HEPPNER, F. L. 2013. Microglia actions in Alzheimer's disease. *Acta neuropathologica,* 126**,** 461-477.

PUGH, C. R., KUMAGAWA, K., FLESHNER, M., WATKINS, L. R., MAIER, S. F. & RUDY, J. W. 1998. Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning. *Brain, behavior, and immunity,* 12**,** 212-229.

QIN, H. & BENVENISTE, E. N. 2012. ELISA methodology to quantify astrocyte production of cytokines/chemokines in vitro. *Astrocytes.* Springer.

RANSOHOFF, R. M. & BROWN, M. A. 2012. Innate immunity in the central nervous system. *The Journal of clinical investigation,* 122**,** 1164-1171.

REED-GEAGHAN, E. G., SAVAGE, J. C., HISE, A. G. & LANDRETH, G. E. 2009. CD14 and toll-like receptors 2 and 4 are required for fibrillar Aβ-stimulated microglial activation. *Journal of Neuroscience,* 29**,** 11982-11992.

RETH, M. & BRUMMER, T. 2004. Feedback regulation of lymphocyte signalling. *Nature Reviews Immunology,* 4**,** 269-278.

RICHWINE, A. F., SPARKMAN, N. L., DILGER, R. N., BUCHANAN, J. B. & JOHNSON, R. W. 2009. Cognitive deficits in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide. *Brain, behavior, and immunity,* 23**,** 794-802.

RODRIGUEZ, J., WITTON, J., OLABARRIA, M., NORISTANI, H. & VERKHRATSKY, A. 2010. Increase in the density of resting microglia precedes neuritic plaque formation and microglial activation in a transgenic model of Alzheimer's disease. *Cell death & disease,* 1**,** e1-e1.

ROSSI, D. & VOLTERRA, A. 2009. Astrocytic dysfunction: insights on the role in neurodegeneration. *Brain research bulletin,* 80**,** 224-232.

ROSSNE, S., LANGE-DOHNA, C., ZEITSCHEL, U. & PEREZ-POLO, J. R. 2005. Alzheimer's disease β-secretase BACE1 is not a neuron-specific enzyme. *Journal of neurochemistry,* 92**,** 226-234.

ROUGERIE, P., MISKOLCI, V. & COX, D. 2013. Generation of membrane structures during phagocytosis and chemotaxis of macrophages: role and regulation of the actin cytoskeleton. *Immunological reviews,* 256**,** 222-239.

RYMAN, D. C., ACOSTA-BAENA, N., AISEN, P. S., BIRD, T., DANEK, A., FOX, N. C., GOATE, A., FROMMELT, P., GHETTI, B. & LANGBAUM, J. B. 2014. Symptom onset in autosomal dominant Alzheimer disease: a systematic review and meta-analysis. *Neurology,* 83**,** 253-260.

SALMINEN, A. & KAARNIRANTA, K. 2009. Siglec receptors and hiding plaques in Alzheimer's disease. *Journal of molecular medicine,* 87**,** 697.

SARMA, J. D. 2014. Microglia-mediated neuroinflammation is an amplifier of virus-induced neuropathology. *Journal of neurovirology,* 20**,** 122-136.

SASMITA, A. O., KURUVILLA, J. & LING, A. P. K. 2018. Harnessing neuroplasticity: modern approaches and clinical future. *International Journal of Neuroscience,* 128**,** 1061-1077.

SCEMES, E. 2000. Components of astrocytic intercellular calcium signaling. *Molecular neurobiology,* 22**,** 167-179.

SCHEFFLER, K., STENZEL, J., KROHN, M., LANGE, C., HOFRICHTER, J., SCHUMACHER, T., BRUNING, T., PLATH, A.-S., WALKER, L. & PAHNKE, J. 2011. Determination of spatial and temporal distribution of microglia by 230nm-high-resolution, high-throughput automated analysis reveals different amyloid plaque populations in an APP/PS1 mouse model of Alzheimer's disease. *Current Alzheimer research,* 8**,** 781-788.

SCHIPKE, C. G., BOUCSEIN, C., OHLEMEYER, C., KIRCHHOFF, F. & KETTENMANN, H. 2002. Astrocyte Ca2+ waves trigger responses in microglial cells in brain slices. *The FASEB Journal,* 16**,** 255-257.

SCHWARZ, F., FONG, J. J. & VARKI, A. 2015. Human-specific evolutionary changes in the biology of siglecs. *Biochemical Roles of Eukaryotic Cell Surface Macromolecules.* Springer.

SCUDERI, C., STECCA, C., IACOMINO, A. & STEARDO, L. 2013. Role of astrocytes in major neurological disorders: the evidence and implications. *IUBMB life,* 65**,** 957-961.

SCUDERI, C., STECCA, C., VALENZA, M., RATANO, P., BRONZUOLI, M., BARTOLI, S., STEARDO, L., POMPILI, E., FUMAGALLI, L. & CAMPOLONGO, P. 2014. Palmitoylethanolamide controls reactive gliosis and exerts neuroprotective functions in a rat model of Alzheimer's disease. *Cell death & disease,* 5**,** e1419-e1419.

SERRANO-POZO, A., MIELKE, M. L., GÓMEZ-ISLA, T., BETENSKY, R. A., GROWDON, J. H., FROSCH, M. P. & HYMAN, B. T. 2011. Reactive glia not only associates with plaques but

also parallels tangles in Alzheimer's disease. *The American journal of pathology,* 179**,** 1373-1384.
SHALLIE, O. F., DALLE, E. & MABANDLA, M. V. 2020. Memory decline correlates with increased plasma cytokines in amyloid-beta (1–42) rat model of Alzheimer's disease. *Neurobiology of Learning and Memory,* 169**,** 107187.
SHASTRI, A., BONIFATI, D. M. & KISHORE, U. 2013. Innate immunity and neuroinflammation. *Mediators of inflammation,* 2013.
SHERWOOD, C. C., STIMPSON, C. D., RAGHANTI, M. A., WILDMAN, D. E., UDDIN, M., GROSSMAN, L. I., GOODMAN, M., REDMOND, J. C., BONAR, C. J. & ERWIN, J. M. 2006. Evolution of increased glia–neuron ratios in the human frontal cortex. *Proceedings of the National Academy of Sciences,* 103**,** 13606-13611.
SHI, L., BAIRD, A. L., WESTWOOD, S., HYE, A., DOBSON, R., THAMBISETTY, M. & LOVESTONE, S. 2018. A decade of blood biomarkers for Alzheimer's disease research: an evolving field, improving study designs, and the challenge of replication. *Journal of Alzheimer's Disease,* 62**,** 1181-1198.
SHOJI, H., TAKAO, K., HATTORI, S. & MIYAKAWA, T. 2014. Contextual and cued fear conditioning test using a video analyzing system in mice. *JoVE (Journal of Visualized Experiments)***,** e50871.
SHU, B., ZHANG, X., DU, G., FU, Q. & HUANG, L. 2018. MicroRNA-107 prevents amyloid-β-induced neurotoxicity and memory impairment in mice. *International journal of molecular medicine,* 41**,** 1665-1672.
SIDORYK-WEGRZYNOWICZ, M., WEGRZYNOWICZ, M., LEE, E., BOWMAN, A. B. & ASCHNER, M. 2011. Role of astrocytes in brain function and disease. *Toxicologic pathology,* 39**,** 115-123.
SIMARD, A. R., SOULET, D., GOWING, G., JULIEN, J.-P. & RIVEST, S. 2006. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron,* 49**,** 489-502.
SKELLY, D. T., HENNESSY, E., DANSEREAU, M.-A. & CUNNINGHAM, C. 2013. A systematic analysis of the peripheral and CNS effects of systemic LPS, IL-1β, TNF-α and IL-6 challenges in C57BL/6 mice. *PloS one,* 8**,** e69123.
SOCHOCKA, M., DINIZ, B. S. & LESZEK, J. 2017. Inflammatory response in the CNS: friend or foe? *Molecular neurobiology,* 54**,** 8071-8089.
SOFRONIEW, M. V. 2015. Astrogliosis. *Cold Spring Harbor perspectives in biology,* 7**,** a020420.
SOFRONIEW, M. V. & VINTERS, H. V. 2010. Astrocytes: biology and pathology. *Acta neuropathologica,* 119**,** 7-35.
SRIVAREERAT, M., TRAN, T. T., ALZOUBI, K. H. & ALKADHI, K. A. 2009. Chronic psychosocial stress exacerbates impairment of cognition and long-term potentiation in β-amyloid rat model of Alzheimer's disease. *Biological psychiatry,* 65**,** 918-926.
STEARDO JR, L., BRONZUOLI, M. R., IACOMINO, A., ESPOSITO, G., STEARDO, L. & SCUDERI, C. 2015. Does neuroinflammation turn on the flame in Alzheimer's disease? Focus on astrocytes. *Frontiers in neuroscience,* 9.
STEENSBERG, A., FISCHER, C. P., KELLER, C., MØLLER, K. & PEDERSEN, B. K. 2003. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology And Metabolism,* 285**,** E433-E437.
STOCKLEY, J. & O'NEILL, C. 2008. Understanding BACE1: essential protease for amyloid-β production in Alzheimer's disease. *Cellular and molecular life sciences,* 65**,** 3265.
STOPFORD, C. L., THOMPSON, J. C., NEARY, D., RICHARDSON, A. M. & SNOWDEN, J. S. 2012. Working memory, attention, and executive function in Alzheimer's disease and frontotemporal dementia. *Cortex,* 48**,** 429-446.
STRATMAN, N., MATHEWS, W., BUHL, A., CARTER, D., TOMASSELLI, A., PARODI, L., HEINRIKSON, R., GURNEY, M., HF, F. N. & HONG, J. 1: Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science. 1999 Oct.

STRLE, K., ZHOU, J.-H., SHEN, W.-H., BROUSSARD, S. R., JOHNSON, R. W., FREUND, G. G., DANTZER, R. & KELLEY, K. W. 2001. lnterleukin-10 in the brain. *Critical Reviews™ in Immunology,* 21.

SUDDUTH, T. L., SCHMITT, F. A., NELSON, P. T. & WILCOCK, D. M. 2013. Neuroinflammatory phenotype in early Alzheimer's disease. *Neurobiology of aging,* 34**,** 1051-1059.

SWARDFAGER, W., LANCTÔT, K., ROTHENBURG, L., WONG, A., CAPPELL, J. & HERRMANN, N. 2010. A meta-analysis of cytokines in Alzheimer's disease. *Biological psychiatry,* 68**,** 930-941.

SWARTZ, K. R., LIU, F., SEWELL, D., SCHOCHET, T., CAMPBELL, I., SANDOR, M. & FABRY, Z. 2001. Interleukin-6 promotes post-traumatic healing in the central nervous system. *Brain research,* 896**,** 86-95.

SWEENEY, W. A., LUEDTKE, J., MCDONALD, M. P. & OVERMIER, J. B. 1997. Intrahippocampal injections of exogenous β-amyloid induce postdelay errors in an eight-arm radial maze. *Neurobiology of learning and memory,* 68**,** 97-101.

TAKAHASHI, K., ROCHFORD, C. D. & NEUMANN, H. 2005. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *The Journal of experimental medicine,* 201**,** 647-657.

TAKATA, K., KITAMURA, Y., YANAGISAWA, D., MORIKAWA, S., MORITA, M., INUBUSHI, T., TSUCHIYA, D., CHISHIRO, S., SAEKI, M. & TANIGUCHI, T. 2007. Microglial transplantation increases amyloid-β clearance in Alzheimer model rats. *FEBS letters,* 581**,** 475-478.

TAMBOLI, I. Y., BARTH, E., CHRISTIAN, L., SIEPMANN, M., KUMAR, S., SINGH, S., TOLKSDORF, K., HENEKA, M. T., LÜTJOHANN, D. & WUNDERLICH, P. 2010. Statins promote the degradation of extracellular amyloid β-peptide by microglia via stimulation of exosome-associated insulin-degrading enzyme (IDE) secretion. *Journal of biological chemistry,* 285**,** 37405-37414.

TAN, L., YU, J.-T., TAN, M.-S., LIU, Q.-Y., WANG, H.-F., ZHANG, W., JIANG, T. & TAN, L. 2014. Genome-wide serum microRNA expression profiling identifies serum biomarkers for Alzheimer's disease. *Journal of Alzheimer's Disease,* 40**,** 1017-1027.

TANG, B. L. 2009. Neuronal protein trafficking associated with Alzheimer disease: from APP and BACE1 to glutamate receptors. *Cell adhesion & migration,* 3**,** 118-128.

TERRANDO, N., ERIKSSON, L. I., KYU RYU, J., YANG, T., MONACO, C., FELDMANN, M., JONSSON FAGERLUND, M., CHARO, I. F., AKASSOGLOU, K. & MAZE, M. 2011. Resolving postoperative neuroinflammation and cognitive decline. *Annals of neurology,* 70**,** 986-995.

THAL, D. R. 2012. The role of astrocytes in amyloid β-protein toxicity and clearance. *Experimental neurology,* 236**,** 1-5.

THAL, D. R., VON ARNIM, C., GRIFFIN, W. S. T., YAMAGUCHI, H., MRAK, R. E., ATTEMS, J. & UPADHAYA, A. R. 2013. Pathology of clinical and preclinical Alzheimer's disease. *European archives of psychiatry and clinical neuroscience,* 263**,** 137-145.

THORED, P., HELDMANN, U., GOMES-LEAL, W., GISLER, R., DARSALIA, V., TANEERA, J., NYGREN, J. M., JACOBSEN, S. E. W., EKDAHL, C. T. & KOKAIA, Z. 2009. Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia,* 57**,** 835-849.

TUPPO, E. E. & ARIAS, H. R. 2005. The role of inflammation in Alzheimer's disease. *The international journal of biochemistry & cell biology,* 37**,** 289-305.

TURCHINOVICH, A., SAMATOV, T. R., TONEVITSKY, A. G. & BURWINKEL, B. 2013. Circulating miRNAs: cell–cell communication function? *Frontiers in genetics,* 4**,** 119.

TURNBULL, I. R., GILFILLAN, S., CELLA, M., AOSHI, T., MILLER, M., PICCIO, L., HERNANDEZ, M. & COLONNA, M. 2006. Cutting edge: TREM-2 attenuates macrophage activation. *The Journal of Immunology,* 177**,** 3520-3524.

ULEP, M. G., SARAON, S. K. & MCLEA, S. 2018. Alzheimer disease. *The Journal for Nurse Practitioners,* 14**,** 129-135.

ULLAND, T. K. & COLONNA, M. 2018. TREM2—a key player in microglial biology and Alzheimer disease. *Nature Reviews Neurology,* 14**,** 667-675.

ULRICH, J. D., FINN, M. B., WANG, Y., SHEN, A., MAHAN, T. E., JIANG, H., STEWART, F. R., PICCIO, L., COLONNA, M. & HOLTZMAN, D. M. 2014. Altered microglial response to Aβ plaques in APPPS1-21 mice heterozygous for TREM2. *Molecular neurodegeneration,* 9**,** 20.

ULRICH, J. D., ULLAND, T. K., COLONNA, M. & HOLTZMAN, D. M. 2017. Elucidating the role of TREM2 in Alzheimer's disease. *Neuron,* 94**,** 237-248.

ULYANOVA, T., BLASIOLI, J., WOODFORD-THOMAS, T. A. & THOMAS, M. L. 1999. The sialoadhesin CD33 is a myeloid-specific inhibitory receptor. *European journal of immunology,* 29**,** 3440-3449.

VAN STRIEN, N., CAPPAERT, N. & WITTER, M. 2009. The anatomy of memory: an interactive overview of the parahippocampal–hippocampal network. *Nature Reviews Neuroscience,* 10**,** 272.

VARKI, A. & ANGATA, T. 2006. Siglecs—the major subfamily of I-type lectins. *Glycobiology,* 16**,** 1R-27R.

VASSAR, R., BENNETT, B. D., BABU-KHAN, S., KAHN, S., MENDIAZ, E. A., DENIS, P., TEPLOW, D. B., ROSS, S., AMARANTE, P. & LOELOFF, R. 1999. β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *science,* 286**,** 735-741.

VAZIRI, N. D. & RODRÍGUEZ-ITURBE, B. 2006. Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. *Nature Clinical Practice Nephrology,* 2**,** 582.

VERGE, G. M., MILLIGAN, E. D., MAIER, S. F., WATKINS, L. R., NAEVE, G. S. & FOSTER, A. C. 2004. Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. *European Journal of Neuroscience,* 20**,** 1150-1160.

VERGHESE, P. B., CASTELLANO, J. M. & HOLTZMAN, D. M. 2011. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *The Lancet Neurology,* 10**,** 241-252.

VERKHRATSKY, A., NEDERGAARD, M. & HERTZ, L. 2015. Why are astrocytes important? *Neurochemical research,* 40**,** 389-401.

VERKHRATSKY, A., RODRÍGUEZ, J. J. & STEARDO, L. 2014. Astrogliopathology: a central element of neuropsychiatric diseases? *The Neuroscientist,* 20**,** 576-588.

VINCENT, A. J., GASPERINI, R., FOA, L. & SMALL, D. H. 2010. Astrocytes in Alzheimer's disease: emerging roles in calcium dysregulation and synaptic plasticity. *Journal of Alzheimer's Disease,* 22**,** 699-714.

VOGEL, C. & MARCOTTE, E. M. 2012. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature reviews genetics,* 13**,** 227-232.

VON BERNHARDI, R., TICHAUER, J. E. & EUGENÍN, J. 2010. Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. *Journal of neurochemistry,* 112**,** 1099-1114.

VORHEES, C. V. & WILLIAMS, M. T. 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols,* 1**,** 848.

WAKE, H., MOORHOUSE, A. J., JINNO, S., KOHSAKA, S. & NABEKURA, J. 2009. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *Journal of Neuroscience,* 29**,** 3974-3980.

WALSH, D. M. & SELKOE, D. J. 2007. Aβ oligomers–a decade of discovery. *Journal of neurochemistry,* 101**,** 1172-1184.

WALSH, D. M., TOWNSEND, M., PODLISNY, M. B., SHANKAR, G. M., FADEEVA, J. V., EL AGNAF, O., HARTLEY, D. M. & SELKOE, D. J. 2005. Certain inhibitors of synthetic amyloid β-peptide (Aβ) fibrillogenesis block oligomerization of natural Aβ and thereby rescue long-term potentiation. *Journal of Neuroscience,* 25**,** 2455-2462.

WALTER, R. B., RADEN, B. W., ZENG, R., HÄUSERMANN, P., BERNSTEIN, I. D. & COOPER, J. A. 2008. ITIM-dependent endocytosis of CD33-related Siglecs: role of intracellular domain, tyrosine phosphorylation, and the tyrosine phosphatases, Shp1 and Shp2. *Journal of leukocyte biology,* 83**,** 200-211.

WANG, R., ZHANG, Y., LI, J. & ZHANG, C. 2017. Resveratrol ameliorates spatial learning memory impairment induced by Aβ1–42 in rats. *Neuroscience,* 344**,** 39-47.

WANG, T., CHEN, K., LI, H., DONG, S., SU, N., LIU, Y., CHENG, Y., DAI, J., YANG, C. & XIAO, S. 2015a. The feasibility of utilizing plasma MiRNA107 and BACE1 messenger RNA gene expression for clinical diagnosis of amnestic mild cognitive impairment. *The Journal of clinical psychiatry,* 76**,** 135-141.

WANG, W.-X., RAJEEV, B. W., STROMBERG, A. J., REN, N., TANG, G., HUANG, Q., RIGOUTSOS, I. & NELSON, P. T. 2008. The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of β-site amyloid precursor protein-cleaving enzyme 1. *Journal of Neuroscience,* 28**,** 1213-1223.

WANG, W.-X., WILFRED, B. R., MADATHIL, S. K., TANG, G., HU, Y., DIMAYUGA, J., STROMBERG, A. J., HUANG, Q., SAATMAN, K. E. & NELSON, P. T. 2010. miR-107 regulates granulin/progranulin with implications for traumatic brain injury and neurodegenerative disease. *The American journal of pathology,* 177**,** 334-345.

WANG, W.-Y., TAN, M.-S., YU, J.-T. & TAN, L. 2015b. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Annals of translational medicine,* 3.

WANG, Y., CELLA, M., MALLINSON, K., ULRICH, J. D., YOUNG, K. L., ROBINETTE, M. L., GILFILLAN, S., KRISHNAN, G. M., SUDHAKAR, S. & ZINSELMEYER, B. H. 2015c. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. *Cell,* 160**,** 1061-1071.

WATSON, C. & PAXINOS, G. 1986. *The rat brain in stereotaxic coordinates*, Academic press San Diego, CA;.

WEBSTER, S. J., BACHSTETTER, A. D., NELSON, P. T., SCHMITT, F. A. & VAN ELDIK, L. J. 2014. Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. *Frontiers in genetics,* 5**,** 88.

WEGGEN, S., DIEHLMANN, A., BUSLEI, R., BEYREUTHER, K. & BAYER, T. 1998. Prominent expression of presenilin-1 in senile plaques and reactive astrocytes in Alzheimer's disease brain. *Neuroreport,* 9**,** 3279-3283.

WELDON, D. T., ROGERS, S. D., GHILARDI, J. R., FINKE, M. P., CLEARY, J. P., O'HARE, E., ESLER, W. P., MAGGIO, J. E. & MANTYH, P. W. 1998. Fibrillar β-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo. *Journal of Neuroscience,* 18**,** 2161-2173.

WHO 2011. World health statistics 2011. 2011. *Geneva: World Health Organization*.

WILSON, C. J., FINCH, C. E. & COHEN, H. J. 2002. Cytokines and cognition—the case for a head-to-toe inflammatory paradigm. *Journal of the American Geriatrics Society,* 50**,** 2041-2056.

WILTGEN, B. J., SANDERS, M. J., ANAGNOSTARAS, S. G., SAGE, J. R. & FANSELOW, M. S. 2006. Context fear learning in the absence of the hippocampus. *Journal of Neuroscience,* 26**,** 5484-5491.

WINBLAD, B., AMOUYEL, P., ANDRIEU, S., BALLARD, C., BRAYNE, C., BRODATY, H., CEDAZO-MINGUEZ, A., DUBOIS, B., EDVARDSSON, D. & FELDMAN, H. 2016. Defeating Alzheimer's disease and other dementias: a priority for European science and society. *The Lancet Neurology,* 15**,** 455-532.

WINKLER, J., CONNOR, D. J., FRAUTSCHY, S. A., BEHL, C., WAITE, J. J., COLE, G. M. & THAL, L. J. 1994. Lack of long-term effects after β-amyloid protein injections in rat brain. *Neurobiology of aging,* 15**,** 601-607.

WOJSIAT, J., ZOLTOWSKA, K. M., LASKOWSKA-KASZUB, K. & WOJDA, U. 2018. Oxidant/antioxidant imbalance in Alzheimer's disease: therapeutic and diagnostic prospects. *Oxidative medicine and cellular longevity,* 2018.

WYSS-CORAY, T. & ROGERS, J. 2012. Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature. *Cold Spring Harbor perspectives in medicine,* 2**,** a006346.

YAMADA, K., TANAKA, T., HAN, D., SENZAKI, K., KAMEYAMA, T. & NABESHIMA, T. 1999. Protective effects of idebenone and α-tocopherol on β-amyloid-(1–42)-induced learning and memory deficits in rats: implication of oxidative stress in β-amyloid-induced neurotoxicity in vivo. *European Journal of Neuroscience,* 11**,** 83-90.

YANG, L.-B., LINDHOLM, K., YAN, R., CITRON, M., XIA, W., YANG, X.-L., BEACH, T., SUE, L., WONG, P. & PRICE, D. 2003. Elevated β-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nature medicine,* 9**,** 3-4.

YANG, X., YAO, C., TIAN, T., LI, X., YAN, H., WU, J., LI, H., PEI, L., LIU, D. & TIAN, Q. 2018. A novel mechanism of memory loss in Alzheimer's disease mice via the degeneration of entorhinal–CA1 synapses. *Molecular psychiatry,* 23**,** 199.

YANKNER, B. A., DAWES, L. R., FISHER, S., VILLA-KOMAROFF, L., OSTER-GRANITE, M. L. & NEVE, R. L. 1989. Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science,* 245**,** 417-420.

YAUGER, Y. J., BERMUDEZ, S., MORITZ, K. E., GLASER, E., STOICA, B. & BYRNES, K. R. 2019. Iron accentuated reactive oxygen species release by NADPH oxidase in activated microglia contributes to oxidative stress in vitro. *Journal of neuroinflammation,* 16**,** 41.

YEH, F. L., WANG, Y., TOM, I., GONZALEZ, L. C. & SHENG, M. 2016. TREM2 binds to apolipoproteins, including APOE and CLU/APOJ, and thereby facilitates uptake of amyloid-beta by microglia. *Neuron,* 91**,** 328-340.

YIANNOPOULOU, K. G. & PAPAGEORGIOU, S. G. 2013. Current and future treatments for Alzheimer's disease. *Therapeutic advances in neurological disorders,* 6**,** 19-33.

YILMAZ, Ş. G., ERDAL, M. E., ÖZGE, A. A. & SUNGUR, M. A. 2016. Can peripheral MicroRNA expression data serve as epigenomic (upstream) biomarkers of Alzheimer's disease? *Omics: a journal of integrative biology,* 20**,** 456-461.

YU, Y., HE, J., ZHANG, Y., LUO, H., ZHU, S., YANG, Y., ZHAO, T., WU, J., HUANG, Y. & KONG, J. 2009. Increased hippocampal neurogenesis in the progressive stage of Alzheimer's disease phenotype in an APP/PS1 double transgenic mouse model. *Hippocampus,* 19**,** 1247-1253.

YUYAMA, K., SUN, H., MITSUTAKE, S. & IGARASHI, Y. 2012. Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia. *Journal of Biological Chemistry,* 287**,** 10977-10989.

ZANETTI, O., SOLERTE, S. & CANTONI, F. 2009. Life expectancy in Alzheimer's disease (AD). *Archives of gerontology and geriatrics,* 49**,** 237-243.

ZELIKOWSKY, M., HERSMAN, S., CHAWLA, M. K., BARNES, C. A. & FANSELOW, M. S. 2014. Neuronal ensembles in amygdala, hippocampus, and prefrontal cortex track differential components of contextual fear. *Journal of neuroscience,* 34**,** 8462-8466.

ZHANG, B., GAITERI, C., BODEA, L.-G., WANG, Z., MCELWEE, J., PODTELEZHNIKOV, A. A., ZHANG, C., XIE, T., TRAN, L. & DOBRIN, R. 2013. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell,* 153**,** 707-720.

ZHANG, Z., YANG, T. & XIAO, J. 2018. Circular RNAs: promising biomarkers for human diseases. *EBioMedicine,* 34**,** 267-274.

ZHAO, J., O'CONNOR, T. & VASSAR, R. 2011. The contribution of activated astrocytes to Aβ production: implications for Alzheimer's disease pathogenesis. *Journal of neuroinflammation,* 8**,** 150.

ZHAO, Y. & LUKIW, W. J. 2018. Bacteroidetes neurotoxins and inflammatory neurodegeneration. *Molecular neurobiology,* 55**,** 9100-9107.

ZHAO, Y., WU, X., LI, X., JIANG, L.-L., GUI, X., LIU, Y., SUN, Y., ZHU, B., PIÑA-CRESPO, J. C. & ZHANG, M. 2018. TREM2 is a receptor for β-amyloid that mediates microglial function. *Neuron,* 97**,** 1023-1031. e7.

ZIRNHELD, A. L., SHETTY, V., CHERTKOW, H., SCHIPPER, H. M. & WANG, E. 2016. Distinguishing mild cognitive impairment from Alzheimer's disease by increased expression of key circulating microRNAs. *Curr Neurobiol,* 7**,** 38-50.

ZIV, Y., RON, N., BUTOVSKY, O., LANDA, G., SUDAI, E., GREENBERG, N., COHEN, H., KIPNIS, J. & SCHWARTZ, M. 2006. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nature neuroscience,* 9**,** 268-275.

PROLOGUE

Chapter 2 of this study examines the level of reactivity of glial cells such as microglia and astrocytes in the hippocampus, and the correlation between these activated glial cells and lipid peroxidation, a marker of oxidative stress as evident in the circulation of a progressive $A\beta_{(1-42)}$ rat model of AD. This chapter is prepared in manuscript format, currently under review in the Journal of Inflammation and the format used is according to the journal specification.

CHAPTER 2

Progressive interdependence between neuroinflammation and oxidative stress in an amyloid-beta (1–42) rat model of Alzheimer's disease

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ABSTRACT

Cellular players involved in the neuroinflammatory process associated with Alzheimer's disease (AD) include astrocytes and microglia. Together with oxidative stress, these activated cellular players are key in the development of neuroinflammation. In this study, we examine the level of reactivity of glial cells in the hippocampus, and the interdependency between these glial cells and lipid peroxidation, as evident in the circulation of a progressive $A\beta_{(1-42)}$ model of AD. Eighty male Sprague-Dawley rats were randomly divided into two groups, euthanised on days 3, 7, 10, and 14 post-lesion, following stereotaxic infusion of amyloid-beta (5 µg/5 µl) for the AD group and phosphate-buffered saline infusion for the control group. Reactive levels of astrocytes and microglia were measured using immunofluorescence and oxidative stress level was assessed by quantification of lipid peroxidation and reduced glutathione concentration. Increased expression of ionized calcium-binding adaptor molecule 1 (IBA-1) and glial fibrillary acidic protein (GFAP) proteins in the hippocampus was observed, as marker for microglia and astrocytes respectively. This study shows a progressive and prolonged activation of glial cells, resulting in reciprocal interaction between microglia and astrocytes. We also observed a positive relationship between the activated glial cells and lipid peroxidation, a marker of oxidative stress. This interaction may facilitate the understanding of the pathogenesis of AD, resulting in a strategy directed at controlling neuroinflammation and oxidative stress in developing therapeutics for AD.

Keywords: Neuroinflammation; Alzheimer's disease; Amyloid-Beta; Microglia; Astrocytes; Oxidative stress

1. Introduction

Neuroinflammation represents an active process in the pathogenesis of Alzheimer's disease (AD), as supported by the functional deficit observed following glial reactivity associated with the progression of the disease (Krabbe et al., 2013a, Olabarria et al., 2010). Its contribution is equal to or greater than that imposed by the amyloid plaques and neurofibrillary tangles in the advancement of the disease (Zhang et al., 2013).

The cellular players involved in this process are the microglia and astrocytes, found closely associated with the amyloid plaques in AD animal models (Heneka et al., 2015, Olabarria et al., 2010). Although microglia are the resident neuroimmune cells primarily concerned with phagocytosis of pathogens and are the first line of defense, the contributions of astrocytes in maintaining normal neuronal functions cannot be underestimated (Sarma, 2014, Jiang and Cadenas, 2014, Kabba et al., 2018). Just like microglia, they can discern and magnify signals of inflammation, thereby initiating a cascade of inflammatory responses, which destroy the connection between nerve cells hence eliciting neuronal functional deficit (Vincent et al., 2010, Carrero et al., 2012, Lim et al., 2013).

The presence of amyloid-beta is the main driver for microglial activation and migration to the plaques which results in phagocytosis (Baik et al., 2016, Yuyama et al., 2012, Tamboli et al., 2010). However, prolonged activation leads to this phagocytotic power being subdued and microglia being unable to clear amyloid-beta, thereby leading to increased accumulation and exacerbation of AD pathology (Kinney et al., 2018, Hickman et al., 2008). This prolonged activation of microglial cells results into the production of cytokines, as reported in our previous study (Shallie et al., 2020). Activated expression of microglia is marked by the protein ionized calcium-binding adaptor molecule 1 (IBA-1), which distinctively classifies cells engaged in routine surveillance from those activated in response to inflammation (Korzhevskii and Kirik, 2016). On the other hand, astrocytes being the most abundant cells in the brain also perform essential functions and their pathological response is represented by reactive astrogliosis, characterized by increased glial fibrillary acidic protein (GFAP) (Sofroniew, 2015, Messing and Brenner, 2003, Sochocka et al., 2017).

During the inflammatory process, the activated phagocytic cells produce large amounts of reactive oxygen species (ROS) and reactive nitrogen and chlorine species including superoxide, hydroxyl free radical, nitric oxide, hydrogen peroxide, and hypochlorous acid to kill the invading agents (Fialkow et al., 2007). These activated phagocytotic cells may incite exaggerated generation of reactive species and some of those reactive species diffuse out of the phagocytic cells and can induce localized oxidative stress and tissue injury (Fialkow et al., 2007). A major contributor to oxidative stress in the brain are the microglia (Loane and Kumar, 2016). However, apart from the direct production of reactive species by the professional phagocytic cells like microglia, the non-phagocytic cells can also produce reactive

species in response to pro-inflammatory cytokines (Li et al., 2015). Studies support an interdependent relationship between inflammation and oxidative stress (Mittal et al., 2014, Yauger et al., 2019).

The amyloid-beta (1-42) ($A\beta_{(1-42)}$) model of AD has been investigated by several studies, with emphasis on the hippocampus due to its vulnerability to neurodegeneration (Facchinetti et al., 2018, Frautschy et al., 1996, Cioanca et al., 2014, Karthick et al., 2019). The hippocampus plays a role in the acquisition and retrieval of information (Wiltgen et al., 2006, Yang et al., 2018). However, to the best of our knowledge, no study has elucidated the progressive reactivity of these glial cells and their relationship to oxidative stress in a progressive model of AD.

Therefore, the current study examines the level of reactivity of glial cells in the hippocampus and the interdependency between these glial cells and the incited oxidative stress as evident in circulation of a progressive $A\beta_{(1-42)}$ model of AD.

2. Materials and methods

2.1. Study Design and Animal Grouping

The experimental protocol for this study was per the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethical Review Board of the University of KwaZulu-Natal (AREC/015/018D). Eighty male Sprague–Dawley rats obtained from the Biomedical Resource Unit of the University of KwaZulu-Natal were used in this study. They were housed under standard laboratory conditions and a 12-h light/dark cycle (lights on at 06h00). Food and water were available *ad libitum*. The animals were randomly divided into a vehicle group of rats (n = 40) that was infused with phosphate-buffered saline and an $A\beta_{(1–42)}$ group (n = 40) that was infused with the $A\beta_{(1–42)}$ peptide. Each group was further sub-divided into four groups (Day 3 group: animals euthanized 3 days after infusion; Day 7 group: animals euthanized 7 days after infusion; Day 10 group: animals euthanized 10 days after infusion, and Day 14 group: animals euthanized 14 days after infusion) (n = 10/group). The group size is based on findings extrapolated from the pilot study, as statistical power distinguishes an actual effect from one of chance. The animals were weighed before all experimentsal intervention and were brought to the holding area outside the experimentation room, 1 hour before commencing behavioural tests.

2.2. Drugs and Reagents

Procurement of $A\beta_{(1–42)}$ peptide was from DLD Scientific (Durban North, KZN, South Africa). Ketamine, xylazine, and temgesic were obtained from Sigma (ST. Louis, MO, USA). Vectafluor Excel R.T.U Antibody kit DyLight 488 Anti-Mouse IgG (DK-2488) was purchased from Vector Laboratories

(ST. Louis, MO, USA), Anti-IBA-1(SAB2702364) was acquired from Sigma (ST. Louis, MO, USA) and Anti-GFAP antibody (ab10062) was purchased from AbCam (Cambridge, MA, USA).

2.3. $A\beta_{(1–42)}$ rat model of AD

Before infusion of the $A\beta_{(1–42)}$ peptide, rats were anesthetized with ketamine (100 mg/Kg, intraperitoneally) and xylazine (5 mg/Kg, intraperitoneally) solution (Bagheri et al., 2011). The head of the animal was shaved and carefully positioned in the stereotaxic apparatus (David Kopf instrument, Tujunga, USA). Biotane was used to disinfect the skin covering the scalp before a midline incision was made to expose the skull. Following drilling of a hole into the skull, Hamilton syringe was used to infuse $A\beta_{(1–42)}$ (5 μg/5 μl) dissolved in 0.01M of Phosphate-buffered saline into the dorsal hippocampus bilaterally at the following co-ordinates: anteroposterior (AP) = −4.8 mm; mediolateral (ML) = ±3.4 mm; dorsoventral (DV) = −3.0 mm) over 10 minutes (Paxinos and Watson, 2006). Lesion sites were confirmed by physical examination at the point of sacrifice followed by cresly violet counterstaining. To maximize the diffusion of the neurotoxin $A\beta_{(1–42)}$, the needle was kept in this region of the dorsal hippocampus for 1 minute before the injection and for 2 minutes following the injection. The incision was sutured and cleaned before the animals were placed under a heating lamp to prevent hypothermia during recovery. Thereafter, the animals were injected with Temgesic (0.05 mg/kg subcutaneously), a postoperative analgesic before being returned to their home cages. The same procedure was followed for the phosphate buffered saline group.

2.4. Euthanization and Sample Preparation

All animals were sacrificed by deep anesthesia with pentobarbital (80mg/kg, i.p.). The brains were transcardially perfused and fixed with phosphate buffered saline (PBS) and 10% neutral buffered formalin (NBF) (Alese and Mabandla, 2019). Whole blood sample (3 ml) acquired aseptically from both the vehicle and $A\beta_{(1–42)}$ lesioned group of rats was collected into ethylenediaminetetraacetic acid (EDTA) coated tubes. The blood samples were centrifuged at a speed of 2000 x g for 10 minutes at 4 °C in a refrigerated centrifuge (Z326, Lasec, South Africa). Plasma samples were transferred into cryo-tubes and stored at -80 °C after blood sample collection.

2.5. Immunofluorescence of IBA1 and GFAP

Immunofluorescence of IBA1 and GFAP was performed according to the protocol of Im et al. (Im et al., 2019). Excised whole brain fixed in 10% NBF were histologically processed using the automated tissue processor (Leica TP1020, Wetzlar, Hesse, Germany) to ensure adequate dehydration, clearing, and infiltration and subsequently embedded in paraffin wax. Coronal sections (3 μm thick) of paraffin blocked hippocampal regions were cut using a rotary microtome (Leica RM2145 Wetzlar, Hesse, Germany), placed in a water bath (Leica HI1210, Wetzlar, Hesse, Germany) set at 37 °C to produce

sections free of folds. The sections were transferred to pre-coated slides and gently blotted to avoid formation of wrinkles and the slides were finally heated on the hot plate (Leica HI1220, Wetzlar, Hesse, Germany) set at 54 °C to ensure proper adherence of tissue sections unto the slide, hence hindering possible fall-off during subsequent treatments (Guo et al., 2016). Sections were deparaffinised and rehydrated through xylene and descending series of ethanol and immersed into preheated Vector antigen unmasking solution H-3300, incubated for 20 minutes at 97 °C, and allowed to cool at room temperature. Sections were then washed in 0.01 M phosphate buffered saline (PBS) for 5 minutes. Protein blocking was performed by incubating sections for 20 minutes with 2.5% Normal horse serum, with the excess serum from sections being tipped off. This was followed by incubation with primary antibodies, mouse Anti-IBA-1 for I hour and Anti-GFAP overnight, diluted at 1:150 and 1:100, respectively. Sections were washed for 5 minutes in PBS and incubated for 15 minutes with amplifier antibody. The sections were re-washed for 5 minutes in PBS and incubated for 30 minutes with VectaFluor reagent. Sections were then washed for 5 minutes twice in PBS and mounted in VectaShield mounting media containing 4',6-diamidino-2-phenylindole (DAPI), and then allowed to cure at room temperature. Negative controls were processed likewise but the incubation with primary antibodies was omitted.

2.6. Photomicrography and Image quantification

Sections were viewed and images captured with an Axioscope A1 microscope (Carl Zeiss, Jena, Germany) around the CA3 region of the hippocampus. Photomicrographs were analysed using custom-written scripts for Fiji/ImageJ (NIH, Bethesda, MD, USA). The corrected total cell fluorescence (CTCF) was calculated using the formula: CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings) (Guichet et al., 2016). A tissue section was analysed per animal and a total of 10 sections were assessed in each experimental group.

2.7. Oxidative Stress Biomarkers

2.7.1. Determination of lipid peroxidation levels

Lipid peroxidation levels of the samples were determined by measuring thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalent. Using the protocol by Chowdhury and Soulsby, and Janero (Chowdhury and Soulsby, 2002, Janero, 1990). One hundred microliters of the plasma samples were mixed with an equal volume of 8.1% sodium dodecyl sulfate (SDS) solution, 375 µL of 20% acetic acid, 1 mL of 0.25% thiobarbituric acid (TBA), and 425 µL of distilled water. The reconstituted mixture was heated at 95°C for 1 h in a water bath. Thereafter, 200 µL of the heated mixture was pipetted into 96-well plate, and absorbance read at 532 nm. Samples were

run in duplicate and a standard curve was generated from the absorbance of standard MDA, from which the TBARS concentration was extrapolated.

2.7.2. Determination of reduced glutathione (GSH) concentration

An important cellular antioxidant that responds to lipid peroxidation (LP) is the reduced form of glutathione (GSH). Reduced glutathione (GSH) concentration was determined by the Ellman's method (Ellman, 1959), which is based on the oxidation of GSH by 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB). Reduced glutathione standards were prepared from GSH stock in increasing concentrations of 0.025, 0.05, 0.1, 0.2 and 0.4 mM in duplicates. Their absorbance was read at 415 nm and plotted against the concentration to get the standard curve. The plasma samples were deproteinize with an equal volume of 10% TCA and then centrifuged at 3500 rpm for 5 mins at 25^{O}C. Thereafter, 200 µL of the supernatant was pipetted into a 96 well plate. 50 µL of Ellman reagent was then added and allowed to stand for 5 mins. Absorbance was read at 415 nm. The GSH concentration was then extrapolated from the standard curve of plotted GSH concentrations.

2.8. Statistical analysis

Data were analysed using Student t-test, one-way or two-way ANOVA followed by Tukey's post-hoc test where applicable using the GraphPad Prism version 7.0 (GraphPad Inc, USA) statistical software package. Pearson's correlation coefficient was used to assess the correlation between data sets, where "r" indicates the strength of the relationship. Shapiro-wilk test showed that all data were normally distributed. Results are expressed as mean ± SEM and $p < 0.05$ was considered significant.

3. Results

3.1. IBA-1 expression in the CA3 region of the hippocampus.

There was a significant ($p < 0.0001$) increase in corrected total cell fluorescence of positive IBA-1 microglia in the hippocampus of $A\beta_{(1–42)}$ groups when compared with the vehicle-infused groups irrespective of the post-lesion day. One-way ANOVA showed a positive significant [$F(3, 36) = 18.92$, $p < 0.0001$] difference between post-lesion days. Day effects were observed with specific positive significant ($p < 0.0001$) differences between the post-lesion $A\beta_{(1–42)}$ day 3 versus day 10 & day 14 and between the post-lesion $A\beta_{(1–42)}$ day 7 versus day 10 & day 14 as shown in figures 1 and 2.

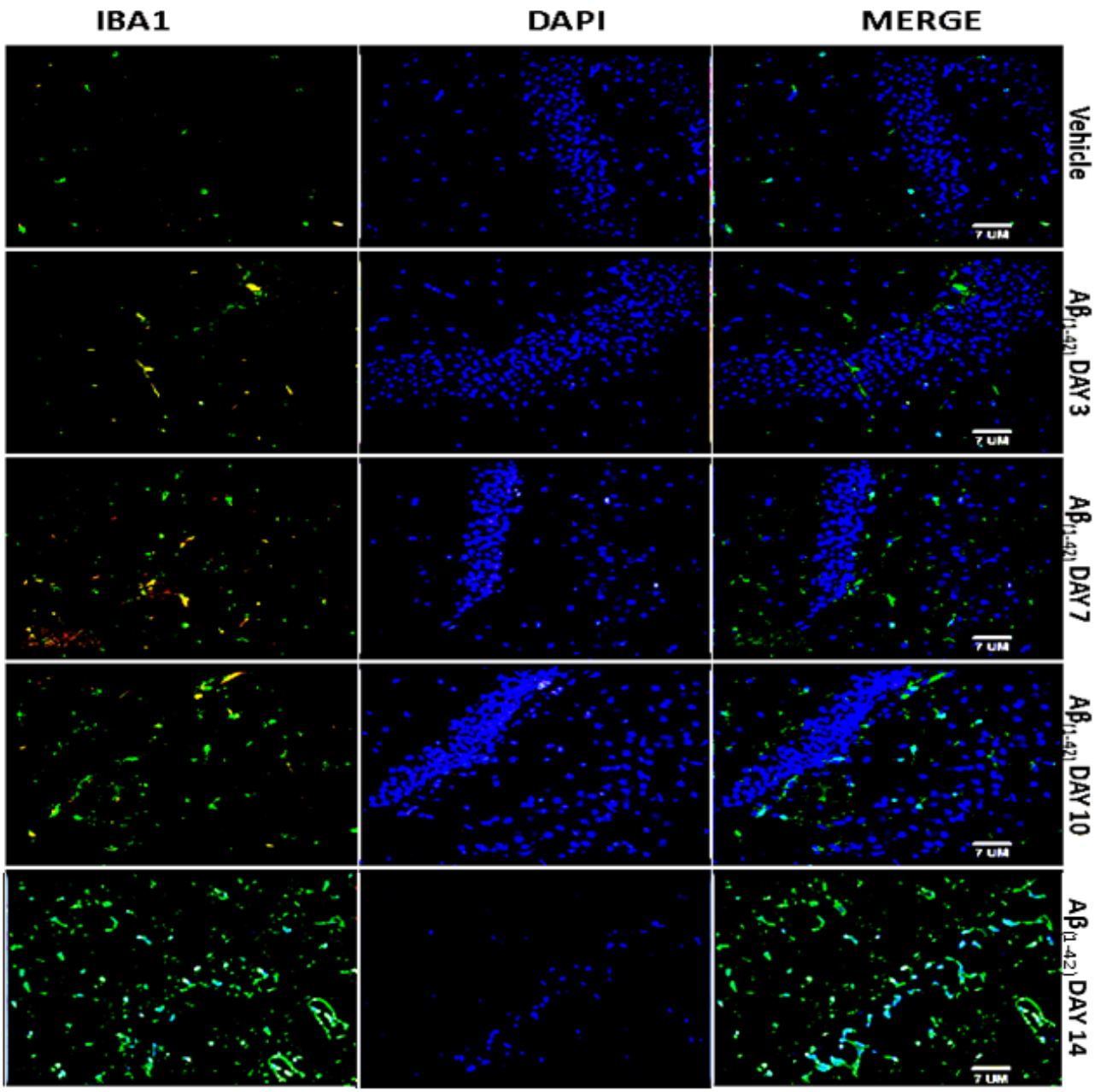

Figure 1: Immunofluorescence in the rat CA3 region of the hippocampus of Vehicle-infused and the $A\beta_{(1–42)}$ lesioned rat model of AD. Representative images of positively stained microglia with IBA-1 in the Vehicle, $A\beta_{(1-42)}$ Day 3, $A\beta_{(1-42)}$ Day 7, $A\beta_{(1-42)}$ Day 10 and $A\beta_{(1-42)}$ Day 14 groups. (LPO

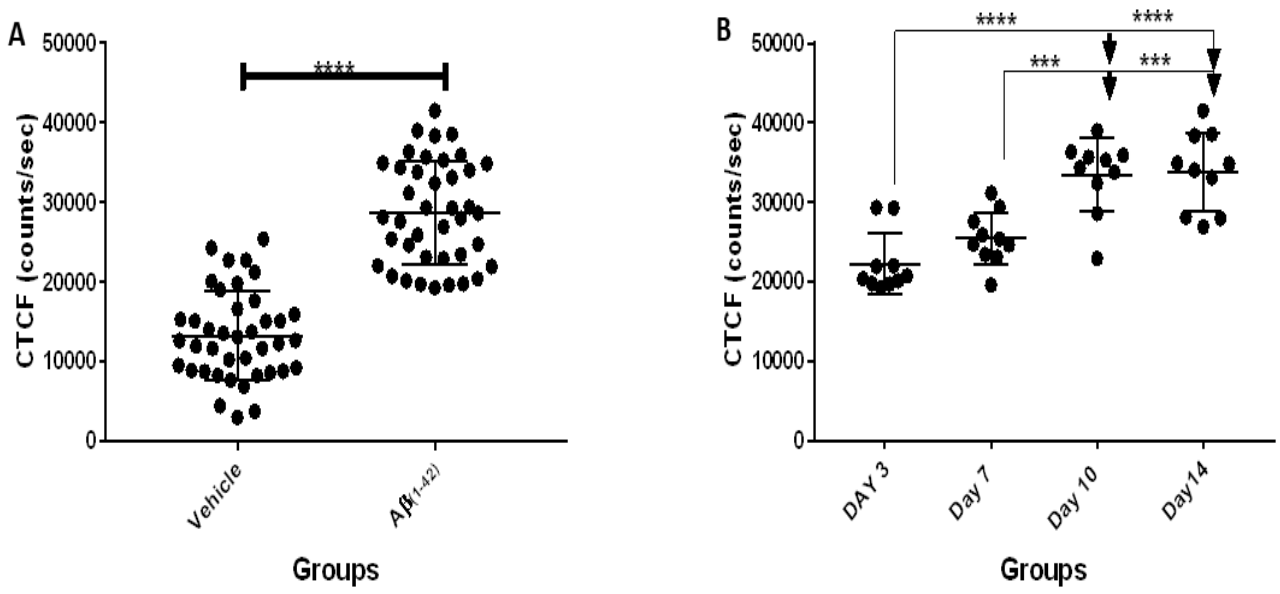

Figure 2: Mean corrected total cell immunofluorescence of IBA-1 in the hippocampus of (A) Vehicle-infused and the $A\beta_{(1–42)}$ lesioned rat model of AD irrespective of the days (B) $A\beta_{(1–42)}$ rat model of AD across post-lesion days. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, (student t-test and one-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM (n = 10/ group).

3.2. GFAP expression in the CA3 region of the hippocampus.

There was a significant ($p < 0.0001$) increase in corrected total cell fluorescence of positive GFAP astrocytes in the hippocampus of $A\beta_{(1–42)}$ groups when compared with the vehicle groups irrespective of the post-lesion days. One-way ANOVA showed a significant [$F(3, 36) = 18.9$, $p < 0.0001$] difference between post-lesion days. Day effects were observed with specific significant differences between post-lesion $A\beta_{(1–42)}$ day 3 versus day 10 & day 14 ($p < 0.0001$); between post-lesion $A\beta_{(1–42)}$ day 7 versus day 10 ($p < 0.01$); between post-lesion $A\beta_{(1–42)}$ day 7 versus day 14 ($p < 0.0001$) as shown in figures 3 and 4.

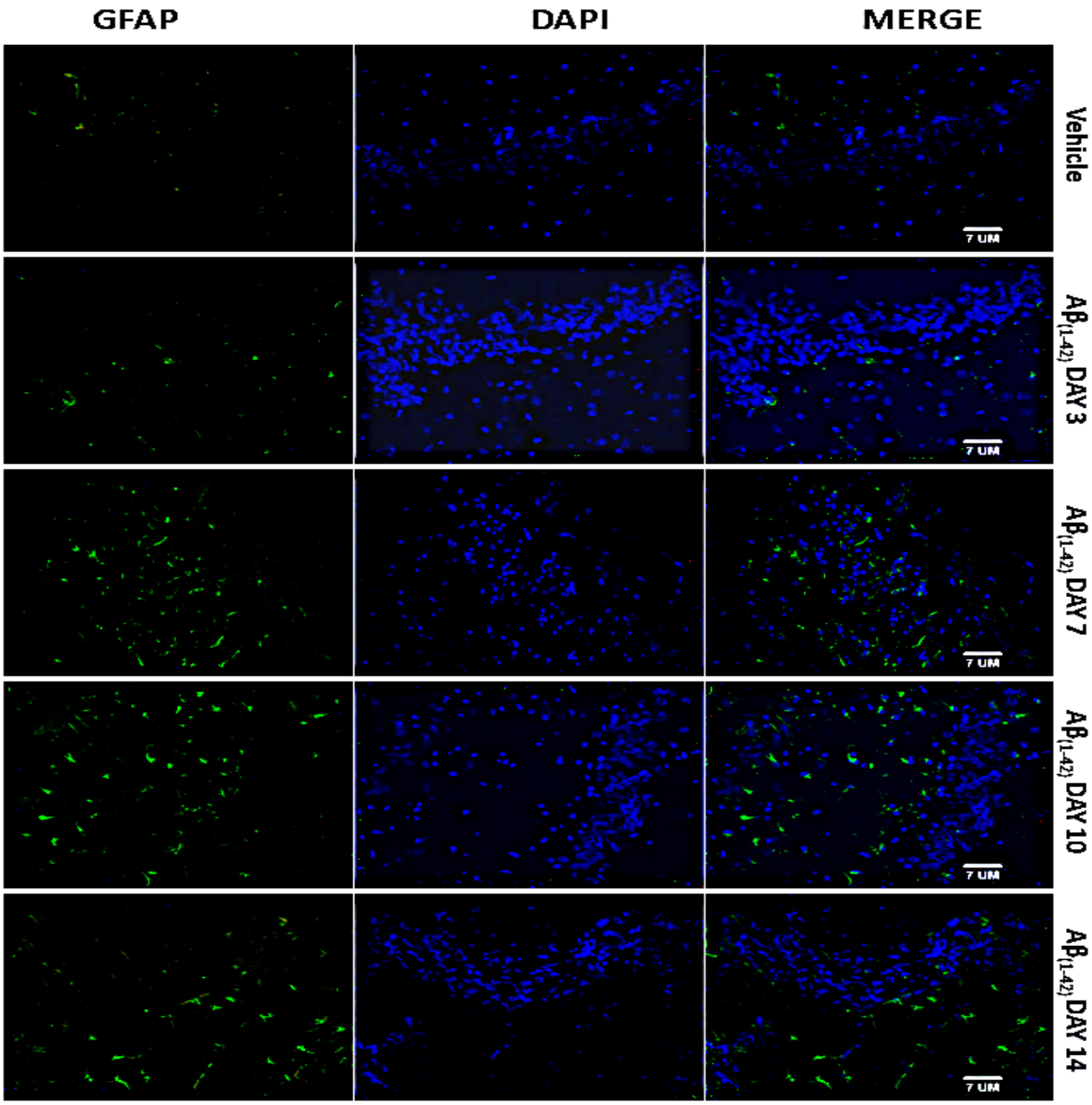

Figure 3: Immunofluorescence in the rat CA3 region of the hippocampus of Vehicle-infused and the $A\beta_{(1–42)}$ lesioned rat model of AD. Representative images of positively stained astrocytes with GFAP in the Vehicle, $A\beta_{(1-42)}$ Day 3, $A\beta_{(1-42)}$ Day 7, $A\beta_{(1-42)}$ Day 10 and $A\beta_{(1-42)}$ Day 14 groups.

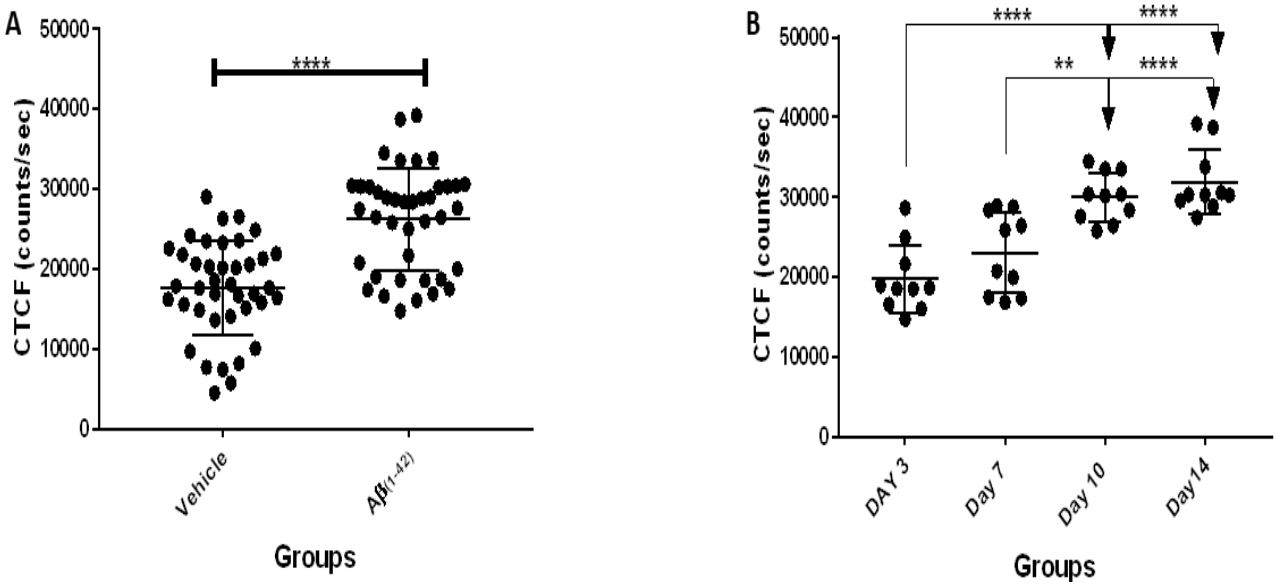

Figure 4: Mean corrected total cell immunofluorescence of GFAP in the hippocampus of (A) Vehicle-infused and the $A\beta_{(1–42)}$ lesioned rat model of AD irrespective of the days (B) $A\beta_{(1–42)}$ rat model of AD across post-lesion days. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, (student t-test and one-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 10/ group).

3.3. Correlative expression of IBA1 and GFAP in the CA3 region of the hippocampus

Strong positive correlations were observed between the corrected total cell fluorescence (CTCF) of IBA1 and GFAP on post-lesion day 3 ($r = 0.8980$, $p = 0.0004$) (Fig. 5a); post-lesion day 7 ($r = 0.8635$, $p = 0.0013$) (Fig. 5b); post-lesion day 10 ($r = 0.7852$, $p = 0.0071$) (Fig. 5c) and post-lesion day 14 ($r = 0.7728$, $p = 0.0008$) (Fig. 5d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Fig. 5).

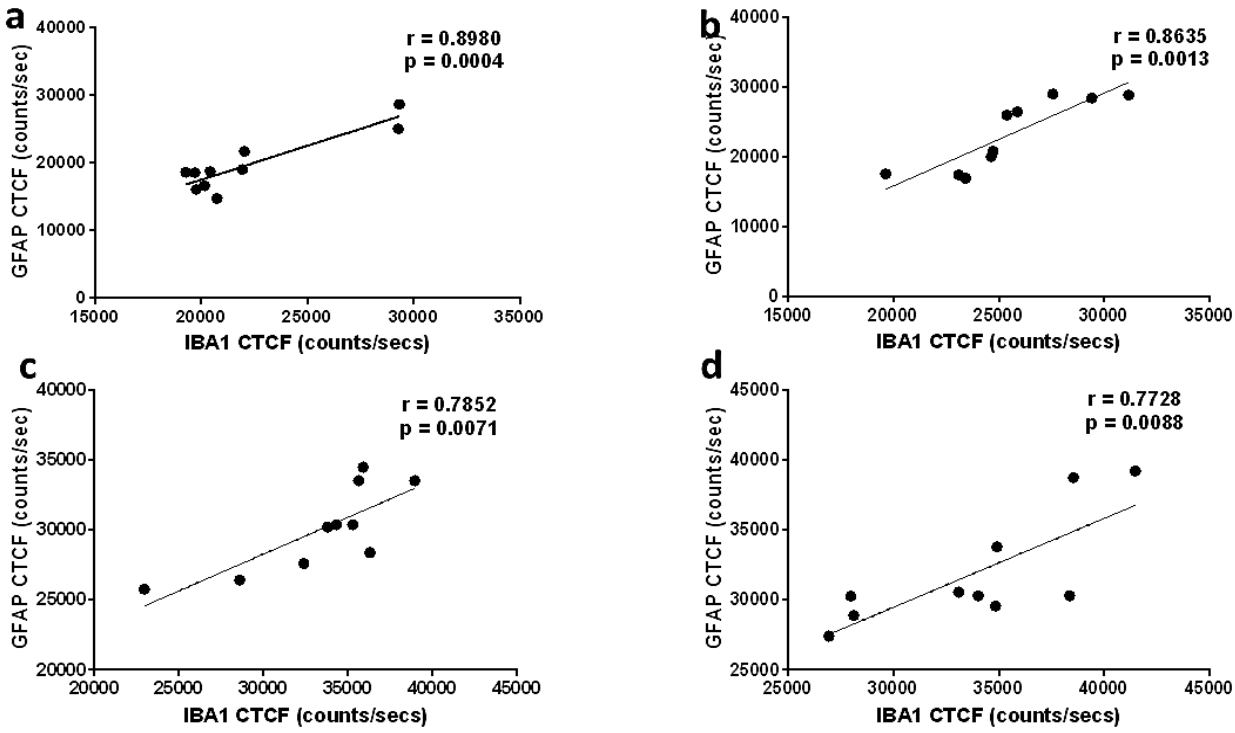

Figure 5: XY scatters plots of corrected total cell immunofluorescence in the hippocampus of positively stained cells with IBA-1 and GFAP on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 in the $A\beta_{(1\text{-}42)}$ rat model of AD. "r" indicates strength of the relationship. A value of $p < 0.05$ was considered as statistically significant for the number of rats ($n = 10$) in each group.

3.4. Lipid peroxidation (MDA) concentration in plasma

We observed a significant interaction [$F(3, 48) = 21.59$, $p < 0.0001$] between lesion and day. A lesion effect was found for lipid peroxidation quantified as MDA concentration, as this marker increased in plasma of $A\beta_{(1–42)}$ lesioned groups [$F(1, 48) = 194.8$, $p < 0.0001$] when compared to the corresponding vehicle groups, with specific significant increase on post-lesion day 7 (0.0058), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$). A day effect was observed as lipid peroxidation quantified as MDA concentration increased progressively in post-lesion day 3 to day 14 of $A\beta_{(1–42)}$ lesioned groups [$F(3, 48) = 3.175$, $p = 0.0324$], with specific significant increase ($p < 0.0001$) on post-lesion day 3 versus 14 group, day 7 versus day 14 ($p < 0.0001$), day 3 versus day 10 ($p = 0.0086$), day 10 versus day 14 ($p = 0.0339$) (Figure 6).

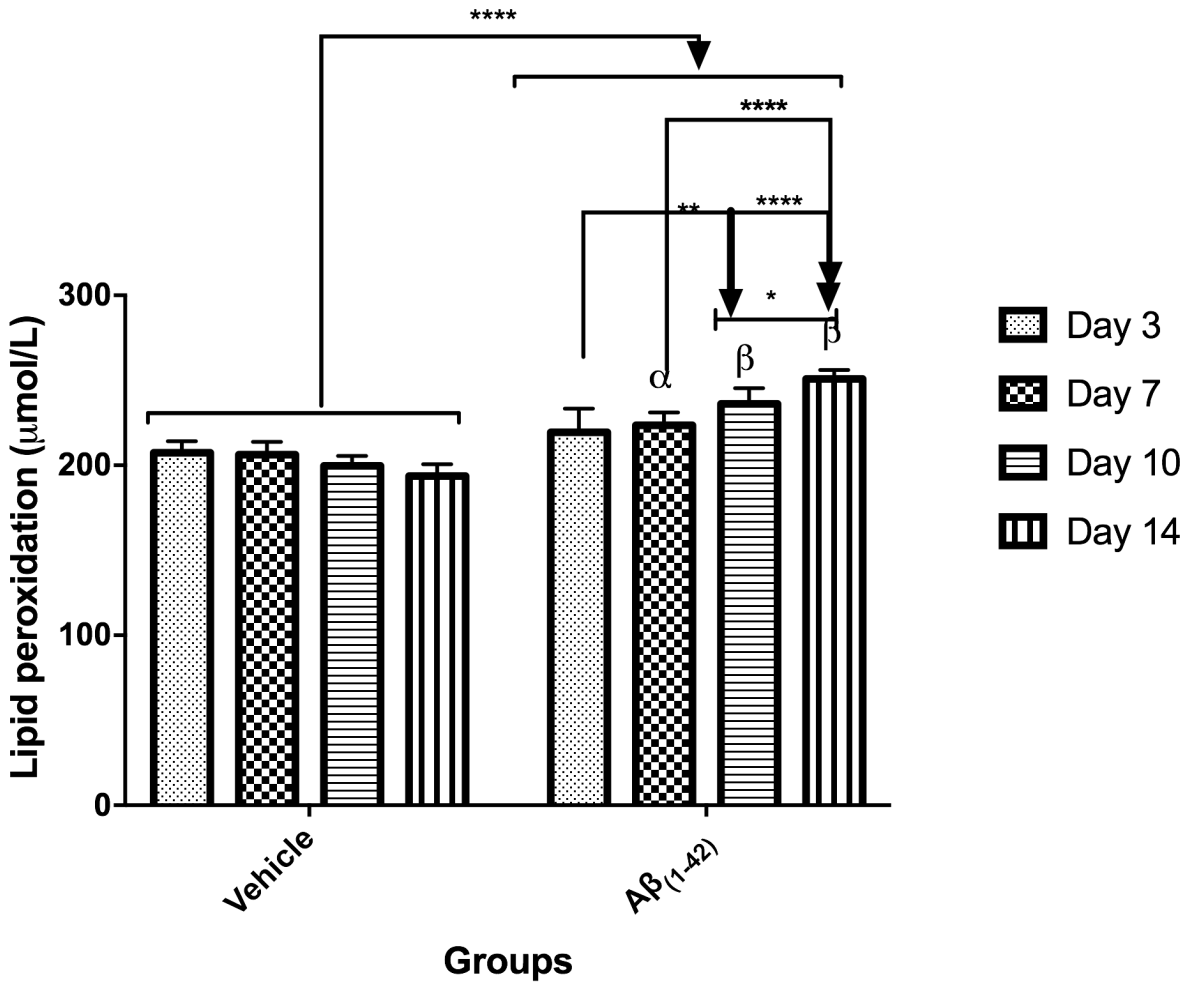

Figure 6: Plasma lipid peroxidation levels in vehicle and $A\beta_{(1–42)}$ lesioned rats at post-lesion day3, 7, 10 and 14. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, α = ** , β = **** (two-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 7/ group).

3.5. Reduced Glutathione concentration in plasma

We observed a significant interaction [$F (3, 48) = 48.56$, $p < 0.0001$] between lesion and day. A lesion effect was found for plasma reduced glutathione concentration, as this antioxidant was reduced in plasma of $A\beta_{(1–42)}$ lesioned groups [$F (1, 48) = 492.1$, $p < 0.0001$] when compared to the corresponding vehicle groups, with specific significant decrease ($p < 0.0001$) on post-lesion day 7, day 10 and day 14 groups. A significant day effect was also observed [$F (3, 48) = 28.51$, $p < 0.0001$] for plasma reduced glutathione level in the vehicle group (Figure 7).

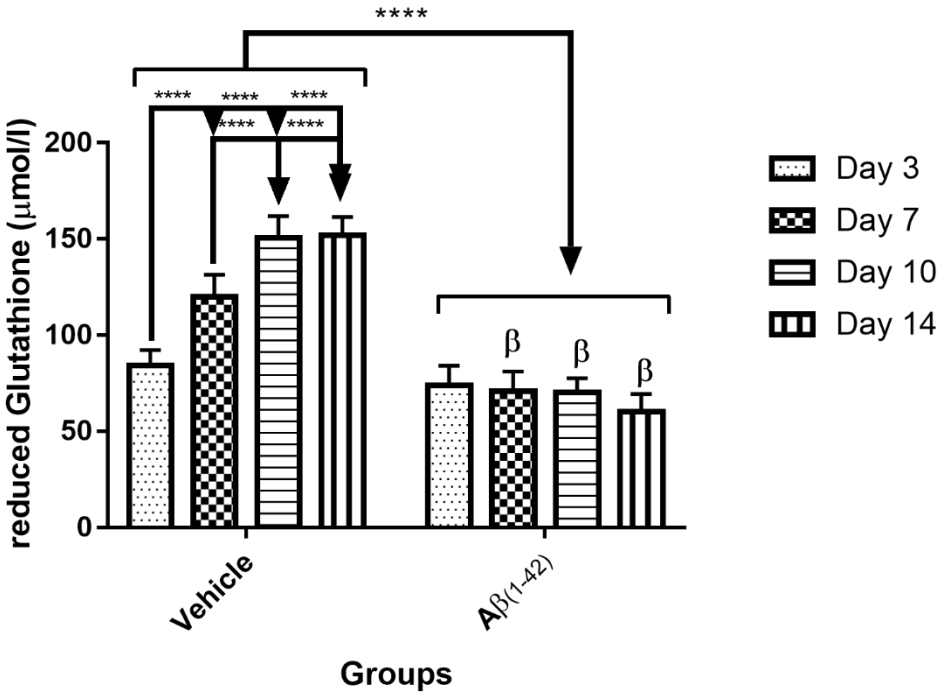

Figure 7: Plasma reduced glutathione levels in vehicle and $A\beta_{(1–42)}$ lesioned rats at post-lesion day3, 7, 10 and 14. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, β = **** (two-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 7/ group).

3.6. Correlation between IBA1 expression and plasma lipid peroxidation concentration in $A\beta_{(1–42)}$ lesioned rats

Positive correlations were observed between IBA1 expression in the hippocampus and lipid peroxidation level in plasma on post-lesion day 3 ($r = 0.9292$, $p = 0.0095$) (Figure 8a); post-lesion day 7 ($r = 0.9893$, $p < 0.0001$) (Figure 8b); post-lesion day 10 ($r = 0.8228$, $p = 0.0230$) (Figure 8c) and post-lesion day 14 ($r = 0.9671$, $p = 0.0004$) (Figure 8d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Figure 8).

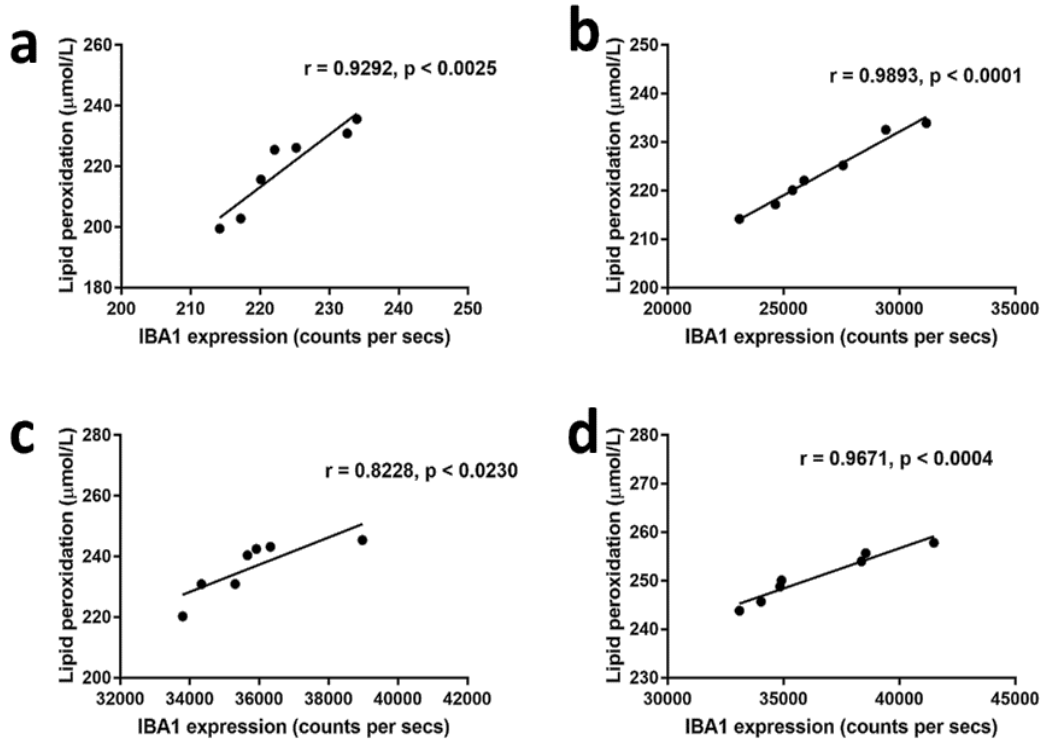

Figure 8: XY scatters plots of IBA-1 expression and lipid peroxidation level on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 in the $A\beta_{(1-42)}$ rat model of AD. "r" indicates strength of the relationship. A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 10) in each group.

4. Discussion

In this study, we examined the level of reactivity of glial cells in the CA3 region of the hippocampus by evaluating the expression of ionized calcium-binding adaptor molecule 1 (IBA-1) and glial fibrillary acidic protein (GFAP) proteins in a progressive $A\beta_{(1–42)}$ model of AD. The protein IBA-1 is a marker of activated microglia, it distinctively classifies cells engaged in routine surveillance from those activated in response to inflammation. On the other hand, the GFAP protein is a standard stain for reactive astrocytes., characterized by increased glial fibrillary acidic protein. We further evaluated oxidative stress levels in plasma and correlated its expression with activated microglia cells which are the resident immune cells in the brain. Although, the CA1 region of the dorsal hippocampus was lesioned, we however analysed the reactivity of glial cells in the CA3 region to avoid bias that could result from mechanical damage during stereotaxic surgery to this region. Besides, the CA3 region plays specific role in memory processes and is susceptible to neurodegeneration (Cherubini and Miles, 2015).

We observed a significant increase in the reactivity of microglia and astrocytes in the $A\beta_{(1-42)}$ model of AD when compared to their respective vehicle-infused groups. This increase is probably due to the toxic impact of aberrant deposits of the extracellular protein Aβ, which activates these glial cells and

initiates neuroinflammation (Reed-Geaghan et al., 2009). The progressive increase observed in this study at all timepoints post-lesion is facilitated by the ongoing formation of Aβ and possible positive feedback loops between inflammation and Aβ, which compromises the cessation of inflammation and exacerbates further accumulation of Aβ (Krabbe et al., 2013a). Activated microglia trigger the recruitment and proliferation of astrocytes that actively bolster the inflammatory response to extracellular amyloid beta deposits (Matsuoka et al., 2001, Murgas et al., 2012). This neuroinflammatory response in AD is further characterized by a local pro-inflammatory cytokine accumulation. All these factors, either alone or in concert, contribute to neuronal dysfunction and the eventual death that occurs in AD. Previous studies have shown the appearance of activated microglia and reactive astrocytes under pathological conditions (Matsuoka et al., 2001, Murgas et al., 2012).

Although both glial cells were activated at all timepoints and correlated positively in this study, we however, observed an overall 4.7% increase in expression of IBA-1 compared to GFAP. These results shed light on the relationship present in the process of their activation. Being the resident immune cells of the brain, microglia are activated earlier than astrocytes (Krabbe et al., 2013a). They are responsible for immune surveillance function as they can detect danger and instruct nearby astrocytes through the release of cytokines like interleukin-1. Release of this cytokine activates astrocytes and recruits more microglia in a feed-forward manner that leads to neuroinflammation (Giulian et al., 1994, Shallie et al., 2020, Liu et al., 2011).

The increase in microglia expression can also be explained from a functional point of view, as microglia represent a hybrid between immune-competent cells and glial cells with neuronal-supporting functions (Wake et al., 2009). Although the nature and function of the interactions between microglia and neuronal circuits are only hypothesized, studies have demonstrated that microglia do not represent only ‘‘dormant’’ resting macrophages, but actively exert neuroprotective actions on neuronal population (Battista et al., 2006, Ziv et al., 2006, Ekdahl et al., 2009). This surveying role is likely to be an important factor in homeostasis maintenance during ‘‘microdamage’’ that commonly occurs in the brain (Ekdahl et al., 2009). It has been proposed that, in the adult brain, microglia take part in synapse remodeling and, probably, in neurogenesis. In vivo evidence also suggests microglial regulation of neurogenesis in the hippocampus (Battista et al., 2006, Ziv et al., 2006) and in the adult subventricular zone, at least after stroke (Thored et al., 2009). Recent studies highlight the molecular constituents of neuro-immune system interaction reciprocally shared between the two systems (Chavan et al., 2017, Shallie et al., 2020). These constituents include microglia, astrocytes, toll-like receptors (TLRs), the expression of pattern recognition receptors (PRRs) receptors, and cytokine receptors. All these provide a molecular substrate for concurrent regulation of immune and neuronal function by cytokines, pathogen-associated molecular patterns (PAMPs), and other immune molecules. This repository is

deployed in the integration of neural circuits and immune response set-off by infection or injury (Chavan et al., 2017, Shallie et al., 2020).

Microglia have been implicated as the major contributor to oxidative stress in the CNS via the release of a number of reactive species (Cotran et al., 2004). We therefore, determined oxidative stress by quantifying plasma MDA, a marker of lipid peroxidation (LPO) and reduced glutathione level, a primary antioxidant defense of the central nervous system in our model of AD. Our results showed that $A\beta_{(1–42)}$ lesioned rats had higher lipid peroxidation levels than vehicle rats at all post-lesion intervals. The significantly higher MDA levels indicate the implication of ROS-mediated damage (Cotran et al., 2004). These results are in agreement with (Krishnan and Rani, 2014) who showed that plasma lipid peroxidation levels were elevated in AD patients who also indicated an age-dependent increase in the levels of MDA. We also found that reduced glutathione concentration, an important cellular antioxidant that responds to LPO was decreased in the $A\beta_{(1–42)}$ lesioned rats while we observed increasing levels in the vehicle rat over the post-lesion day intervals. The reduction in levels of GSH in the $A\beta_{(1–42)}$ lesioned rats could be attributed to the detrimental effect of excess reactive oxygen species, since GSH system effectively maintains the redox balance and is also important in scavenging of hydroperoxides independently (Nazıroğlu, 2009). The initial reduction seen in the vehicle on Day 3 could be due to the stress induced by stereotaxic surgical procedure. This reduction is seen to be followed by an increase in the subsequent days as the effect of the stress wanes.

We correlated activated microglia and lipid peroxidation to further understand the association between neuroinflammation and oxidative stress. Our results showed a positive relationship. This indicates that an increase in IBA1 expression leads to increase in lipid peroxidation level as microglia can modulate the inflammatory response through reactive oxygen species (ROS) (Loane and Kumar, 2016). We therefore postulate that the possible initial impact of neuroinflammation is followed by oxidative stress, which will further accentuate neuroinflammation. Conversely, if oxidative stress is the primary event, neuroinflammation will develop as a consequence which will further exaggerate oxidative stress (Vaziri and Rodríguez-Iturbe, 2006). Therefore, identification of primary abnormality and the confounding factor/s could be of great clinical importance, as the treatment of the primary disorder is likely to ensure a sustained relief from the problem.

5. Conclusion

Neuroinflammation in the $A\beta_{(1–42)}$ model of AD was initiated due to the prolonged and progressive activation of the glial cells, resulting in a bidirectional interaction between neuroinflammation and oxidative stress. This interaction may facilitate the understanding of the pathogenesis of AD, resulting in a strategy directed at controlling neuroinflammation and oxidative stress in developing therapeutics for AD.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgements

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References

1. Krabbe, G., et al., *Functional impairment of microglia coincides with Beta-amyloid deposition in mice with Alzheimer-like pathology.* PloS one, 2013. **8**(4): p. e60921.
2. Olabarria, M., et al., *Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease.* Glia, 2010. **58**(7): p. 831-838.
3. Zhang, B., et al., *Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease.* Cell, 2013. **153**(3): p. 707-720.
4. Heneka, M.T., et al., *Neuroinflammation in Alzheimer's disease.* The Lancet Neurology, 2015. **14**(4): p. 388-405.
5. Sarma, J.D., *Microglia-mediated neuroinflammation is an amplifier of virus-induced neuropathology.* Journal of neurovirology, 2014. **20**(2): p. 122-136.
6. Jiang, T. and E. Cadenas, *Astrocytic metabolic and inflammatory changes as a function of age.* Aging cell, 2014. **13**(6): p. 1059-1067.
7. Kabba, J.A., et al., *Microglia: housekeeper of the central nervous system.* Cellular and molecular neurobiology, 2018. **38**(1): p. 53-71.
8. Vincent, A.J., et al., *Astrocytes in Alzheimer's disease: emerging roles in calcium dysregulation and synaptic plasticity.* Journal of Alzheimer's Disease, 2010. **22**(3): p. 699-714.
9. Carrero, I., et al., *Oligomers of beta-amyloid protein (Aβ1-42) induce the activation of cyclooxygenase-2 in astrocytes via an interaction with interleukin-1beta, tumour necrosis factor-alpha, and a nuclear factor kappa-B mechanism in the rat brain.* Experimental neurology, 2012. **236**(2): p. 215-227.
10. Lim, D., et al., *Amyloid beta deregulates astroglial mGluR5-mediated calcium signaling via calcineurin and Nf-kB.* Glia, 2013. **61**(7): p. 1134-1145.
11. Baik, S.H., et al., *Microglia contributes to plaque growth by cell death due to uptake of amyloid β in the brain of Alzheimer's disease mouse model.* Glia, 2016. **64**(12): p. 2274-2290.
12. Yuyama, K., et al., *Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia.* Journal of Biological Chemistry, 2012. **287**(14): p. 10977-10989.
13. Tamboli, I.Y., et al., *Statins promote the degradation of extracellular amyloid β-peptide by microglia via stimulation of exosome-associated insulin-degrading enzyme (IDE) secretion.* Journal of biological chemistry, 2010. **285**(48): p. 37405-37414.
14. Kinney, J.W., et al., *Inflammation as a central mechanism in Alzheimer's disease.* Alzheimer's & Dementia: Translational Research & Clinical Interventions, 2018. **4**: p. 575-590.
15. Hickman, S.E., E.K. Allison, and J. El Khoury, *Microglial dysfunction and defective β-amyloid clearance pathways in aging Alzheimer's disease mice.* Journal of Neuroscience, 2008. **28**(33): p. 8354-8360.
16. Shallie, O.F., E. Dalle, and M.V. Mabandla, *Memory decline correlates with increased plasma cytokines in amyloid-beta (1–42) rat model of Alzheimer's disease.* Neurobiology of Learning and Memory, 2020. **169**: p. 107187.

17. Korzhevskii, D. and O. Kirik, *Brain microglia and microglial markers.* Neuroscience and Behavioral Physiology, 2016. **46**(3): p. 284-290.
18. Choi, S.S., et al., *Human astrocytes: secretome profiles of cytokines and chemokines.* PloS one, 2014. **9**(4): p. e92325.
19. Sofroniew, M.V. and H.V. Vinters, *Astrocytes: biology and pathology.* Acta neuropathologica, 2010. **119**(1): p. 7-35.
20. Malarkey, E.B. and V. Parpura, *Mechanisms of glutamate release from astrocytes.* Neurochemistry international, 2008. **52**(1-2): p. 142-154.
21. Sofroniew, M.V., *Astrogliosis.* Cold Spring Harbor perspectives in biology, 2015. **7**(2): p. a020420.
22. Messing, A. and M. Brenner, *GFAP: functional implications gleaned from studies of genetically engineered mice.* Glia, 2003. **43**(1): p. 87-90.
23. Sochocka, M., B.S. Diniz, and J. Leszek, *Inflammatory response in the CNS: friend or foe?* Molecular neurobiology, 2017. **54**(10): p. 8071-8089.
24. Fialkow, L., Y. Wang, and G.P. Downey, *Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function.* Free Radical Biology and Medicine, 2007. **42**(2): p. 153-164.
25. Loane, D.J. and A. Kumar, *Microglia in the TBI brain: the good, the bad, and the dysregulated.* Experimental neurology, 2016. **275**: p. 316-327.
26. Li, J., et al., *Reciprocal activation between IL-6/STAT3 and NOX4/Akt signalings promotes proliferation and survival of non-small cell lung cancer cells.* Oncotarget, 2015. **6**(2): p. 1031.
27. Mittal, M., et al., *Reactive oxygen species in inflammation and tissue injury.* Antioxidants & redox signaling, 2014. **20**(7): p. 1126-1167.
28. Yauger, Y.J., et al., *Iron accentuated reactive oxygen species release by NADPH oxidase in activated microglia contributes to oxidative stress in vitro.* Journal of neuroinflammation, 2019. **16**(1): p. 41.
29. Facchinetti, R., M.R. Bronzuoli, and C. Scuderi, *An animal model of Alzheimer disease based on the intrahippocampal injection of amyloid β-peptide (1–42)*, in *Neurotrophic Factors*. 2018, Springer. p. 343-352.
30. Frautschy, S.A., et al., *Rodent models of Alzheimer's disease: rat Aβ infusion approaches to amyloid deposits.* Neurobiology of aging, 1996. **17**(2): p. 311-321.
31. Cioanca, O., et al., *Inhalation of coriander volatile oil increased anxiolytic–antidepressant-like behaviors and decreased oxidative status in beta-amyloid (1–42) rat model of Alzheimer's disease.* Physiology & behavior, 2014. **131**: p. 68-74.
32. Karthick, C., et al., *Time-dependent effect of oligomeric amyloid-β (1–42)-induced hippocampal neurodegeneration in rat model of Alzheimer's disease.* Neurological research, 2019. **41**(2): p. 139-150.
33. Wiltgen, B.J., et al., *Context fear learning in the absence of the hippocampus.* Journal of Neuroscience, 2006. **26**(20): p. 5484-5491.
34. Yang, X., et al., *A novel mechanism of memory loss in Alzheimer's disease mice via the degeneration of entorhinal–CA1 synapses.* Molecular psychiatry, 2018. **23**(2): p. 199.
35. Bagheri, M., et al., *Genistein ameliorates learning and memory deficits in amyloid β (1–40) rat model of Alzheimer's disease.* Neurobiology of learning and memory, 2011. **95**(3): p. 270-276.
36. Paxinos, G. and C. Watson, *The rat brain in stereotaxic coordinates: hard cover edition*. 2006: Elsevier.
37. Ijomone, O.K., P.D. Shallie, and T. Naicker, *Nω-nitro-l-arginine methyl model of pre-eclampsia elicits differential IBA1 and EAAT1 expressions in brain.* Journal of chemical neuroanatomy, 2019. **100**: p. 101660.
38. Guo, Y., Y. Xiang, and Z.-W. Yang, *Drying paraffin sections on hotplate unadvisable.* Journal of Histology & Histopathology, 2016. **3**(1): p. 4.
39. Guichet, P.-O., et al., *Asymmetric distribution of GFAP in glioma multipotent cells.* PloS one, 2016. **11**(3): p. e0151274.

40. Chowdhury, P. and M. Soulsby, *Lipid peroxidation in rat brain is increased by simulated weightlessness and decreased by a soy-protein diet.* Annals of Clinical & Laboratory Science, 2002. **32**(2): p. 188-192.
41. Janero, D.R., *Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury.* Free Radical Biology and Medicine, 1990. **9**(6): p. 515-540.
42. Ellman, G.L., *Tissue sulfhydryl groups.* Archives of Biochemistry and Biophysics, 1959. **82**(1): p. 70-77.
43. Reed-Geaghan, E.G., et al., *CD14 and toll-like receptors 2 and 4 are required for fibrillar Aβ-stimulated microglial activation.* Journal of Neuroscience, 2009. **29**(38): p. 11982-11992.
44. Matsuoka, Y., et al., *Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer's disease.* The American journal of pathology, 2001. **158**(4): p. 1345-1354.
45. Murgas, P., B. Godoy, and R. Von Bernhardi, *Aβ potentiates inflammatory activation of glial cells induced by scavenger receptor ligands and inflammatory mediators in culture.* Neurotoxicity research, 2012. **22**(1): p. 69-78.
46. Giulian, D., et al., *The impact of microglia-derived cytokines upon gliosis in the CNS.* Developmental neuroscience, 1994. **16**(3-4): p. 128-136.
47. Liu, W., Y. Tang, and J. Feng, *Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system.* Life sciences, 2011. **89**(5-6): p. 141-146.
48. Wake, H., et al., *Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals.* Journal of Neuroscience, 2009. **29**(13): p. 3974-3980.
49. Ekdahl, C., Z. Kokaia, and O. Lindvall, *Brain inflammation and adult neurogenesis: the dual role of microglia.* Neuroscience, 2009. **158**(3): p. 1021-1029.
50. Battista, D., et al., *Neurogenic niche modulation by activated microglia: transforming growth factor β increases neurogenesis in the adult dentate gyrus.* European Journal of Neuroscience, 2006. **23**(1): p. 83-93.
51. Ziv, Y., et al., *Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood.* Nature neuroscience, 2006. **9**(2): p. 268-275.
52. Thored, P., et al., *Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke.* Glia, 2009. **57**(8): p. 835-849.
53. Chavan, S.S., V.A. Pavlov, and K.J. Tracey, *Mechanisms and therapeutic relevance of neuro-immune communication.* Immunity, 2017. **46**(6): p. 927-942.
54. Cotran, R.S., V. Kumar, and R. Stanley, *Robbins pathologic basis of disease*. 2004: WB Saunders CompHny, Philadelphia, USA.
55. Krishnan, S. and P. Rani, *Evaluation of selenium, redox status and their association with plasma amyloid/tau in Alzheimer's disease.* Biological trace element research, 2014. **158**(2): p. 158-165.
56. Nazıroğlu, M., *Role of selenium on calcium signaling and oxidative stress-induced molecular pathways in epilepsy.* Neurochemical research, 2009. **34**(12): p. 2181-2191.
57. Vaziri, N.D. and B. Rodríguez-Iturbe, *Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension.* Nature Clinical Practice Nephrology, 2006. **2**(10): p. 582.

PROLOGUE

Chapter 3 elucidates the effect of amyloid-beta (1-42) on memory and how cytokines as inflammatory markers respond to this model of AD. Formats used in this chapter are according to the journal specifications. This manuscript has been published in the journal of Neurobiology of Learning and Memory. See appendix IV.

CHAPTER 3

Memory decline correlates with increased plasma cytokines in amyloid-beta (1-42) rat model of Alzheimer's disease

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ABSTRACT

Dysregulation of inflammatory markers like cytokines is implicated in the pathophysiology of Alzheimer's disease (AD). Altered level of these cytokines show that pathogenesis of AD is beyond dysfunction of neurons resulting from amyloid beta accumulation but involves neuroinflammatory mechanisms elicited by the neuroimmune cell. In this study, we investigated the effect of amyloid-beta (1-42) ($A\beta_{(1–42)}$) on memory and how inflammatory markers respond to this model of AD. Male Sprague-Dawley rats were used for this study. The animals were randomly divided into four groups euthanized on day 3, 7, 10 and 14 post-lesion with amyloid-beta (5 μg/ 5 μl) while corresponding control groups were stereotaxically injected with a vehicle (5 μl of 0.01 M phosphate- buffered saline). The Morris water maze (MWM) test to access learning and memory was conducted pre and post-lesion and blood was collected through cardiac puncture on day 3, 7, 10 and 14 post lesion. Multiplex immunoassay was performed to determine the plasma levels of IL-1β, IL-6, IL-10 and TNF-α. Our results showed impaired spatial memory and elevated plasma levels of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) with a concomitantly lowered level of the anti-inflammatory cytokine (IL-10) in the $A\beta_{(1–42)}$ lesioned rats when compared to the vehicle groups. This study shows a negative correlation between the performance in spatial memory task and plasma levels of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and positive correlation between performance in spatial memory task and the anti-inflammatory cytokine IL-10. In conclusion, this study most importantly demonstrated an association between progressive decline in spatial memory and increased plasma cytokine level induced by the infusion of $A\beta_{(1-42)}$.

Keywords: Alzheimer's disease; Amyloid beta; Glial cells; Inflammatory cytokines; Memory; Neuroinflammation

1. Introduction

Alzheimer's disease (AD) is an escalating neurodegenerative disease that represents a major factor for the prevalence of dementia in the aged population worldwide (Mohajeri et al., 2015). Dementia currently affects an estimated number of 50 million people worldwide, and this population has been projected to rise to 152 million by 2050, thereby making it an indubitable public health mandate (Organization, 2018). Cumulative evidence indicates that Alzheimer's disease is caused by the toxicity resulting from the buildup of amyloid beta (Hardy and Selkoe, 2002, Eckman and Eckman, 2007). However, neuroinflammation is presently being proposed as a possible mechanism for neuronal death observed in the disease progression (McKenzie et al., 2017, Ahmad et al., 2018). This is supported by the presence of abundantly activated glial cells observed in postmortem evaluation of AD brains and by the recurrent failure of drugs that only target amyloid plaques in the disease (Kametani and Hasegawa, 2018, Hopperton et al., 2018). The hippocampus is the main seat of memory and one of the earliest parts of the brain affected in this disease (Morris and Baddeley, 1988, Bondi et al., 2008, Webster et al., 2014). Therefore, an impaired memory that deteriorates with disease progression is a core clinical manifestation in AD (Perry et al., 2000, Stopford et al., 2012). Neuropathological characterization of AD is based on the occurrence of intracellular neurofibrillary tangles and extracellular plaques, while the main component of this plaque is amyloid beta-protein (Domingues et al., 2017). Amyloid-beta 1-42 ($A\beta_{1-42}$) is the most toxic form of this peptide, it is derived from β-amyloid precursor protein (βAPP), and triggers AD pathogenesis by quickening age-related memory decline (Ferreira et al., 2015, Sasmita et al., 2018).

Cytokines are inflammatory markers that contribute significantly to the pathophysiology of AD (Alam et al., 2016). They are synthesized in response to inflammation by microglia, the resident neuroimmune cells tasked with maintaining homeostatic balance in the brain (Ransohoff and Brown, 2012, Norris and Kipnis, 2019). Deposition of amyloid-beta 1-42 ($A\beta_{1-42}$) in the brain activates the microglia, which in turn incites the surrounding brain tissue and triggers the expression of inflammatory mediators including cytokines such as interleukin (IL)-1β, IL-6, IL-10 and tumor necrosis factor-α (TNF-α). These secreted cytokines in turn act to either exacerbate or attenuate the inflammatory state and progression of the disease (Block and Hong, 2005, Glass et al., 2010, Shastri et al., 2013). Their action is dependent on one another, as no particular cytokine can mediate inflammation alone (Platzer et al., 1995, Steensberg et al., 2003, Donzis and Tronson, 2014). Our aim was therefore to investigate the effect of $A\beta_{(1-42)}$ on memory and to assess cytokine response to this model of AD, also, we further examined the response of these markers in association with the state of memory in $A\beta_{(1-42)}$ lesioned rat model of AD.

2. Materials and methods

2.1. Animals

All procedures were reviewed and approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (AREC/015/018D). Fifty-six (56) male Sprague-Dawley rats weighing between 300 and 350g (8/9 weeks of age) were obtained from the Biomedical Research Unit of the University of KwaZulu- Natal and kept under standard laboratory conditions with food (Rodent Ripe Pellets, Meadow, South Africa) and water freely available. The animals were randomly divided into four groups (Day 3 group: animals euthanized 3 days after lesioning; Day 7 group: animals euthanized 7 days after lesioning; Day 10 group: animals euthanized 10 days after lesioning, and Day 14 group: animals euthanized 14 days after lesioning) (n = 14/group). Each group was further sub-divided into a vehicle group of rats (n = 7) that were injected with phosphate buffered saline and an $A\beta_{(1–42)}$ group (n = 7) that were injected with the neurotoxin $A\beta_{(1–42)}$ peptide (Lonappan et al., 2017). The animals were weighed prior to all experiments and were brought to the experimentation room 1 hour before commencing behavioral tests.

2.2. Drugs and reagents

$A\beta_{(1–42)}$ peptide was purchased from DLD Scientific (Durban North, South Africa). Ketamine, xylazine, and temgesic were obtained from Sigma (ST. Louis MO, USA). Bio-Plex Pro™ Rat Cytokine assay kit (catalogue number: 10014905) was purchased from Bio-Rad Laboratories (CA, USA).

2.3. Behavioral test

The Morris water maze (MWM) test commonly used to assess spatial learning and memory in rodents was used in this study (Morris, 1984, Garthe and Kempermann, 2013, Cassim et al., 2015). The procedure was performed as previously described by (Cassim et al., 2015). Animals were trained in a water maze (diameter: 1m) filled with water (23 ± 1 °C). The pool was divided into four virtual quadrants, each with a cue to assist the rat in finding the hidden platform, the platform was submerged in the middle of one of the four quadrants. Each rat was placed in a quadrant other than where the hidden platform is located, facing the wall of the pool and was given 120 seconds to find the platform and 15 seconds to stay on it. Animals that did not find the platform were gently guided and placed on it during the learning test. Learning was assessed pre-lesion, it measures the time taken for the animal to locate the hidden platform (escape latency) while the time spent in the quadrant of the hidden platform is regarded as the ability to remember (memory), which was assessed post-lesion.

2.4. Alzheimer's disease rat model ($A\beta_{(1–42)}$ lesion)

Rats were anesthetized with ketamine (100 mg/Kg, intraperitoneally) and xylazine (5 mg/Kg, intraperitoneally) solution before being placed in the stereotaxic apparatus (David Kopf instrument, Tujunga, USA) (Bagheri et al., 2011). After complete anaesthesia was observed following the absence of hind paw reflex on pinching, animals were bilaterally injected using a Hamilton syringe coupled to

a 25 G needle, with either 10 µL (5 µL on each side) solution of $A\beta_{(1–42)}$) peptide or an equal volume of phosphate buffered saline solution (Vehicle) into the CA1 field of the dorsal hippocampus (dCA1) according to the following stereotaxic co-ordinates referenced in millimeters from bregma: Anteroposterior (AP) = −4.8 mm; mediolateral (ML) = ±3.4 mm; dorsoventral (DV) = −3.0 mm) (Watson and Paxinos, 1986, Paxinos and Watson, 2006). The needle was kept in the dCA1 for 1 min prior to the injection and for 2 min following the injection to maximize diffusion. The incision was thereafter sutured, cleaned, and the animals were placed under a heating lamp to prevent hypothermia during recovery. Animals were injected with Temgesic (0.05 mg/kg subcutaneously) a post-operative analgesic before being returned to their home cages. Post-lesion behavioral test was conducted before the animals were euthanized on days 3, 7, 10 and 14, according to previous study in our laboratory (Lonappan et al., 2017).

2.5. Decapitation and neurochemistry

All rats were euthanized on post-lesion days 3, 7, 10 and 14 by halothane overdose. Blood (4 ml) acquired aseptically through cardiac puncture from both the vehicle and $A\beta_{(1–42)}$ lesioned rats were collected into ethylenediaminetetraacetic acid (EDTA) coated tubes, which were then centrifuged at 1000 × g for 15 minutes at 4 °C in a refrigerated centrifuge (Z326, Lasec, South Africa). In order to completely remove platelets and precipitates, the acquired plasma sample was centrifuged again at 10,000 × g for 10 minutes at 4 °C. Plasma were transferred into Eppendorf vials, quickly snap frozen in liquid nitrogen and stored at – 80 °C until analysis.

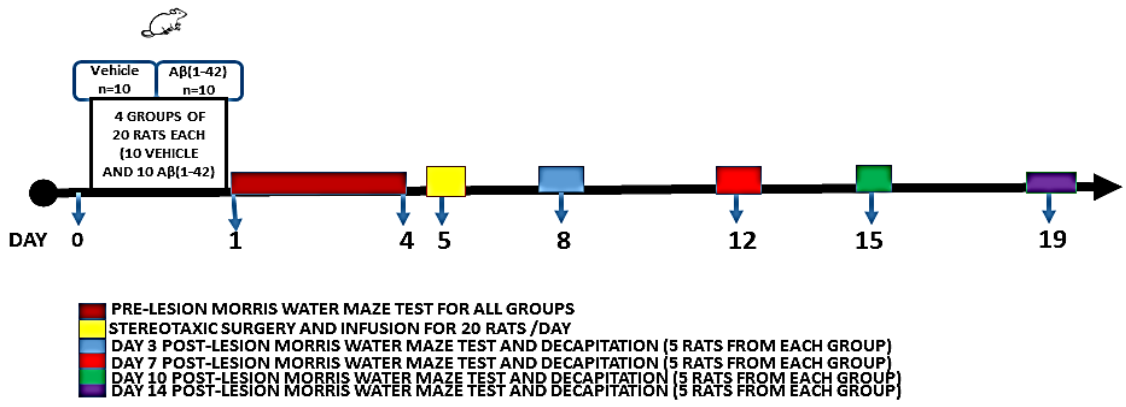

Fig. 1. Experimental timeline. Rats received bilateral intra-dCA1 infusion of $A\beta_{(1–42)}$ or vehicle solution after pre-lesion MWM test. On 3,7,10 and 14 days post-lesion, separate cohorts of rats belonging to $A\beta_{(1–42)}$ or vehicle-infused groups were tested for spatial memory function and euthanized 1 hour after each probe trial depending on the post-lesion group.

2.6. Multiplex assay

Plasma samples stored at -80 °C were thawed and used for the measurement of the cytokine levels (IL-1β, IL-6, IL-10, and TNF-α). Multiplex immunoassay was performed using a Bio-Plex Pro™ Rat Cytokine assay kit (catalog number: 10014905) in accordance with the manufacturer's instructions. Using a 96-well flat bottom assay plate, coupled magnetic beads (50 µl) were added into each well of the assay plate followed by washing twice using Bio-plex wash buffer (100 µl). Four-fold serial dilutions of both the standard and sample (50 µl) were then prepared and dispensed into designated wells followed by washing thrice using Bio-plex wash buffer (100 µl). Incubation on shaker at 850 ± 50 rpm at room temperature for 1 hour allowed for the interaction of the antigen with the coupled magnetic beads. Biotinylated detection antibodies (25 µl) (IL-1β, IL-6, IL-10, and TNF-α) were also added to the plates with subsequent incubation on shaker at 850 ± 50 rpm at room temperature for 30 minutes and washed thrice using Bio-plex wash buffer (100 µl). The addition of streptavidin-phycoerythrin (SAPE) (50 µl) incubated on shaker at 850 ± 50 rpm at room temperature for 10 minutes to complete the reaction was also concluded with washing thrice using Bio-plex wash buffer (100 µl). Plates were suspended in assay buffer (125 µl) on a shaker at 850 ± 50 rpm at room temperature for 30 seconds and read on a Bio-Plex® MAGPIX™ Multiplex system (Bio-Rad Laboratories Inc., USA). Data from the multiplex analysis was obtained using the Bio-Plex Manager™ version 4.1 software.

2.7. Statistical analysis

GraphPad Prism (version 7, Software Inc.) was used to analyze the data. Descriptive statistics for continuous data were reported as mean ± standard error of mean (SEM). Shapiro-Wilk normality test was used to assess distribution of data. Two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons were used to assess statistical significance within the study population. Pearson's correlation coefficient was used to assess the correlation between spatial memory and interleukin level, where "r" indicates the strength of the relationship. A value of $p < 0.05$ was considered statistically significant in all analyses.

3. Results

3.1. Spatial learning and memory

There was a significant day effect on spatial learning [$F(3, 208) = 337.9$, $p < 0.0001$] indicated by a decrease in time to locate the hidden platform (escape latency) over the training days for all groups (Fig. 2a). We also observed a significant day effect [$F(3, 208) = 6.404$, $p = 0.0004$] with specific significance between day 3 vs. day 10 ($p = 0.036$) and between day 7 vs. day 10 ($p = 0.0037$). Significant day effect within groups were observed: In Day 3 group: On day 1 vs. day 3 ($p < 0.0001$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 3 ($p = 0.002$); on day 2 vs. day 4 ($p < 0.0001$) and on day 3 vs. day

4 ($p = 0.0012$). In Day 7 group: On day 1 vs. day 3 ($p = 0.0031$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 3 ($p = 0.021$); on day 2 vs. day 4 ($p < 0.0001$) and on day 3 vs. day 4 ($p < 0.0001$). In Day 10 group: On day 1 vs. day 3 ($p = 0.0005$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 4 ($p = 0.0003$) and on day 3 vs. day 4 ($p = 0.0031$). In Day 14 group: On day 1 vs. day 3 ($p = 0.0015$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 3 ($p = 0.0006$); on day 2 vs. day 4 ($p < 0.0001$) and on day 3 vs. day 4 ($p = 0.0234$) (Fig. 2a).

Fig. 2b shows a significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on spatial memory [$F(1, 48) = 234.2$, $p < 0.0001$] with decreased time spent in the quadrant of the hidden platform on post-lesion day 3 ($p < 0.01$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the corresponding vehicle injected groups. A significant day effect was observed within the $A\beta_{(1–42)}$ lesioned groups [$F(3, 48) = 2.2$, $p = 0.0954$] differing with increasing significance from post-lesioned day 3 vs. day 7 ($p = 0.0176$), day 3 vs. day 10 ($p = 0.0097$) and day 3 vs. day 14 ($p = 0.0052$). We also observed a significant interaction [$F(3, 48) = 5.7$, $p = 0.0020$] between lesion and day (Fig. 2b).

Fig. 2c shows a significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on time taken to locate the quadrant of the hidden platform [$F(1, 48) = 684.7$, $p < 0.0001$] between the vehicle and their corresponding $A\beta_{(1–42)}$ lesioned groups. A significant day effect was also observed within the $A\beta_{(1–42)}$ lesioned groups [$F(3, 48) = 27.42$, $p = 0.0001$]. With specific significance between ($Day3_{A\beta(1–42)}$ vs. $Day7_{A\beta(1–42)}$, $p < 0.0001$), ($Day3_{A\beta(1–42)}$ vs. $Day10_{A\beta(1–42)}$, $p = 0.0001$), ($Day3_{A\beta(1–42)}$ vs. $Day14_{A\beta(1–42)}$, $p < 0.0001$), ($Day7_{A\beta(1–42)}$ vs. $Day14_{A\beta(1–42)}$, $p = 0.0001$) and ($Day3_{A\beta(1–42)}$ vs. $Day14_{A\beta(1–42)}$, $p = 0.0328$).

Table 1 shows the swimming speed and duration of time spent in opposite quadrant, expressed in cm/sec ± SEM and sec ± SEM respectively.

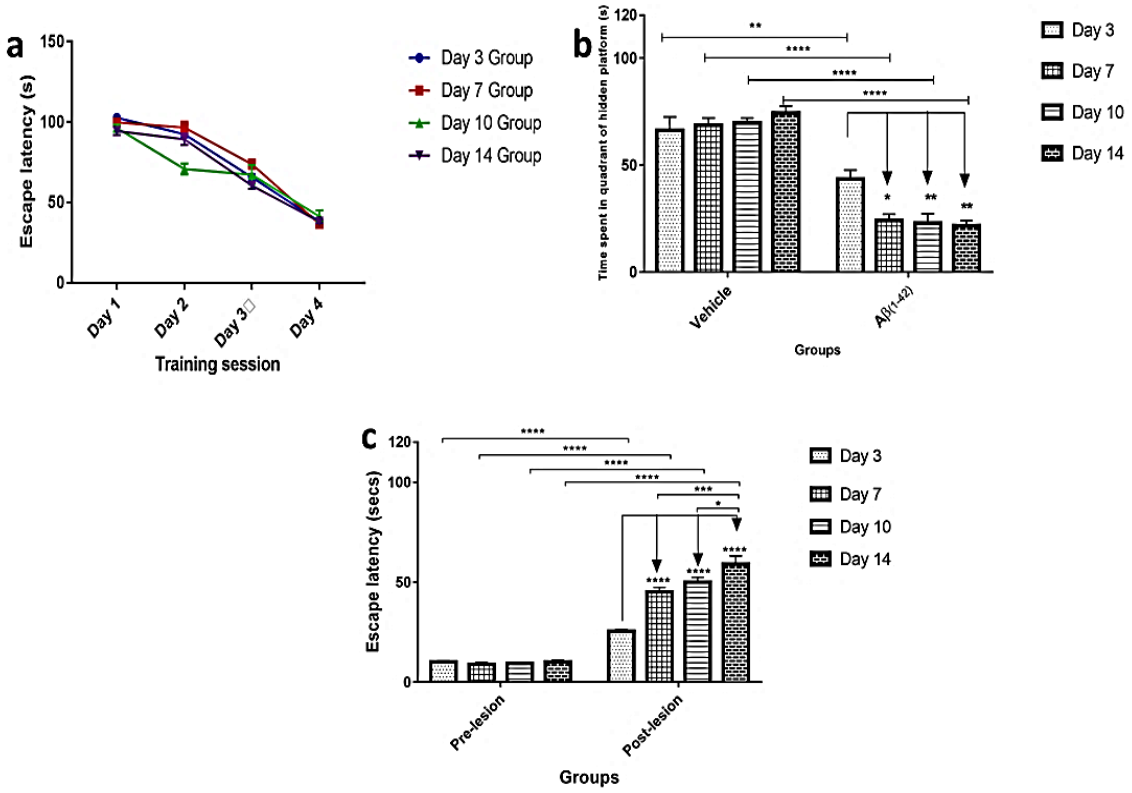

Fig. 2. (a) Time taken to locate the hidden platform for all rats of day 3, 7, 10 and 14 groups pre-lesion in the MWM. Values are expressed as Mean ± SEM (n = 14/group). **(b)** Time spent in the quadrant of the hidden platform for all groups at post-lesion days 3, 7, 10 and 14 in the MWM. **(Day3$_{vehicle}$ vs. Day3$_{A\beta(1-42)}$, $p < 0.01$), ****(Day7$_{vehicle}$ vs. Day7$_{A\beta(1-42)}$, $p < 0.0001$), ****(Day10$_{vehicle}$ vs. Day10$_{A\beta(1-42)}$, $p < 0.0001$), ****(Day14$_{vehicle}$ vs. Day14$_{A\beta(1-42)}$, $p < 0.0001$), *(Day3$_{A\beta(1-42)}$ vs. Day7$_{A\beta(1-42)}$, $p = 0.0176$), **(Day3$_{A\beta(1-42)}$ vs. Day10$_{A\beta(1-42)}$, $p = 0.0097$), **(Day3$_{A\beta(1-42)}$ vs. Day14$_{A\beta(1-42)}$, $p = 0.0052$). **(c)** Time taken to locate the quadrant of the hidden platform for all groups between pre-lesion and post-lesion days 3, 7, 10 and 14 in the MWM is significant at **** ($p < 0.0001$). ****(Day3$_{A\beta(1-42)}$ vs. Day7$_{A\beta(1-42)}$, $p < 0.0001$), ****(Day3$_{A\beta(1-42)}$ vs. Day10$_{A\beta(1-42)}$, $p = 0.0001$), ****(Day3$_{A\beta(1-42)}$ vs. Day14$_{A\beta(1-42)}$, $p < 0.0001$), ***(Day7$_{A\beta(1-42)}$ vs. Day14$_{A\beta(1-42)}$, $p = 0.0001$), **(Day3$_{A\beta(1-42)}$ vs. Day14$_{A\beta(1-42)}$, $p = 0.0328$). Statistical analysis made by two-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.

		Speed (cm/sec) ± SEM	Duration in the opposite Quadrant (sec) ± SEM
Day 3 group	$A\beta_{(1–42)}$	18.82 ± 1.21	36.5 ± 4.33
	Vehicle	17.69 ± 0.60	40.8 ± 3.75
Day 7 group	$A\beta_{(1-42)}$	17.97 ± 0.98	19.4 ± 2.65
	Vehicle	17.69 ± 0.60	38.9 ± 3.07
Day 10 group	$A\beta_{(1-42)}$	18.32 ± 0.76	21.3 ± 1.21
	Vehicle	17.69 ± 0.60	42.6 ± 3.15
Day 14 group	$A\beta_{(1–42)}$	19.03 ± 0.87	34.4 ± 4.21
	Vehicle	19.54 ± 1.03	44.5 ± 3.80

Table 1. Speed to reach the target quadrant and the time spent in the quadrant opposite to the target quadrant. Data represents the average swimming speed (cm/sec ± SEM) and the average time spent (sec ± SEM) in the quadrant opposite to the target quadrant in the Morris water maze during the probe tests conducted 3 days, 7 days, 10 day and 14 days post infusion.

3.2. Cytokine levels in the plasma

Pro-inflammatory cytokines level in the plasma are illustrated in Figure 3a-c.

A lesion effect was found for pro-inflammatory cytokine IL-1β, as this cytokine increased in plasma of $A\beta_{(1–42)}$ lesioned groups [$F(1, 48) = 75.17$, $p < 0.0001$] when compared to the corresponding vehicle groups, with specific significant increase ($p < 0.0001$) on post-lesion day 3 and 7 groups. A day effect was found as IL-1β levels decreased progressively in post-lesion day 3 to day 14 of $A\beta_{(1–42)}$ lesioned groups [$F(3, 48) = 17.86$, $p < 0.0001$], with specific day decline between day 3 versus 10 ($p < 0.0001$), day 3 versus 14 ($p < 0.0001$), day 7 versus 10 ($p = 0.0009$) and day 7 versus 14 ($p < 0.0001$) (See Fig. 3a). We also observed a significant interaction [$F(3, 48) = 13.38$, $p < 0.0001$] between lesion and day. Similarly, a lesion effect was found in plasma IL-6 levels as $A\beta_{(1–42)}$ lesioned groups [$F(1, 48) = 29.43$, $p < 0.0001$] increased when compared to the vehicle groups, with specific significant ($p < 0.0001$) increase in post-lesion $A\beta_{(1–42)}$ day 14 compared to its control. A significant day effect [$F(3, 48) = 27.97$, $p < 0.0001$] was also found with specific significant ($p < 0.0001$) differences between post-lesion $A\beta_{(1–42)}$ days 3, 7, 10 versus day 14 (See Fig. 3b). A significant interaction [$F(3, 48) = 22.28$, $p < 0.0001$] was found between lesion and day for IL-6 levels in plasma. We also found a significant lesion effect in plasma TNF-α levels as the $A\beta_{(1-42)}$ lesioned groups [$F(1, 48) = 38.1$, $p < 0.0001$] increased when compared to the vehicle groups, with specific significant ($p < 0.0001$ & $p = 0.0093$) increase in post-lesion $A\beta_{(1–42)}$ day 3 compared to its control and day 10 compared to its control respectively. A significant day effect [$F(3, 48) = 3.39$, $p = 0.0253$] was also observed. A significant interaction was observed [$F(3, 48) = 7.32$, $p < 0.0004$] between lesion and day for TNF-α levels (See Fig. 3c).

Anti-inflammatory cytokine level in plasma is illustrated in Figure 3d.

A significant lesion effect was observed in plasma IL-10 levels as $A\beta_{(1–42)}$ lesioned groups [F (1, 48) = 141.8, $p < 0.0001$] decreased when compared to the vehicle groups, with significant decrease ($p < 0.0001$) observed in post-lesion $A\beta_{(1–42)}$ days 7 and 14 groups when compared to their respective controls. A significant day effect [F (3, 48) = 172.7, $p < 0.0001$] showed progressive increase of IL-10 on days 3-14 post-lesion in both vehicle and $A\beta_{(1–42)}$ injected groups, with a significant ($p < 0.0001$) increase in Day 3 versus Day 7, Day 10, and Day 14; as well as Day 7 versus Day 14; and Day 10 versus Day 14 in the vehicle injected rats; and Day 3 versus 10; Day 3 versus 14; Day 7 versus 10 and Day 7 versus 14 in the $A\beta_{(1–42)}$ lesioned rats. (See Fig. 3d). We observed a significant interaction [F (3, 48) = 57.63, $p < 0.0001$] between lesion and day for IL-10 levels in plasma.

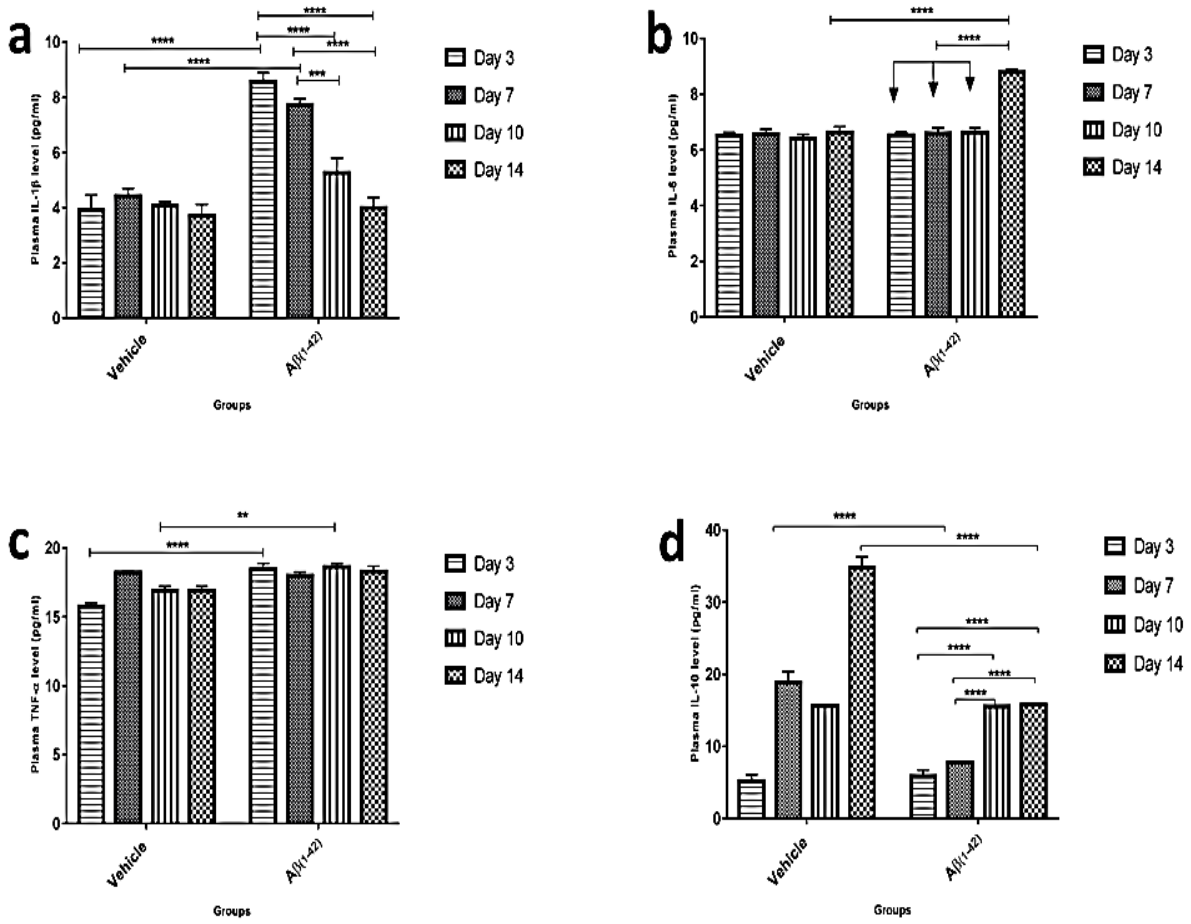

Fig. 3. (a) Plasma IL-1β levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($Day3_{vehicle}$ vs $Day3_{A\beta(1–42),}$ $p < 0.0001$), **** ($Day7_{vehicle}$ vs $Day7_{A\beta(1–42),}$ $p < 0.0001$), ****($Day3_{A\beta(1–42)}$ vs $Day10_{A\beta(1–42),}$ $p < 0.0001$), ****($Day3_{A\beta(1–42)}$ vs $Day14_{A\beta(1–42),}$ $p = 0.0014$), ***($Day7_{A\beta(1–42)}$ vs $Day10_{A\beta(1–42),}$ $p = 0.0009$), ****($Day7_{A\beta(1–42)}$ vs $Day14_{A\beta(1–42),}$ $p < 0.0001$). **(b)** Plasma IL-6 levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($Day14_{vehicle}$ vs. $Day14_{A\beta(1–42),}$ $p < 0.0001$), ****($Day3_{A\beta(1–42)}$ vs. $Day14_{A\beta(1–42),}$ $p < 0.0001$), ****($Day7_{A\beta(1–42)}$ vs. $Day14_{A\beta(1–42),}$ $p < 0.0001$), ***($Day10_{A\beta(1–42)}$ vs. $Day14_{A\beta(1–42),}$ $p < 0.0001$). **(c)** Plasma TNF-α levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($D3_{vehicle}$ vs $D3_{A\beta(1–42),}$ $p < 0.0001$), **($D10_{vehicle}$vs $D10_{A\beta(1–42),}$ $p = 0.0093$). **(d)** Plasma IL-10 levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($Day7_{vehicle}$ vs. $Day7_{A\beta(1–42),}$ $p < 0.0001$), ****($Day14_{vehicle}$ vs. $Day14_{A\beta(1–42),}$ $p <$

0.0001), ****(Day7$_{A\beta(1-42)}$ vs. Day14$_{A\beta(1-42)}$, $p < 0.0001$), ***(Day10$_{A\beta(1-42)}$ vs. Day14$_{A\beta(1-42)}$, $p < 0.0001$). Values are expressed as Mean ± SEM (n = 7/group). Statistical analysis made by two-way ANOVA followed by Tukey's post-hoc test for multiple comparison.

3.3. Correlation between memory decline and plasma cytokine levels in $A\beta_{(1-42)}$ lesioned rats.

The results presented in Fig. 4a-d depicts the correlation between memory decline expressed as time spent in the quadrant of the hidden platform of the Morris water maze and the plasma levels of cytokines in $A\beta_{(1-42)}$ lesioned rats. This statistical analysis was performed in order to determine whether there was a relationship between the progression of the disease with respect to memory decline and the fluctuations in the plasma cytokine levels.

A strong negative correlation was observed between the time spent in the quadrant of the hidden platform and plasma IL-1β level ($r = -0.6772$, $p < 0.0001$) in the $A\beta_{(1-42)}$ lesioned rat model of AD (Fig. 4a). Negative significant correlation was also observed between the time spent in the quadrant of the hidden platform and plasma IL-6 levels ($r = -0.5027$, $p = 0.0064$), in the $A\beta_{(1-42)}$ lesioned rat model of AD (Fig. 4b). Furthermore, there was a strong significant negative correlation between the time spent in the quadrant of the hidden platform and plasma TNF-α level ($r = -0.646$, $p = 0.0002$) in the $A\beta_{(1-42)}$ lesioned rat model of AD (Fig. 4c). However, we observed positive significant correlation between the time spent in the quadrant of the hidden platform and plasma levels of IL-10 ($r = 0.7780$, $p = 0.0001$) in the $A\beta_{(1-42)}$ lesioned rat model of AD (Fig. 4d).

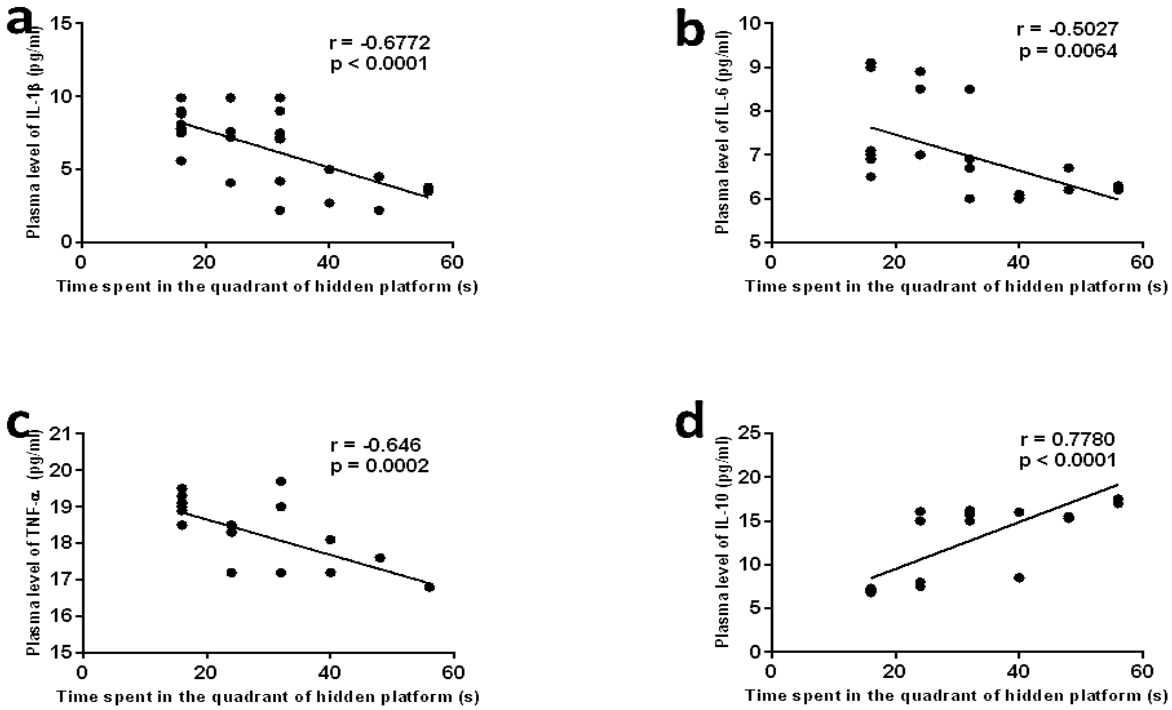

Fig. 4. (a) XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-1β for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ lesioned rat model of AD. **(b)** XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-6 for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ rat model of AD. **(c)** XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of TNF-α for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ rat model of AD. **(d)** XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-10 for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ rat model of AD. The degree and nature of the correlation between the time spent in the quadrant of the hidden platform and the plasma parameter are given by the value of r (Pearson's correlation coefficient). A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 28) in each group.

3.4. Correlation between memory decline and plasma cytokine levels in the vehicle-infused (Control) rats.

The results presented in Fig. 5 depicts the correlation between memory decline expressed as time spent in the quadrant of the hidden platform of the Morris water maze and the plasma levels of cytokines in the vehicle-infused (Control) rats. This statistical analysis was performed in order to determine whether the relationship observed in $A\beta_{(1-42)}$ lesioned rats with respect to memory decline and the fluctuations in the plasma cytokine levels was not due to the stress associated with the stereotaxic surgery.

Weak correlations were observed between the time spent in the quadrant of the hidden platform and: **(5a)** plasma IL-1β level ($r = -0.08508$, $p < 0.6669$) in the vehicle-infused (Control) rats; **(Fig. 5b)** plasma IL-6 levels ($r = -0.09363$, $p = 0.6356$) in the vehicle-infused (Control) rats; **(Fig. 5c)** plasma TNF-α

level (r = -0.08631, p = 0.6623) in the vehicle-infused (Control) rats; **(Fig. 5d)** plasma levels of IL-10 (r = -0.05747, p = 0.7714) in the vehicle-infused (Control) rats.

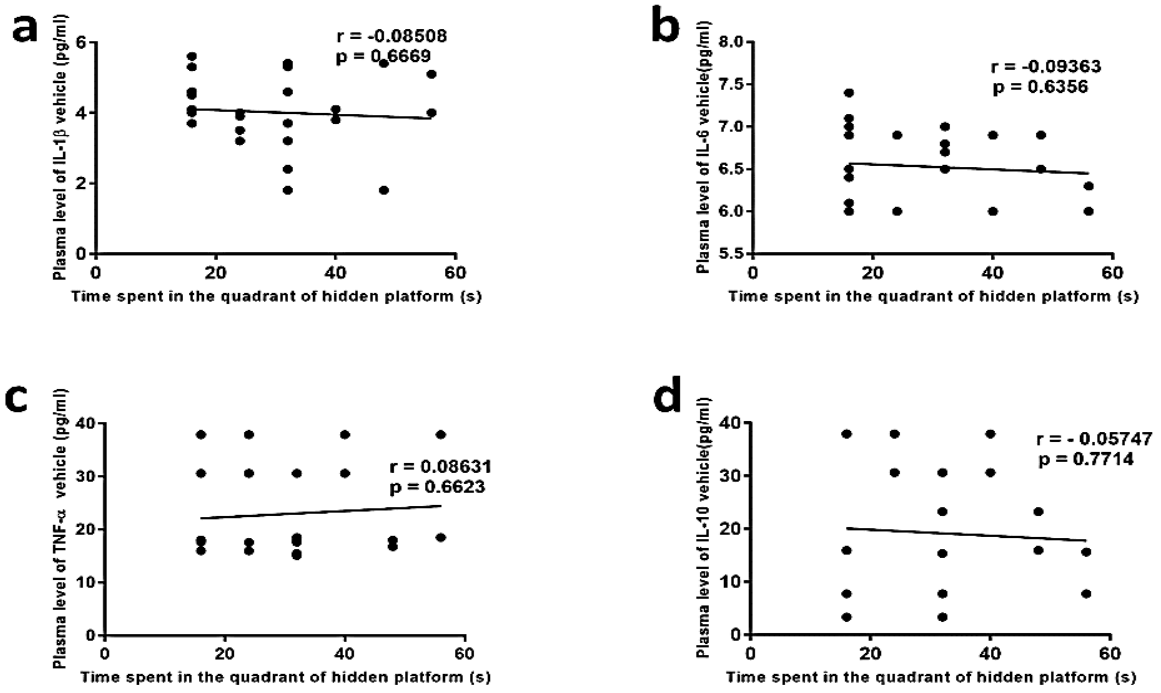

Fig. 5. **(a)** XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-1β for post-lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. **(b)** XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-6 for post-lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. **(c)** XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of TNF-α for post-lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. (**d)** XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-10 for post-lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. The degree and nature of the correlation between the time spent in the quadrant of the hidden platform and the plasma parameter are given by the value of r (Pearson's correlation coefficient). A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 28) in each group.

4. Discussion

This study aimed to mimic Alzheimer's disease progression following $A\beta_{(1-42)}$ infusion into the hippocampal region of rat. $A\beta_{(1-42)}$ infusion into the hippocampal area of the brain has been shown to elicit cognitive deficits as a result of its deleterious role in memory processing (Wang et al., 2017, Christensen et al., 2008). In our study we firstly investigated the effect of $A\beta_{(1-42)}$ on memory and secondly evaluated how $A\beta_{(1-42)}$ influences plasma levels of pro- and anti-inflammatory markers. We further concluded by highlighting the relationship between the decline in spatial memory and plasma levels of these inflammatory markers.

In the MWM test, we observed that all rats learnt to locate the hidden platform as indicated by a decreased escape latency over the training period of days, this shows that spatial learning was not affected pre-lesion. We however observed a lesion effect following infusion of $A\beta_{(1-42)}$, as the rats spent lesser time in the quadrant of the hidden platform indicating a deficit in memory. The lesion effect was prolonged as the post-lesion day 14 group of rats also showed memory deficits. Our findings are in agreement with studies that showed that intrahippocampal $A\beta_{(1-42)}$ infusion results to impaired spatial memory (Wang et al., 2017, Christensen et al., 2008). Cognitive impairment was observed in our study following a single bilateral intrahippocampal injection of $A\beta_{(1–42)}$, resulting into a time-dependent impairment, thereby establishing our model. We also observed differences in time spent in quadrant of hidden platform between the vehicle and $A\beta_{(1–42)}$ lesioned rats in the post-lesion Day 3, 7, 10 and 14 groups. Impairment of spatial memory in animals tested at delayed time-points following $A\beta_{(1–42)}$ injection into the dorsal hippocampus has been reported by some studies (Hruska and Dohanich, 2007, Christensen et al., 2008).

Markers of neuroinflammation such as IL-1β, IL-6, TNF-α and IL-10 have been shown to have direct effects on cognition and memory (Gibertini et al., 1995, Swardfager et al., 2010, Khemka et al., 2014). In order to illuminate the interaction between these cytokines and how the resultant synergistic effect modulates memory, we engaged the use of multiplex assay and found that $A\beta_{(1–42)}$ infusion led to IL-1β, IL-6 and TNF-α elevation in the plasma of the post-lesion day 3, 7, 10 and 14 group of rats when compared to the respective vehicle group of rats. The elevated plasma levels of these pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) observed in this study, is an indication of the unguarded inflammatory response in the progression of the disease. This is perhaps due to the induced expression of pro-inflammatory genes, which could result into loss of neurons as well as the clustering of phosphorylated tau (Von Bernhardi et al., 2010).

We propose that the initial upsurge in plasma level of IL-1β may be due to its role in initiating immune response to neuro-inflammatory cascade and this initial stimulus for elevation is likely the result of microglia activation to the lesioning effect of $A\beta_{(1–42)}$ (Simard et al., 2006). The plasma level of IL-6 showed a clear upsurge as observed in the post-lesion day 14 group suggestive of its role as a critical cytokine controlling the transition from innate to acquired immunity, which is imperative for dealing properly with injured or infected CNS tissue (Swartz et al., 2001). Studies also suggest that the detrimental influence of IL-6 on memory processes is potentiated over a progressive period of time in animals, this may therefore account for the late elevation of this cytokine as observed in this study. This age-dependent effect is demonstrated by showing progressive memory decline in mice that had increased levels of IL-6, while a gradual memory improvement is observed in mice with reduced levels of IL-6 (Heyser et al., 1997, Braida et al., 2004). TNF-α levels in the plasma of $A\beta_{(1–42)}$ lesioned rats was also elevated, causing a decline in memory, although this effect is less robust than the effects of

IL-1β and IL-6. This statement is true of our findings as there was a transient increase in the TNF-α levels in the lesioned rats compared to the vehicle injected rats. The process through which TNF-α mediates decline in long term potentiation (LTP) is quite alike to the process involved in the deleterious effect of IL-1β on LTP. Just like IL-1β, TNF-α prompts the activation of p38 mitogen-activated protein (MAP) kinase in the dentate gyrus (Butler et al., 2004). The observed significance in the day 3 $A\beta_{(1–42)}$ lesioned rats when compared to the days 7, 10 and 14 groups can be associated with the ability of p38 mitogen -activated protein (MAP) kinase to only moderates primarily the initial inhibitory effect of TNF-α on LTP. Another suggestive cause for the observed fluctuations in plasma TNF-α level is the TNF receptor deprivation and attenuation of its expression mediated by IL-10 in a bid to reduce cellular response.

Our results are in agreement with studies by (Wang et al., 2015b) that demonstrated that peripheral inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, traverse the blood brain barrier to prompt neuro-inflammatory reactions under pathologic conditions. However, the observed decline in plasma level of IL-1β across the post-lesion day 3, 7, 10 and 14 groups is suggestive of the counteracting effect of IL-10 to limit inflammation by reducing the synthesis of IL-1β, subduing expression of the receptor for IL-1β and by hindering activation of the associated receptor in the brain (Strle et al., 2001).

Concomitantly, we found that the plasma level of anti-inflammatory cytokine IL-10 decreased in the lesioned rats from post-lesion day 7 to the post-lesion day 14 groups. IL-10 is an anti-inflammatory cytokine that brings to bear surfeit immunomodulatory functions during an inflammatory response. The degree of pathology in the brain determines IL-10 expression, with the aim of promoting survival of neurons and glial cells, and dampening of inflammatory responses through signalling pathways (Strle et al., 2001, Garcia et al., 2017). IL-10 limits inflammation by reducing the expression of cytokine receptors and inhibiting activation of receptors (Strle et al., 2001, Garcia et al., 2017). However, the significant increase in the plasma levels of IL-10 in the vehicle-infused groups could be due to cellular stress in this case suggestive of induced by traumatic brain injury (TBI) resulting from the stereotaxic surgery. Previous studies have described overexpression of IL-10 in association with cellular stress (Le Moine et al., 1996). Our results showed a progressive time-based increased in the level of IL-10 in the vehicle groups from day 3 to day 7 followed by decrease in the day 10 group, with the uptrend continuing in day 14 group. The increase recorded in the vehicle-infused groups may be associated with increase expression of IL-10 mRNA immediately following TBI, which correlated with increase in IL-10 protein as reported by (Kamm et al., 2006).

These findings suggest that the surge in IL-10 levels may be due to an increase in local IL-10 synthesis rather than systemic IL-10 entering through a compromised blood-brain barrier (BBB). Early reports found that plasma IL-10 levels peak within the first 3 hrs (Hensler et al., 2002, Hensler et al., 2000),

while a later study showed the peak is between 5 and 6 days post-injury (Helmy et al., 2012). Other studies state that IL-10 levels may remain elevated for up to 22 days (Csuka et al., 1999, Maier et al., 2001) or even up to 6 months (Caplan et al., 2015). Cases of a second peak in concentration have also been reported, this may explain the slight decrease noticed on day 10 giving rise to the second peak on day 14 (Csuka et al., 1999, Hensler et al., 2000). However, there is inconsistency in the literature as to whether IL-10 levels are more increased in the CSF or serum following TBI (Csuka et al., 1999, Maier et al., 2001), rendering it difficult to determine the source of increased IL-10 levels. It has been reported that IL-10 can abrogate memory deficit observed in models of Alzheimer's disease (Kawahara et al., 2012, Kiyota et al., 2012b) probably via downregulating the elevation of the pro-inflammatory cytokines.

Overall, the correlative test showed that increase in the peripheral levels of IL-1β, IL-6 and TNF-α in $A\beta_{(1–42)}$ lesioned rats was associated with a decrease in the time spent in the quadrant of the hidden platform. On the other hand, decrease in anti-inflammatory cytokine IL-10 was associated to a decrease in the time spent in the quadrant of the hidden platform and vice-versa. From what has already been stated from our results, we can infer that plasma cytokine levels reflect the state of the central nervous system. This goes further to indicate that there is a suggestive breakdown of the blood brain barrier, hence the pathway of communication between the central nervous system and peripheral immune system (Wilson et al., 2002, Dantzer et al., 2008).

Based on previous findings by (Faucher et al., 2016), we can infer that the progressive spatial memory decline observed in our study is not due to the occurrence of amyloid plaques but caused by the molecular alterations elicited following the infusion of $A\beta_{(1–42)}$. This suggestive evidence depicted by the altered peripheral cytokine level in this study clearly shows a role for IL-1β, IL-6 and TNF-α in the modulation of memory, despite the variations across groups for each cytokine. As reflected in our results, these cytokines most likely exert an indirect synergistic effect on memory, rather than acting independently as postulated by (Donzis and Tronson, 2014). For instance, the observed increase in IL-1β is not in isolation as increase in IL-6 and TNF-α are also observed along with a decrease in IL-10.

5. Conclusion

This study is consistent with a growing body of literature that implicates inflammatory cytokines in AD pathology. The present study demonstrated that peripheral pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α are over-expressed in $A\beta_{(1-42)}$ lesioned rat, indicating a profound involvement of neuroinflammation in initiating and exacerbating AD pathology. Our results also suggest that the release of brain cytokines is a secondary response to the hallmarks of AD, occurring over a progressive

phase of the disease. This study most importantly demonstrated that memory decline may be associated with cytokine dysregulation.

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Conflict of Interest

The authors confirm that this article content has no conflict of interest.

References

Ahmad, M. H., Fatima, M., & Mondal, A. C. (2018). Influence of microglia and astrocyte activation in the neuroinflammatory pathogenesis of Alzheimer's disease: rational insights for the therapeutic approaches. *Journal of Clinical Neuroscience*.

Alam, Q., Zubair Alam, M., Mushtaq, G., A Damanhouri, G., Rasool, M., Amjad Kamal, M., & Haque, A. (2016). Inflammatory process in Alzheimer's and Parkinson's diseases: central role of cytokines. *Current pharmaceutical design, 22*(5), 541-548.

Bagheri, M., Joghataei, M.-T., Mohseni, S., & Roghani, M. (2011). Genistein ameliorates learning and memory deficits in amyloid β (1–40) rat model of Alzheimer's disease. *Neurobiology of learning and memory, 95*(3), 270-276.

Block, M. L., & Hong, J.-S. (2005). Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Progress in neurobiology, 76*(2), 77-98.

Bondi, M. W., Jak, A. J., Delano-Wood, L., Jacobson, M. W., Delis, D. C., & Salmon, D. P. (2008). Neuropsychological contributions to the early identification of Alzheimer's disease. *Neuropsychology review, 18*(1), 73-90.

Braida, D., Sacerdote, P., Panerai, A. E., Bianchi, M., Aloisi, A. M., Iosuè, S., & Sala, M. (2004). Cognitive function in young and adult IL (interleukin)-6 deficient mice. *Behavioural brain research, 153*(2), 423-429.

Butler, M., O'connor, J., & Moynagh, P. N. (2004). Dissection of tumor-necrosis factor-α inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early—but not late—phase LTP. *Neuroscience, 124*(2), 319-326.

Caplan, B., Bogner, J., Brenner, L., Kumar, R. G., Boles, J. A., & Wagner, A. K. (2015). Chronic inflammation after severe traumatic brain injury: characterization and associations with outcome at 6 and 12 months postinjury. *Journal of Head Trauma Rehabilitation, 30*(6), 369-381.

Cassim, S., Qulu, L., & Mabandla, M. V. (2015). Prenatal stress and early life febrile convulsions compromise hippocampal genes MeCP2/REST function in mid-adolescent life of Sprague-Dawley rats. *Neurobiology of learning and memory, 125*, 195-201.

Christensen, R., Marcussen, A. B., Wörtwein, G., Knudsen, G., & Aznar, S. (2008). Aβ (1–42) injection causes memory impairment, lowered cortical and serum BDNF levels, and decreased hippocampal 5-HT2A levels. *Experimental neurology, 210*(1), 164-171.

Csuka, E., Morganti-Kossmann, M. C., Lenzlinger, P. M., Joller, H., Trentz, O., & Kossmann, T. (1999). IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-α, TGF-β1 and blood–brain barrier function. *Journal of neuroimmunology, 101*(2), 211-221.

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature reviews neuroscience, 9*(1), 46.

Domingues, C., AB da Cruz e Silva, O., & Henriques, A. (2017). Impact of cytokines and chemokines on Alzheimer's disease neuropathological hallmarks. *Current Alzheimer Research, 14*(8), 870-882.

Donzis, E. J., & Tronson, N. C. (2014). Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences. *Neurobiology of learning and memory, 115*, 68-77.

Eckman, C. B., & Eckman, E. A. (2007). An update on the amyloid hypothesis. *Neurologic clinics, 25*(3), 669-682.

Faucher, P., Mons, N., Micheau, J., Louis, C., & Beracochea, D. J. (2016). Hippocampal injections of oligomeric amyloid β-peptide (1–42) induce selective working memory deficits and long-lasting alterations of ERK signaling pathway. *Frontiers in Aging Neuroscience, 7*, 245.

Ferreira, S. T., Lourenco, M. V., Oliveira, M. M., & De Felice, F. G. (2015). Soluble amyloid-b oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. *Frontiers in cellular neuroscience, 9*, 191.

Garcia, J. M., Stillings, S. A., Leclerc, J. L., Phillips, H., Edwards, N. J., Robicsek, S. A., . . . Doré, S. (2017). Role of interleukin-10 in acute brain injuries. *Frontiers in neurology, 8*, 244.

Garthe, A., & Kempermann, G. (2013). An old test for new neurons: refining the Morris water maze to study the functional relevance of adult hippocampal neurogenesis. *Frontiers in neuroscience, 7*, 63.

Gibertini, M., Newton, C., Friedman, H., & Klein, T. W. (1995). Spatial learning impairment in mice infected with Legionella pneumophila or administered exogenous interleukin-1-β. *Brain, behavior, and immunity, 9*(2), 113-128.

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., & Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell, 140*(6), 918-934.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science, 297*(5580), 353-356.

Helmy, A., Antoniades, C. A., Guilfoyle, M. R., Carpenter, K. L., & Hutchinson, P. J. (2012). Principal component analysis of the cytokine and chemokine response to human traumatic brain injury. *PLoS ONE, 7*(6), e39677.

Hensler, T., Sauerland, S., Bouillon, B., Raum, M., Rixen, D., Helling, H.-J., . . . Neugebauer, E. A. (2002). Association between injury pattern of patients with multiple injuries and circulating levels of soluble tumor necrosis factor receptors, interleukin-6 and interleukin-10, and polymorphonuclear neutrophil elastase. *Journal of Trauma and Acute Care Surgery, 52*(5), 962-970.

Hensler, T., Sauerland, S., Riess, P., Hess, S., Helling, H., Andermahr, J., . . . Neugebauer, E. (2000). The effect of additional brain injury on systemic interleukin (IL)-10 and IL-13 levels in trauma patients. *Inflammation Research, 49*(10), 524-528.

Heyser, C. J., Masliah, E., Samimi, A., Campbell, I. L., & Gold, L. H. (1997). Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin 6 in the brain. *Proceedings of the National Academy of Sciences, 94*(4), 1500-1505.

Hopperton, K., Mohammad, D., Trepanier, M., Giuliano, V., & Bazinet, R. (2018). Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: a systematic review. *Molecular psychiatry, 23*(2), 177.

Hruska, Z., & Dohanich, G. P. (2007). The effects of chronic estradiol treatment on working memory deficits induced by combined infusion of β-amyloid (1–42) and ibotenic acid. *Hormones and behavior, 52*(3), 297-306.

Kametani, F., & Hasegawa, M. (2018). Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Frontiers in neuroscience, 12*, 25.

Kamm, K., VanderKolk, W., Lawrence, C., Jonker, M., & Davis, A. T. (2006). The effect of traumatic brain injury upon the concentration and expression of interleukin-1β and interleukin-10 in the rat. *Journal of Trauma and Acute Care Surgery, 60*(1), 152-157.

Kawahara, K., Suenobu, M., Yoshida, A., Koga, K., Hyodo, A., Ohtsuka, H., . . . Nakayama, H. (2012). Intracerebral microinjection of interleukin-4/interleukin-13 reduces β-amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice. *Neuroscience, 207*, 243-260.

Khemka, V. K., Ganguly, A., Bagchi, D., Ghosh, A., Bir, A., Biswas, A., . . . Chakrabarti, S. (2014). Raised serum proinflammatory cytokines in Alzheimer's disease with depression. *Aging and disease, 5*(3), 170.

Kiyota, T., Ingraham, K. L., Swan, R. J., Jacobsen, M. T., Andrews, S. J., & Ikezu, T. (2012). AAV serotype 2/1-mediated gene delivery of anti-inflammatory interleukin-10 enhances neurogenesis and cognitive function in APP+ PS1 mice. *Gene therapy, 19*(7), 724.

Le Moine, O., Stordeur, P., Schandené, L., Marchant, A., De Groote, D., Goldman, M., & Devière, J. (1996). Adenosine enhances IL-10 secretion by human monocytes. *The Journal of immunology, 156*(11), 4408-4414.

Lonappan, A., Dallé, E. R. H. E. a., Afullo, T. J. O., & Daniels, W. M. U. (2017). Analysis of Behaviour Pattern in Sprague-Dawley Rats Using Microwave Techniques. *Journal of Electromagnetic Analysis and Applications, 9*(11), 155.

Maier, B., Schwerdtfeger, K., Mautes, A., Holanda, M., Müller, M., Steudel, W. I., & Marzi, I. (2001). Differential release of interleukines 6, 8, and 10 in cerebrospinal fluid and plasma after traumatic brain injury. *Shock (Augusta, Ga.), 15*(6), 421-426.

McKenzie, J. A., Spielman, L. J., Pointer, C. B., Lowry, J. R., Bajwa, E., Lee, C. W., & Klegeris, A. (2017). Neuroinflammation as a common mechanism associated with the modifiable risk factors for Alzheimer's and Parkinson's diseases. *Current aging science, 10*(3), 158-176.

Mohajeri, M. H., Troesch, B., & Weber, P. (2015). Inadequate supply of vitamins and DHA in the elderly: implications for brain aging and Alzheimer-type dementia. *Nutrition, 31*(2), 261-275.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of neuroscience methods, 11*(1), 47-60.

Morris, R. G., & Baddeley, A. D. (1988). Primary and working memory functioning in Alzheimer-type dementia. *Journal of clinical and experimental neuropsychology, 10*(2), 279-296.

Norris, G. T., & Kipnis, J. (2019). Immune cells and CNS physiology: microglia and beyond. *Journal of Experimental Medicine, 216*(1), 60-70.

Organization, W. H. (2018). Dementia fact sheet. 2017. In.

Paxinos, G., & Watson, C. (2006). *The rat brain in stereotaxic coordinates: hard cover edition*: Elsevier.

Perry, R. J., Watson, P., & Hodges, J. R. (2000). The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: relationship to episodic and semantic memory impairment. *Neuropsychologia, 38*(3), 252-271.

Platzer, C., Meisel, C., Vogt, K., Platzer, M., & Volk, H.-D. (1995). Up-regulation of monocytic IL-10 by tumor necrosis factor-α and cAMP elevating drugs. *International immunology, 7*(4), 517-523.

Ransohoff, R. M., & Brown, M. A. (2012). Innate immunity in the central nervous system. *The Journal of clinical investigation, 122*(4), 1164-1171.

Sasmita, A. O., Kuruvilla, J., & Ling, A. P. K. (2018). Harnessing neuroplasticity: modern approaches and clinical future. *International Journal of Neuroscience, 128*(11), 1061-1077.

Shastri, A., Bonifati, D. M., & Kishore, U. (2013). Innate immunity and neuroinflammation. *Mediators of inflammation, 2013*.

Simard, A. R., Soulet, D., Gowing, G., Julien, J.-P., & Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron, 49*(4), 489-502.

Steensberg, A., Fischer, C. P., Keller, C., Møller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology And Metabolism, 285*(2), E433-E437.

Stopford, C. L., Thompson, J. C., Neary, D., Richardson, A. M., & Snowden, J. S. (2012). Working memory, attention, and executive function in Alzheimer's disease and frontotemporal dementia. *Cortex, 48*(4), 429-446.

Strle, K., Zhou, J.-H., Shen, W.-H., Broussard, S. R., Johnson, R. W., Freund, G. G., . . . Kelley, K. W. (2001). lnterleukin-10 in the brain. *Critical Reviews™ in Immunology, 21*(5).

Swardfager, W., Lanctôt, K., Rothenburg, L., Wong, A., Cappell, J., & Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biological psychiatry, 68*(10), 930-941.

Swartz, K. R., Liu, F., Sewell, D., Schochet, T., Campbell, I., Sandor, M., & Fabry, Z. (2001). Interleukin-6 promotes post-traumatic healing in the central nervous system. *Brain research, 896*(1-2), 86-95.

Von Bernhardi, R., Tichauer, J. E., & Eugenín, J. (2010). Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. *Journal of neurochemistry, 112*(5), 1099-1114.

Wang, R., Zhang, Y., Li, J., & Zhang, C. (2017). Resveratrol ameliorates spatial learning memory impairment induced by Aβ1–42 in rats. *Neuroscience, 344*, 39-47.

Wang, W.-Y., Tan, M.-S., Yu, J.-T., & Tan, L. (2015). Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Annals of translational medicine, 3*(10).

Watson, C., & Paxinos, G. (1986). *The rat brain in stereotaxic coordinates*: Academic press San Diego, CA;.

Webster, S. J., Bachstetter, A. D., Nelson, P. T., Schmitt, F. A., & Van Eldik, L. J. (2014). Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. *Frontiers in genetics, 5*, 88.

Wilson, C. J., Finch, C. E., & Cohen, H. J. (2002). Cytokines and cognition—the case for a head-to-toe inflammatory paradigm. *Journal of the American Geriatrics Society, 50*(12), 2041-2056.

PROLOGUE

Chapter 4 focuses on the impact of microglial genes (CD33 and TREM2) regulating neuroinflammation, and their correlation with post-training recall in a progressive beta-amyloid model of AD. Formats used in this chapter are according to the journal specifications. This manuscript has been published in the journal of Behavioural Brain Research. See appendix V.

CHAPTER 4

Amyloid-beta (1-42) lesion of CA1 rat dorsal hippocampus reduces contextual fear memory and increases expression of microglial genes regulating neuroinflammation

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ABSTRACT

Emerging evidence indicates that the pathogenesis of Alzheimer's disease (AD) is not confined to neuronal disruptions but robustly communicates with the brain's immune system. Genome-wide analysis suggests that several genes, which increase the risk for AD, encode for factors that regulate the glial clearance of misfolded proteins and the inflammatory reaction. This study reappraises the amyloid hypothesis by focusing on the impact of neuroinflammation in a beta-amyloid model of AD, how this possibly exacerbates the progression of the disease and the correlation between genes regulating neuroinflammation (CD33 and TREM2) with post-training recall. Male Sprague-Dawley rats were used for this study, randomly divided into a vehicle group of rats (n = 40) that were injected with phosphate-buffered saline (PBS) and an $A\beta_{(1–42)}$ group (n = 40) that were injected with the neurotoxin $A\beta_{(1–42)}$ peptide. Fear conditioning test (FCT) to assess fear memory was conducted pre and post-lesion. The polymerase chain reaction was performed to determine the expression levels of CD33 and TREM2 genes. Our results show that $A\beta_{(1–42)}$ lesion of the rat CA1 hippocampal subregion significantly reduces contextual fear memory, and this reduction was exacerbated as the post-lesion days increased. We also observed an increase in the expression levels of CD33 and TREM2 genes in the $A\beta_{(1–42)}$ lesioned groups when compared to their corresponding vehicle groups. Taken together, the behavioral and gene expression data provide inferential evidence that $A\beta_{(1–42)}$ infusion impairs contextual memory by disrupting cellular pattern separation processes in the hippocampus, thus linking neuroinflammation to specific neural circuit disruption and cognitive deficit.

Keywords: Alzheimer's disease; Fear conditioning; Cornu ammonis 1; Contextual memory; Microglial genes; Amyloid beta

1. Introduction

The *cornu ammonis* 1 (CA1) is a subregion of the hippocampus, crucially involved in learning, spatial orientation, and different memory functions, including the recall of remote episodic memory and the strength of integrated memories (Bartsch et al., 2011, Amaral and Lavenex, 2007). It represents the primary output of the hippocampus, can thus deliver information about temporally ordered events, such as specific item–position conjunction within the sequence and specific alterations of a known sequence (Barrientos and Tiznado, 2016). Apart from being the primary output of the hippocampus, the CA1 integrates information from the entorhinal cortex (EC) to the CA1; a direct projection and an indirect EC projection through cornu ammonis 3 (CA3) and dentate gyrus (Van Strien et al., 2009, Jarsky et al., 2005, Bittner et al., 2015). The ensued matching or mismatching of information from these two projections elicits a representation of familiar or novel items (Barrientos and Tiznado, 2016).

Studies have shown that CA1 is one of the most affected regions in Alzheimer's disease (AD), mainly at early stages presented as neuronal alterations (Llorens-Martín et al., 2014, Gómez-Isla et al., 1997, Yang et al., 2018). Alzheimer's disease (AD) is the most ubiquitous cause of dementia, accounting for 50% to 70% of all cases of dementia (Winblad et al., 2016). The neuropathology of AD includes the presence of plaques of amyloid-beta (Aβ) peptides and intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated forms of microtubule-associated tau protein in the neurons (Hyman et al., 2012). Aβ is implicated as a central pathogenic event in AD, due to impaired clearance rates in AD patients. In contrast, its production rates remain unaltered when compared with those in healthy controls (Mawuenyega et al., 2010). Due to these changes, synaptic degeneration and death of selected populations of nerve cells take place, being accompanied by pronounced inflammation of the affected brain regions (Martucci et al., 2014, Goedert, 2015).

Genome-wide association studies implicate innate immunity in susceptibility to Alzheimer's disease (AD), with over 30 genetic loci for AD identified, many related to immune response and microglia. Among these are CD33 - Cluster of Differentiation 33 (Bertram and Tanzi, 2008, Hollingworth et al., 2011, Naj et al., 2011) and TREM2 -Triggering Receptor Expressed on Myeloid cells 2 (Guerreiro et al., 2013, Jonsson et al., 2013) which are exclusively expressed in the brain by innate immune cells.

CD33 encodes for Cluster of Differentiation 33, a type I transmembrane protein belonging to the class of sialic acid-binding immunoglobulin-like lectins, which mediates the cell-cell interaction and inhibit normal functions of immune cells such as phagocytosis (Crocker et al., 2007). In the brain, CD33 is mainly expressed on microglial cells, and compelling evidence indicates that CD33 facilitates Aβ-related pathology in AD by impairing microglia-mediated Aβ clearance (Bradshaw et al., 2013b, Malpass, 2013). Increased CD33 expression by microglia appears to result in significantly reduced amyloid-beta (Aβ) peptide phagocytosis as microglia lacking CD33 were able to phagocytose

significantly greater amounts of Aβ peptide than microglia expressing CD33 at normal levels (Griciuc et al., 2013).

TREM2 encodes for the Triggering Receptor Expressed on Myeloid cells 2 (TREM2), a protein highly expressed on the surface of microglia in the brain (Neumann and Takahashi, 2007). It has been suggested that mutated TREM2 is less effective in lipid signaling and thus may induce microglial activation leading to neuroinflammation (Yeh et al., 2016). TREM2 has been ascribed a multitude of cellular functions, including regulation of phagocytosis, inhibition of inflammatory signaling, and promotion of cell survival (Colonna, 2003). Its function in phagocytosis is particularly relevant in AD as a mechanism for clearing of pathogenic proteins, such as Aβ, thereby leading to a reduction in Aβ pool. TREM2 is also important for the microglial response to amyloid pathology and impairment of TREM2 function may alter plaque morphology and increase plaque-associated toxicity (Ulrich et al., 2017). Moreover, data from several animal models show that TREM2 takes part in the microglia response to Aβ plaques (Ulrich et al., 2014, Ulland and Colonna, 2018). These results suggest that both TREM2 and CD33, which are involved in the innate immunological response, are important for AD development. Hence, it confirms the significant role of neuroinflammatory processes in the pathogenesis of AD.

Most studies on the effects of neuroinflammation on cognition have examined the effect on memory acquisition or consolidation. In contrast, there are relatively few reports on whether neuroinflammation affects memory retrieval. Therefore, this study reappraises the amyloid hypothesis by focusing on the impact of neuroinflammation in a beta-amyloid model of AD, how this possibly exacerbates the progression of the disease and the correlation between genes regulating neuroinflammation (CD33 and TREM2) with post-training recall.

2. Materials and methods

2.1 Animals

All protocols involved studies on male Sprague-Dawley rats and were approved by the University of KwaZulu-Natal Animal Care Ethics Committee (AREC/015/018D). The animals were housed under controlled conditions of temperature (25 ± 1°C) and luminosity (12/12 h light/dark cycle, with lights, turned on 7:00 a.m. and turned off 7:00 p.m.). Food and water were provided *ad libitum.* The animals were randomly divided into a vehicle group of rats (n = 40) that were infused with phosphate-buffered saline (PBS) and an $A\beta_{(1–42)}$ group (n = 40) that were infused with the neurotoxin $A\beta_{(1–42)}$ peptide. Each group was further sub-divided into four groups (Day 3 group: animals euthanized 3 days after infusion; Day 7 group: animals euthanized 7 days after infusion; Day 10 group: animals euthanized 10 days after infusion, and Day 14 group: animals euthanized 14 days after infusion) (n = 10/group). Before all procedures, animals were allocated to the experimental room for habituation, and all efforts were made

to minimize pain, suffering, or animal discomfort. All behavioral experiments were conducted in the mornings, beginning at 8:00 a.m.

2.2 $A\beta_{(1–42)}$ lesion of CA1 hippocampal subregion (Neuroinflammatory model of AD)

The relevance of using intrahippocampal $A\beta_{(1–42)}$ as an animal model of AD has been reviewed by (McLarnon and Ryu, 2008, Shallie et al., 2020), and overall procedures employed in the use of this model are detailed by (Jean et al., 2015). After anesthesia with ketamine/xylazine cocktail at 100 mg/kg ketamine and 5 mg/kg xylazine by injecting intraperitoneally (IP), the depth of anesthesia was confirmed by the loss of toe pinch reflex. The animals were subjected to stereotaxic surgery for $A\beta_{(1–42)}$ peptide (5 μg/5 μl) or control 0.01M phosphate-buffered saline (PBS) infusion bilaterally into dorsal hippocampus subregion CA1 from bregma (AP: −4.8 mm; ML: ±3.4 mm and DV: −3.0). The coordinates were chosen according to a rat brain atlas (Paxinos and Watson, 2006), and microinjections were performed using a 10 μL microsyringe (Hamilton Company, Reno, NV, USA) connected to a 26-gauge steel needle into the brain sites. Temgesic was administered subcutaneously for pain relief.

2.3 Drugs and Reagents

$A\beta_{(1–42)}$ peptide was purchased from DLD Scientific (Durban North, South Africa). Ketamine, xylazine, and temgesic were obtained from Sigma (ST. Louis MO, USA). Real-time PCR kits were purchased from Bio-Rad Laboratories (CA, USA).

2.4. Assessment of fear learning and memory

The behavioral paradigm used to assess fear learning and memory in this study is the contextual and cued fear conditioning test (Crawley, 2007, Fanselow and Poulos, 2005, LeDoux, 2000). In this test, animals are exposed to a pairing of an auditory cue (neutral or conditioned stimulus - CS) with an electric foot shock (aversive or unconditioned stimulus - US). They respond to the fear-producing stimulus by displaying freezing behavior, measured as an index of associative fear learning and memory (Maren, 2001, Fanselow, 2000). The contextual and cued Fear Conditioning test was performed as described by (Shoji et al., 2014) in a 26 × 32 × 21 cm operant chamber. In this study, the chamber was equipped with a speaker and a stainless-steel rod floor through which a foot shock was administered.

On day 1, training consisted of placing the rat in a conditioning chamber, and the rats were accorded free access in the chamber for 3 mins. After that, the auditory cue (tone) was presented as a CS for 30 sec, and a (1.0 mA) foot shock was given to the rats as a US during the last 2 sec of the sound. The presentation of the CS-US pairing was repeated three times per session (120, 240, and 360 sec after the beginning of the conditioning) to strengthen the association. The rats were left in the chamber for a length of time (90 sec) after the last presentation to further establish the association between the context

of the chamber and the aversive experience. Context test was measured 24 hours after conditioning (regarded as day 2a, see figure 1); the rats were returned to the same conditioning chamber and allowed to explore the chamber for 3 mins freely without CS and US presentations. They were scored for freezing behavior to measure contextually conditioned fear. The cued test was conducted on the same day (regarded as day 2b, see figure 1) as the context test. In this test, rats were placed into another testing chamber with different properties, providing a new context that was not related to the conditioning chamber for another 3 min. At the end of the initial 3 min, the auditory cue that was presented at the time of conditioning was given to rats for 3 min in the novel context environment. In this protocol, the cued test was performed a few hours after the context test. Rats were given free access in the chamber for 360 sec. During this first 3 min, neither a CS nor US was presented, and after that, a CS (a 55 dB white noise) was presented for the last 3 min. Rats (n = 10/ group) were trained and tested for two consecutive days before lesioning and post-training recall was assessed for all groups (see figure 1).

2.5. Euthanization and Neurochemical Analysis

Animals were euthanized via deep anaesthesia using the halothane chamber. The excised brain tissues were immediately placed in frozen 0.9% saline slush before the hippocampus was carefully dissected out and quickly frozen in a bio-freezer at -80°C.

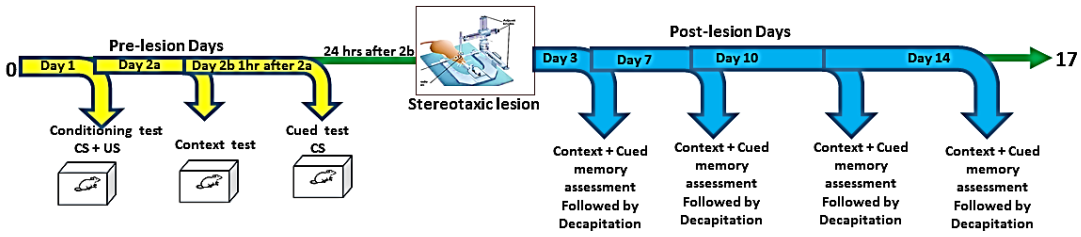

Figure 1. Experimental timeline.

2.6. Real-time quantitative PCR (qPCR) analysis for CD33 and TREM2 mRNA levels

Hippocampal tissue was suspended in lysis buffer at a ratio of 1:6, the samples were homogenized using a Tissue Sonicator (CML-4, Fisher, USA), and total RNA isolation was carried out as per manufacturer's protocol (ZR RNA MiniPrep^{TM}, USA). A NanoDrop Spectrophotometer (A260: A280 ratio), was used to determine the quality and concentration of the total RNA, purity of 1.7–2.1 recommended for use in the construction of cDNA was maintained. Total RNA was reverse-transcribed into cDNA using the iScript^{TM} cDNA synthesis kit and run in duplicate in a 20 µl reaction volume in a 96-well plate format, containing 500 nM of each oligonucleotide primer and SYBR Green PCR Master Mix (Bio-Rad). The primers (Table 1) were synthesized by Inqaba Biotech (Pretoria, South Africa),

with Gapdh serving as the reference gene. Primer sequences were reconstituted in RNA nuclease-free water according to the manufacturer's report and were added to a master mix comprising of SYBR green dye and nuclease-free water. PCR was performed using Lightcycler 96 consisting of denaturation at 95°C for 10 seconds, and then 20 seconds of primer annealing at 60°C (for TREM2), 60°C (for CD33) and 60°C (for Gapdh), followed by final elongation at 72°C for 20 seconds. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to normalize the expression of the target (Guo et al., 2013). We ensured that all qPCR reactions produced a single peak with the melt curve analysis. Relative expression levels were calculated after normalization against the reference gene GAPDH using $2^{-\Delta\Delta CT}$ method, and results were expressed as 2−(gene of interest mean Ct value − control gene mean Ct value) for the qRT-PCR experiments (Livak and Schmittgen, 2001). Our qPCR analyses followed the most recommended criteria for minimal information for publication of quantitative real-time PCR experiments (MIQE) (Bustin et al., 2009).

Gene	Forward	Reverse
TREM2	5′-AAGATGCTGGAGACCTCTGG-3′	5′-GGATGCTGGCTGTAAGAAGC-3′
CD33	5′-ATGAGAGAGCTGGTCCTGGT-3′	5′-CCCATGTGCACTGACAGCTT-3′
GAPDH	5′-TGTGAACGGATTTGGCCGTA-3′	5′-ATGAAGGGGTCGTTGATGGC-3′

Table 1. Nucleotide sequence of primers used for real-time PCR.

2.7. Statistical Analysis

The data were analysed using the software program GraphPad Prism (version 7.0, San Diego, California, USA). The Shapiro-Wilk test was used to test for normality of distribution and where data met requirements, parametric test was used. The main factors in each analysis included lesion and day. Two-way analysis of variance (ANOVA) was used. A p-value < 0.05 was considered significant in all analysis. Significant main effects were followed by Tukey's post hoc test.

3. Results

3.1. $A\beta_{(1–42)}$ lesion of CA1 reduces contextual fear memory

Contextual fear memory was assessed pre-lesion and post-lesion with $A\beta_{(1–42)}$ on days 3, 7, 10 and 14. A significant interaction ($F(3,72) = 5.894$, $p = 0.0016$) was observed between lesion and day, the post-lesioned $A\beta_{(1–42)}$ group of rats showed reduced freezing behavior relative to the pre-lesioned group when placed in the context, as measured by the mean percent of time freezing across all three minutes (main effect of lesion : ($F(1,72) = 82.22$, $p < 0.0001$). Freezing also varied significantly as a function of day across the post-lesion days in the context (main effect of day: ($F(3,72) = 8.806$, $p < 0.0001$) (Figure 2).

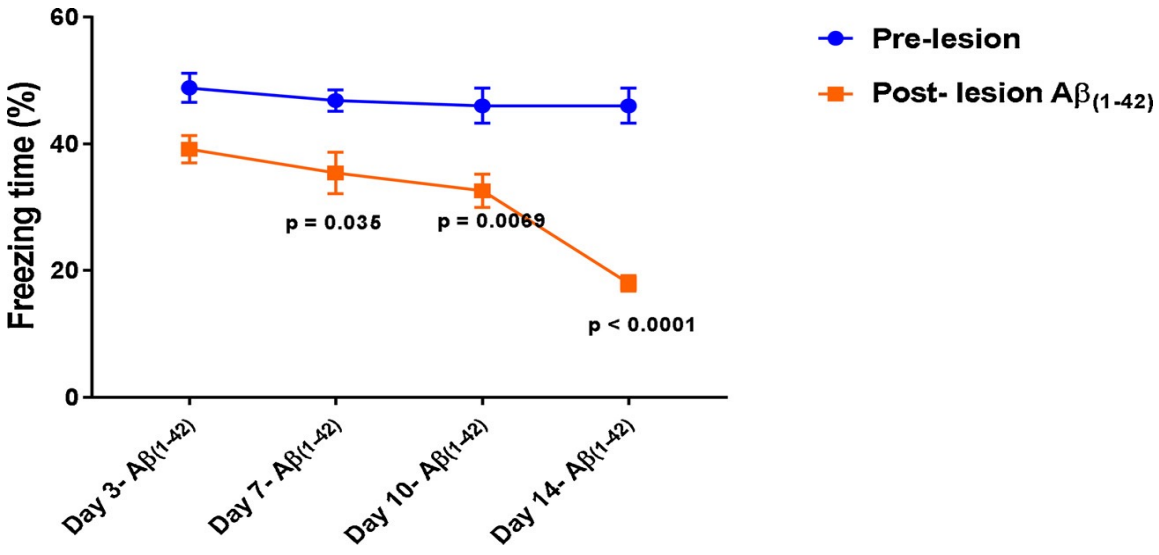

Figure 2. Mean percent freezing time in pre and post-lesion $A\beta_{(1–42)}$ contextual fear test.

3.2. $A\beta_{(1–42)}$ lesion of CA1 unalters cued fear memory

Cued fear memory was assessed on the same day as the contextual fear memory, pre-lesion and post-lesion with $A\beta_{(1–42)}$ on days 3,7,10 and 14. No significant difference in freezing was detected between the $A\beta_{(1–42)}$ post-lesioned group and the pre-lesion group of rats. (main effect of lesion: $F(1,72) = 0.02609$, $p = 0.8724$); main effect of day: $F(3,72) = 0.7217$, $p = 0.5439$), nor an interaction ($F(3,72) = 0.2348$, $p = 0.8717$) was detected, therefore *post hoc* tests within each group were not performed (Figure 3).

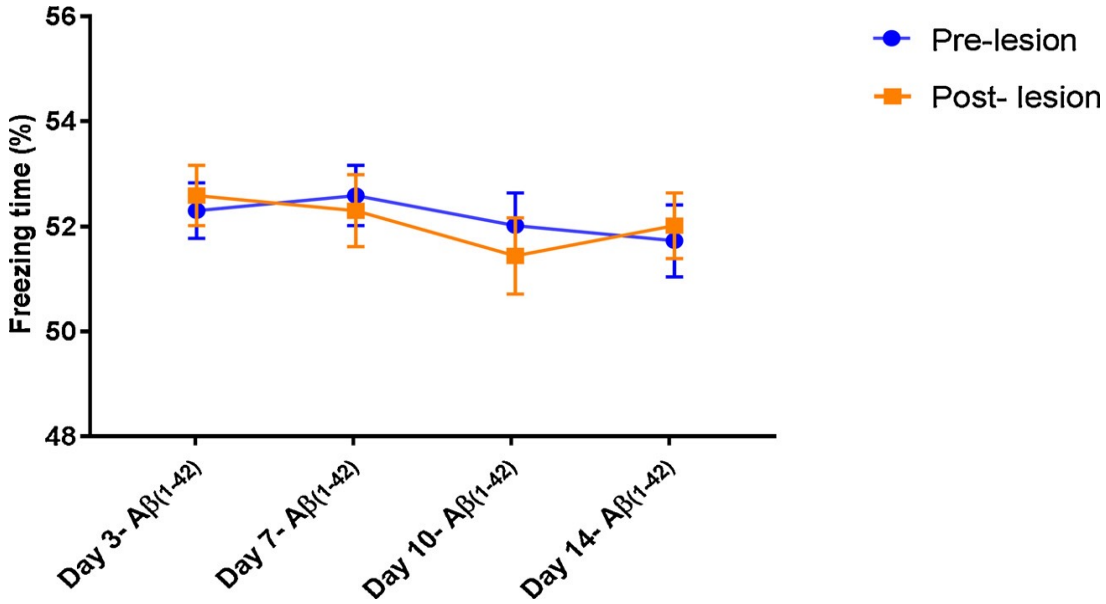

Figure 3. Mean percent freezing time in pre and post-lesion $A\beta_{(1–42)}$ cued fear test.

3.3. Hippocampus modulates contextual but not cued memory in $A\beta_{(1–42)}$ rat model of AD

A positive significant (F (3,72) =9.143, $p < 0.0001$) interaction was observed between the vehicle-treated and $A\beta_{(1–42)}$ lesioned rats in the contextual but not in the cued tests. In addition, we observed a main effect of lesion (F (1,72) = 10.15, $p < 0.0001$) was observed between the vehicle-treated group and the post-lesion $A\beta_{(1–42)}$ group in the context test but not in the cued test, furthermore, a significant day effect (F (3,72) = 105.5, $p < 0.0001$) between the vehicle-treated and the post-lesion $A\beta_{(1–42)}$ group in the context test was observed (Figure 4).

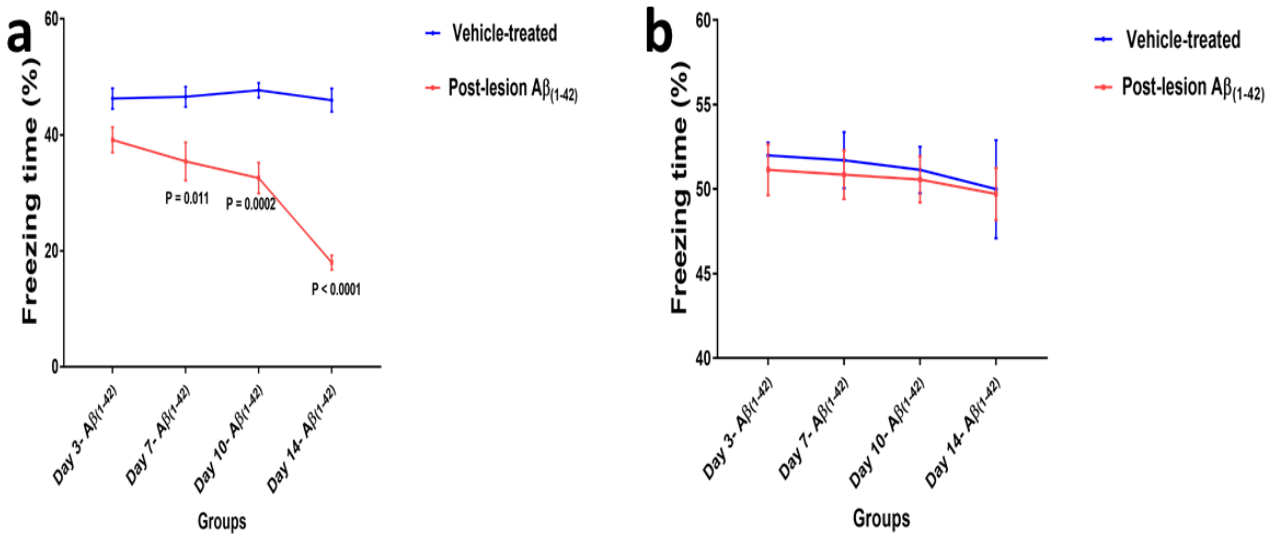

Figure 4. Mean percent freezing time in (a) contextual and (b) cued fear test across post-lesion days 3, 7, 10 and 14 for the vehicle-treated and $A\beta_{(1–42)}$ lesioned groups.

3.4. Upregulated mRNA CD33 and TREM2 expression

Significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on relative CD33 gene expression was observed [F (4, 45) = 90.79, $p < 0.0001$], with upregulated expressions on post-lesion day 3 ($p < 0.0001$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the control(day 14 PBS-lesioned group) group. Significant downregulated day effect was observed within the $A\beta_{(1–42)}$ lesioned groups at post-lesioned day 7 vs day 14 ($p = 0.0483$) and day 3 vs. day 14 ($p = 0.0083$) (Figure 5A). Significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on relative TREM2 gene expression [F (4, 45) = 39.5, $p < 0.0025$] with upregulated expressions on post-lesion day 3 ($p = 0.0002$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the vehicle injected group was observed. Significant upregulated day effect was also observed within the $A\beta_{(1–42)}$ lesioned groups at post-lesioned day 3 vs day 10 ($p < 0.0001$); day 3 vs. day 14 ($p < 0.0001$); day 7 vs day 10 ($p = 0.0004$) and day 7 vs. day 14 ($p < 0.0001$) (Figure 5B).

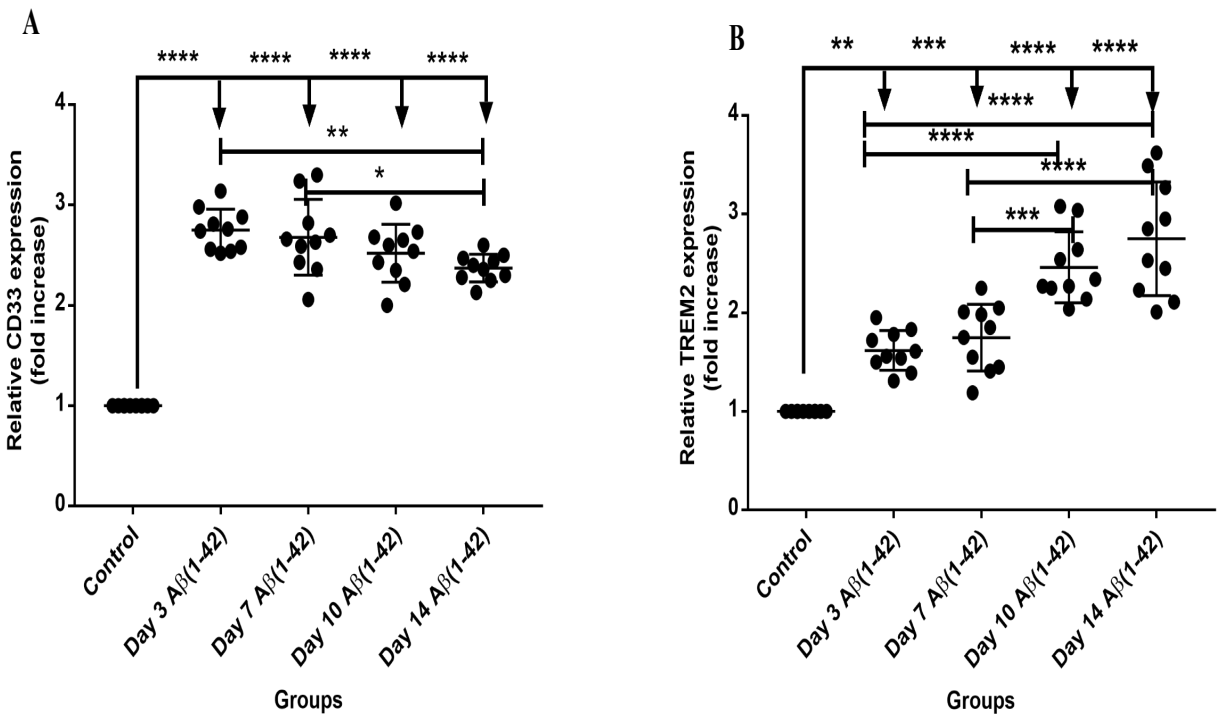

Figure 5(A). Relative CD33 **(B)** TREM2 expression in the hippocampus of $A\beta_{(1-42)}$ lesioned rats across different post-lesion days. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (two-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 10/ group)

3.5. Correlative expressions of CD33 and TREM2 genes

Strong negative correlations were observed between the relative expressions of CD33 and TREM2 genes on post-lesion day 3 ($r = -0.9664$, $p < 0.0001$) (Fig. 6a); post-lesion day 7 ($r = -0.8777$, $p = 0.0008$) (Fig. 6b); post-lesion day 10 ($r = -0.9060$, $p = 0.0003$) (Fig. 6c) and post-lesion day 14 ($r = -0.8649$, $p = 0.0012$) (Fig. 6d) in the $A\beta_{(1-42)}$ lesioned rat model of AD (Figure 6).

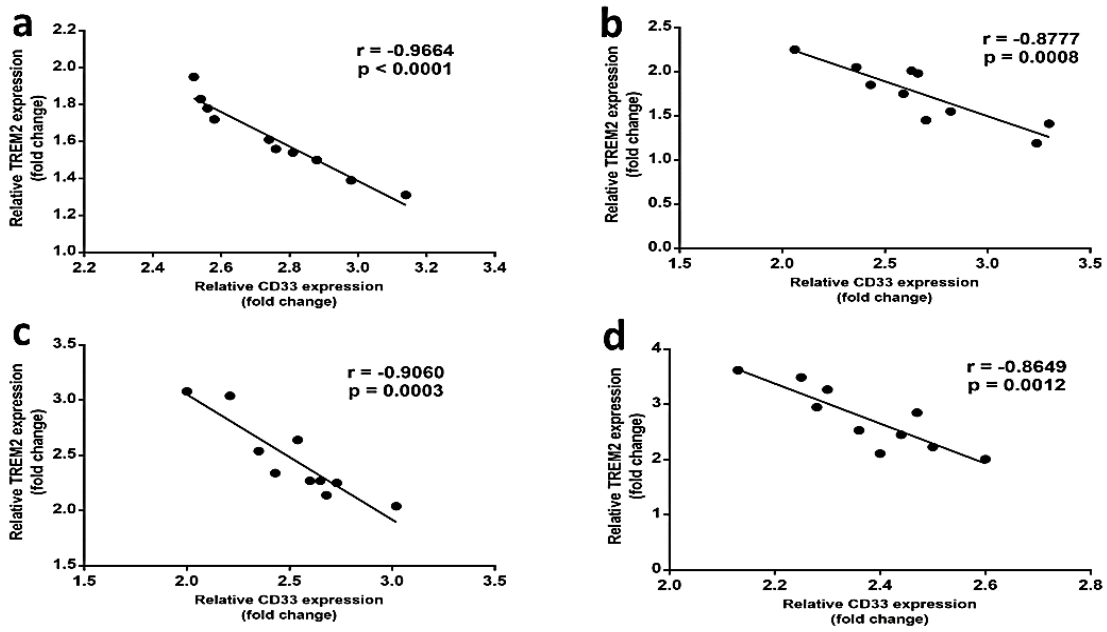

Figure 6. XY scatter plots of the relative expressions of CD33 and TREM2 on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1–42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 10) in each group.

3.6. Correlation between CD33 mRNA expression with contextual freezing time.

Strong negative correlations were observed between the relative expressions of CD33 mRNA and contextual freezing time on post-lesion day 3 ($r = -0.9285$, $p < 0.0001$) (Fig. 7a); post-lesion day 7 ($r = -0.9047$, $p < 0.0003$) (Fig. 7b); post-lesion day 10 ($r = -0.9328$, $p < 0.0001$) (Fig. 7c) and post-lesion day 14 ($r = -0.9339$, $p < 0.0001$) (Fig. 7d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Figure 7).

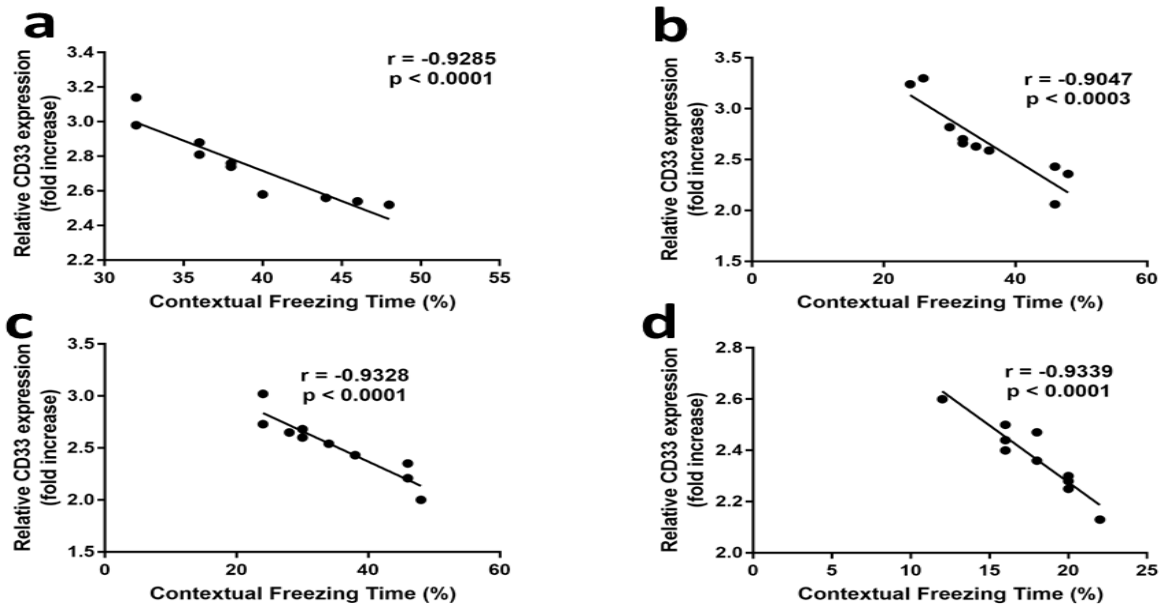

Figure 7. XY scatter plots of the relative expressions of CD33 and contextual freezing time on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1–42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 10) in each group.

3.7. Correlation between TREM2 mRNA expression with contextual freezing time.

Strong positive correlation was observed between the relative expression TREM2 mRNA and contextual freezing time on post-lesion day 3 ($r = 0.9829$, $p < 0.0001$) (Fig. 8a); post-lesion day 7 ($r = 0.9093$, $p < 0.0003$) (Fig. 8b); post-lesion day 10 ($r = 0.8550$, $p < 0.0016$) (Fig. 8c) and post-lesion day 14 ($r = 0.9046$, $p < 0.0003$) (Fig. 8d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Figure 8).

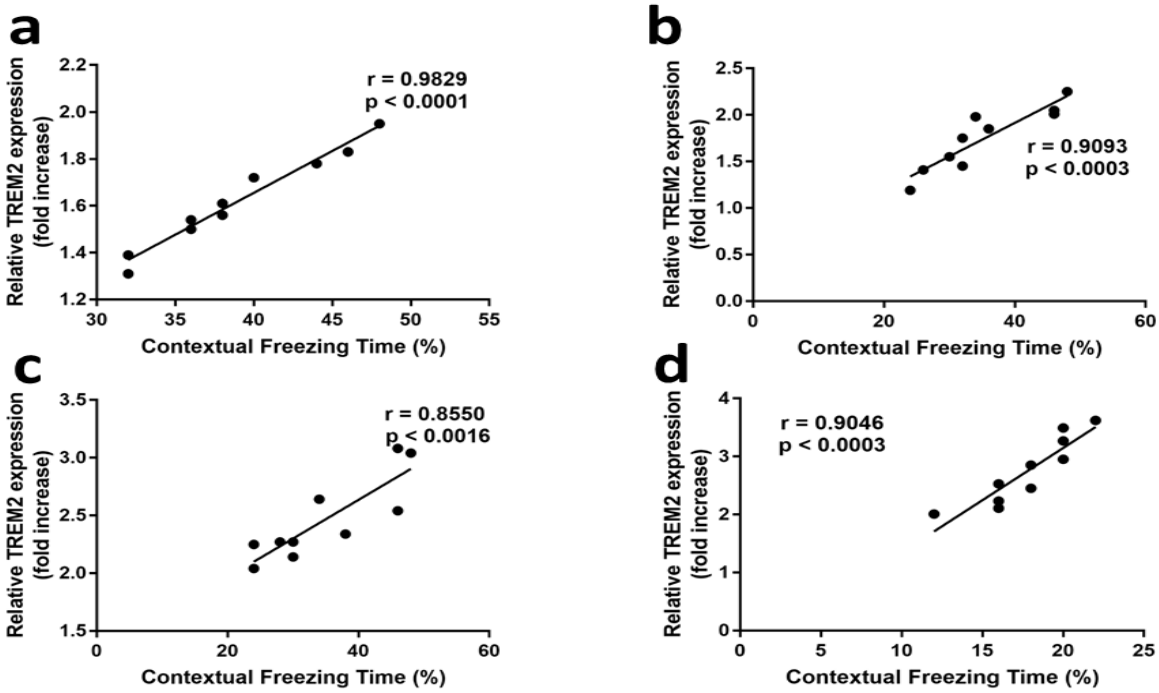

Figure 8. XY scatter plots of the relative expressions of TREM2 and contextual freezing time on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1–42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats ($n = 10$) in each group.

4. Discussion

In this study, we assessed the impact of $A\beta_{(1–42)}$ lesion of CA1 neuronal activity, as a model of AD, on fear memory after acquisition. We further investigated the expression of some microglial genes regulating neuroinflammation, such as CD33 and TREM2 and correlated these expressions to post-training recall. Our observed data provide credible reinforcement for a compelling interaction between immune system activation and neural circuit activity. Notably, this occurred when levels of IL-1β, TNF-α and IL-6 were elevated in the dorsal hippocampus of $A\beta_{(1–42)}$ lesioned rats as opposed to the vehicle infused rats (Shallie et al., 2020), a structure highly mandatory for behavioral context discrimination (Czerniawski and Guzowski, 2014).

We observed a difference in the behavioral outcomes after CA1 lesion at all post-lesion intervals, observed as decreased freezing in context fear memory but not in cued fear memory. This decreased freezing indicates that the $A\beta_{(1–42)}$ lesioned rats have impaired memory for the context test but not the cued test. These results are indicative of the specified actions of CA1 as reported by previous studies, where context fear conditioning has been shown to require the activity of neurons in the hippocampus, specifically in CA1 region, for context encoding (Goshen et al., 2011, Muller et al., 1997, Zelikowsky et al., 2014), while cued fear conditioning requires activity from auditory regions and amygdala

structures but do not usually require the hippocampus (Phillips and LeDoux, 1992, Muller et al., 1997). These latter studies are suggestive reasons for the unaltered cued fear memory in $A\beta_{(1–42)}$ lesioned rats as observed in this study, as both vehicle-infused and $A\beta_{(1–42)}$ lesioned rats exhibited similar levels of memory in the cue test. Previous studies have also shown that neurons in the dorsal CA1 region of rats have strong spatial properties, with well-defined place fields capable of encoding a context (Jung et al., 1994, Moser et al., 2008). Hence, lesions to or inhibition of the dorsal hippocampus impair context fear memory (Hunsaker and Kesner, 2008, Wiltgen et al., 2006), as observed in the dorsal CA1 of this model.

$A\beta_{(1–42)}$ lesion of the CA1 subregion of the hippocampus is known to elicit neuroinflammatory changes as a result of prolonged activation of microglia cells, causing subsequent secretion of inflammatory cytokines (Shallie et al., 2020). Our results showed that mRNA levels of TREM2 and CD33 were increased at all intervals in the hippocampus of $A\beta_{(1–42)}$ lesioned rats relative to the respective vehicle-infused rats. We observed a significant 2.8 fold increase of CD33 expression in the day 3 $A\beta_{(1–42)}$ lesioned group of rats relative to control, with a slight decrease over the subsequent post-lesion intervals, probably due to the counteracting increased expression of TREM2. The increased mRNA CD33 expression in $A\beta_{(1–42)}$ lesioned rats denotes phagocytotic inhibition, indicating that there is an impaired microglial mediated Aβ clearance. Our results of increased expression of CD33 is consistent with the previous study by (Griciuc et al., 2013), which showed increased mRNA levels of CD33 in AD patients. Expression of TREM2, on the other hand, showed a progressive increase across the post-lesion intervals with an initial significant 1.8 fold increase in the $A\beta_{(1–42)}$ lesioned rats relative to the control. TREM2 expression increase in the $A\beta_{(1–42)}$ lesioned rats indicates a heightened state of microgliosis, as microglial cells are over-activated to facilitate phagocytosis. Our results suggest that TREM2 mediates Aβ-induced microglial response, as supported by previous study by (Zhao et al., 2018). Although mRNA levels may not be an accurate estimate of protein level since gene expression is controlled at different stages and in many different ways. However, the ability to quantify the level at which a particular gene is expressed provides much valuable information as reliance on mRNA measurements can be traced both to the relative ease of availability of mRNA compared to protein data (Vogel and Marcotte, 2012, Maier et al., 2009). Moreover, the mRNA expression levels can be used to deduce the functionality of genes with the implicit assumption that differentially expressed mRNAs impact their respective experimental conditions via differences in proteins.

Correlative expression of both genes shows an inverse relationship, depicting an interactive platform and a possible explanation to the crosstalk between these genes, as reported by Griciuc et al., 2019. The correlative mRNA expression of the genes with contextual fear freezing time reflects the role of these genes in memory. The negative correlation of CD33 mRNA expression level with contextual freezing time indicates an impaired memory as mRNA CD33 expression increases. A study by Griciuc *et al*., in

which the T allele of SNP rs3865444, which led to the reduction of CD33 levels in the brain, was found to be linked with decreased amyloid plaque burdens in the brain cortex of AD patients supports our finding (Griciuc et al., 2013). Further corroborating this finding, Bradshaw and colleagues showed that the C allele of SNP rs3865444, which caused the elevation in CD33 levels, was associated with a higher burden of fibrillar amyloid in older asymptomatic individuals (Bradshaw et al., 2013a). Animal models of AD have also provided more direct evidence on the association between CD33 and Aβ pathology, as amyloid precursor protein/presenilin 1 (APP/PS1) transgenic mice lacking CD33 exhibited significant lower Aβ levels as well as reduced amyloid plaque burden in the brain (Griciuc et al., 2013). These observations indicated a pathogenic role of CD33 in the facilitation of Aβ pathology. More so, the deletion of CD33 in APP/PS1 transgenic mice did not alter the APP processing or the levels of pro-inflammatory cytokines in the brain, implying that CD33 contributed to Aβ pathology by interfering with Aβ clearance rather than promoting its generation (Griciuc et al., 2013).

The positive correlation between TREM2 mRNA expression level and contextual freezing time indicates that memory is enhanced as TREM2 mRNA level increases since increased freezing time interprets to greater learning ability, as the better the memory, the more time the animal spends in freezing behavior. Studies supporting our results by Jiang *et al*., using APPswe/PS1dE9 mice compared to wildtype resulted in spatial learning and memory deficit when subjected to the Morris water maze test, were reversed with subsequently observed improved performance on insertion of TREM2 vector into these mice (Jiang et al., 2014, Jiang et al., 2016). Lee *et al*., also validate the role of TREM2 in memory using the 5XFAD mice, which demonstrated impaired contextual memory as opposed to 5XFAD/TREM2 OE and APPPS1/TREM2 OE which had similar performance to the wildtype, indicating that TREM2 overexpression improves cognition in amyloid mouse models since TREM2 overexpression alone did not affect performance (Lee et al., 2018).

5. Conclusion

Our findings lend support to the knowledge that neuroinflammatory response resulting from $A\beta_{(1–42)}$ lesion CA1 impacts both neural circuit activity and expression of microglial genes regulation neuroinflammation, indicating a dynamic interaction between the immune and nervous systems. This integrative approach can be used to query the role of neuroinflammation in memory processes, and, importantly, to identify the possibility of blocking the immune response in the brain to restore cell network activity and cognitive function.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Bartsch, T., et al., *CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness.* Proceedings of the National Academy of Sciences, 2011. **108**(42): p. 17562-17567.
2. Amaral, D. and P. Lavenex, *Hippocampal neuroanatomy.* 2007.
3. Barrientos, S.A. and V. Tiznado, *Hippocampal CA1 Subregion as a Context Decoder.* Journal of Neuroscience, 2016. **36**(25): p. 6602-6604.
4. Van Strien, N., N. Cappaert, and M. Witter, *The anatomy of memory: an interactive overview of the parahippocampal–hippocampal network.* Nature Reviews Neuroscience, 2009. **10**(4): p. 272.
5. Jarsky, T., et al., *Conditional dendritic spike propagation following distal synaptic activation of hippocampal CA1 pyramidal neurons.* Nature neuroscience, 2005. **8**(12): p. 1667.
6. Bittner, K.C., et al., *Conjunctive input processing drives feature selectivity in hippocampal CA1 neurons.* Nature neuroscience, 2015. **18**(8): p. 1133.
7. Llorens-Martín, M., et al., *Selective alterations of neurons and circuits related to early memory loss in Alzheimer's disease.* Frontiers in neuroanatomy, 2014. **8**: p. 38.
8. Gómez-Isla, T., et al., *Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease.* Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 1997. **41**(1): p. 17-24.
9. Yang, X., et al., *A novel mechanism of memory loss in Alzheimer's disease mice via the degeneration of entorhinal–CA1 synapses.* Molecular psychiatry, 2018. **23**(2): p. 199.
10. Winblad, B., et al., *Defeating Alzheimer's disease and other dementias: a priority for European science and society.* The Lancet Neurology, 2016. **15**(5): p. 455-532.
11. Hyman, B.T., et al., *National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease.* Alzheimer's & dementia, 2012. **8**(1): p. 1-13.
12. Mawuenyega, K.G., et al., *Decreased clearance of CNS β-amyloid in Alzheimer's disease.* Science, 2010. **330**(6012): p. 1774-1774.
13. Martucci, M., et al., *Cerebral amyloid angiopathy-related inflammation: imaging findings and clinical outcome.* Neuroradiology, 2014. **56**(4): p. 283-289.
14. Goedert, M., *Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein.* Science, 2015. **349**(6248): p. 1255555.
15. Bertram, L. and R.E. Tanzi, *Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses.* Nature Reviews Neuroscience, 2008. **9**(10): p. 768.
16. Hollingworth, P., et al., *Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease.* Nature genetics, 2011. **43**(5): p. 429.
17. Naj, A.C., et al., *Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease.* Nature genetics, 2011. **43**(5): p. 436.
18. Guerreiro, R., et al., *TREM2 variants in Alzheimer's disease.* New England Journal of Medicine, 2013. **368**(2): p. 117-127.
19. Jonsson, T., et al., *Variant of TREM2 associated with the risk of Alzheimer's disease.* New England Journal of Medicine, 2013. **368**(2): p. 107-116.
20. Crocker, P.R., J.C. Paulson, and A. Varki, *Siglecs and their roles in the immune system.* Nature Reviews Immunology, 2007. **7**(4): p. 255.
21. Bradshaw, E.M., et al., *CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology.* Nature neuroscience, 2013. **16**(7): p. 848.

22. Malpass, K., *Alzheimer disease: functional dissection of CD33 locus implicates innate immune response in Alzheimer disease pathology.* Nature Reviews Neurology, 2013. **9**(7): p. 360.
23. Griciuc, A., et al., *Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta.* Neuron, 2013. **78**(4): p. 631-643.
24. Neumann, H. and K. Takahashi, *Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis.* Journal of neuroimmunology, 2007. **184**(1-2): p. 92-99.
25. Yeh, F.L., et al., *TREM2 binds to apolipoproteins, including APOE and CLU/APOJ, and thereby facilitates uptake of amyloid-beta by microglia.* Neuron, 2016. **91**(2): p. 328-340.
26. Colonna, M., *TREMs in the immune system and beyond.* Nature Reviews Immunology, 2003. **3**(6): p. 445-453.
27. Ulrich, J.D., et al., *Elucidating the role of TREM2 in Alzheimer's disease.* Neuron, 2017. **94**(2): p. 237-248.
28. Ulrich, J.D., et al., *Altered microglial response to Aβ plaques in APPPS1-21 mice heterozygous for TREM2.* Molecular neurodegeneration, 2014. **9**(1): p. 20.
29. Ulland, T.K. and M. Colonna, *TREM2—a key player in microglial biology and Alzheimer disease.* Nature Reviews Neurology, 2018. **14**(11): p. 667-675.
30. McLarnon, J.G. and J.K. Ryu, *Relevance of Aβ 1-42 intrahippocampal injection as an animal model of inflamed Alzheimer's disease brain.* Current Alzheimer Research, 2008. **5**(5): p. 475-480.
31. Shallie, O.F., E. Dalle, and M.V. Mabandla, *Memory decline correlates with increased plasma cytokines in amyloid-beta (1–42) rat model of Alzheimer's disease.* Neurobiology of Learning and Memory, 2020. **169**: p. 107187.
32. Jean, Y.Y., et al., *Stereotaxic infusion of oligomeric amyloid-beta into the mouse hippocampus.* JoVE (Journal of Visualized Experiments), 2015(100): p. e52805.
33. Paxinos, G. and C. Watson, *The rat brain in stereotaxic coordinates: hard cover edition*. 2006: Elsevier.
34. Crawley, J.N., *What's wrong with my mouse?: behavioral phenotyping of transgenic and knockout mice*. 2007: John Wiley & Sons.
35. Fanselow, M.S. and A.M. Poulos, *The neuroscience of mammalian associative learning.* Annu. Rev. Psychol., 2005. **56**: p. 207-234.
36. LeDoux, J.E., *Emotion circuits in the brain.* Annual review of neuroscience, 2000. **23**(1): p. 155-184.
37. Maren, S., *Neurobiology of Pavlovian fear conditioning.* Annual review of neuroscience, 2001. **24**(1): p. 897-931.
38. Fanselow, M.S., *Contextual fear, gestalt memories, and the hippocampus.* Behavioural brain research, 2000. **110**(1-2): p. 73-81.
39. Shoji, H., et al., *Contextual and cued fear conditioning test using a video analyzing system in mice.* JoVE (Journal of Visualized Experiments), 2014(85): p. e50871.
40. Guo, C., S. Liu, and M.-Z. Sun, *Novel insight into the role of GAPDH playing in tumor.* Clinical and Translational Oncology, 2013. **15**(3): p. 167-172.
41. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method.* methods, 2001. **25**(4): p. 402-408.
42. Bustin, S.A., et al., *The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments.* Clinical chemistry, 2009. **55**(4): p. 611-622.
43. Czerniawski, J. and J.F. Guzowski, *Acute neuroinflammation impairs context discrimination memory and disrupts pattern separation processes in hippocampus.* Journal of Neuroscience, 2014. **34**(37): p. 12470-12480.
44. Goshen, I., et al., *Dynamics of retrieval strategies for remote memories.* Cell, 2011. **147**(3): p. 678-689.
45. Muller, J., et al., *Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli.* Behavioral neuroscience, 1997. **111**(4): p. 683.

46. Zelikowsky, M., et al., *Neuronal ensembles in amygdala, hippocampus, and prefrontal cortex track differential components of contextual fear.* Journal of neuroscience, 2014. **34**(25): p. 8462-8466.
47. Phillips, R. and J. LeDoux, *Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning.* Behavioral neuroscience, 1992. **106**(2): p. 274.
48. Jung, M.W., S.I. Wiener, and B.L. McNaughton, *Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat.* Journal of Neuroscience, 1994. **14**(12): p. 7347-7356.
49. Moser, E.I., E. Kropff, and M.-B. Moser, *Place cells, grid cells, and the brain's spatial representation system.* Annu. Rev. Neurosci., 2008. **31**: p. 69-89.
50. Hunsaker, M.R. and R.P. Kesner, *Dissociations across the dorsal–ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear.* Neurobiology of learning and memory, 2008. **89**(1): p. 61-69.
51. Wiltgen, B.J., et al., *Context fear learning in the absence of the hippocampus.* Journal of Neuroscience, 2006. **26**(20): p. 5484-5491.
52. Zhao, Y., et al., *TREM2 is a receptor for β-amyloid that mediates microglial function.* Neuron, 2018. **97**(5): p. 1023-1031. e7.
53. Vogel, C. and E.M. Marcotte, *Insights into the regulation of protein abundance from proteomic and transcriptomic analyses.* Nature reviews genetics, 2012. **13**(4): p. 227-232.
54. Maier, T., M. Güell, and L. Serrano, *Correlation of mRNA and protein in complex biological samples.* FEBS letters, 2009. **583**(24): p. 3966-3973.
55. Bradshaw, E.M., et al., *CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology.* Nature neuroscience, 2013. **16**(7): p. 848-850.
56. Jiang, T., et al., *Upregulation of TREM2 ameliorates neuropathology and rescues spatial cognitive impairment in a transgenic mouse model of Alzheimer's disease.* Neuropsychopharmacology, 2014. **39**(13): p. 2949-2962.
57. Jiang, T., et al., *TREM2 modifies microglial phenotype and provides neuroprotection in P301S tau transgenic mice.* Neuropharmacology, 2016. **105**: p. 196-206.
58. Lee, C.D., et al., *Elevated TREM2 gene dosage reprograms microglia responsivity and ameliorates pathological phenotypes in Alzheimer's disease models.* Neuron, 2018. **97**(5): p. 1032-1048. e5.

PROLOGUE

Chapter 5 investigates the feasibility of microRNA-107 as a plasma biomarker for AD by correlating its expression with BACE1 levels in the brain tissue. Formats used in this chapter are according to the journal specifications. This manuscript has been accepted by the journal of Neurotoxicity Research.

CHAPTER 5

MicroRNA-107 as a possible plasma biomarker in an amyloid-beta (1-42) rat model of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia and one of the fastest-growing major disease burdens, yet there are no available treatments to alter the natural history of this disease. The barriers to effective therapies include the lack of a specific biomarker for the early detection of this disease before the onset of clinically apparent cognitive impairment. This study investigated the feasibility of microRNA-107 (miRNA107) as a plasma biomarker for AD by correlating its expression with Beta-site amyloid precursor protein cleaving enzyme 1(BACE1) levels in brain tissue. The BACE1 enzyme is the enzyme that initiates the generation of amyloid beta, the main component of amyloid plaques found in AD brains which destroys nerve cells. Consequently, BACE1 is an attractive target in AD prevention and treatment. Male Sprague-Dawley rats were used in this study and AD-like symptoms induced via intrahippocampal injection of amyloid-beta 1-42 ($A\beta_{(1–42)}$) while phosphate-buffered saline was administered as the vehicle. The Morris water maze test was used to evaluate spatial learning and memory. BACE1 expression in the CA3 region of the hippocampus and plasma miRNA were measured using immunohistochemistry and real-time PCR techniques respectively. Our results show impaired memory in the $A\beta_{(1–42)}$ model of AD, associated with the upregulated expression of BACE1, which inversely correlated progressively with the downregulated expression of miRNA107 in the plasma. This bi-directional interdependence between BACE1 and miRNA107 makes miRNA107 a potential biomarker for the early diagnosis of Alzheimer's diseases.

Keyword: Biomarkers; Alzheimer's disease; MicroRNAs; Amyloid-beta; BACE1; Hippocampus

1. Introduction

Alzheimer's disease (AD) is the most common form of neurodegenerative disorder leading to dementia (Ulep et al., 2018). The disease currently affects 75 million people worldwide and predicted to increase to 135 million people by 2050 (Shi et al., 2018, Leidinger et al., 2013, Brookmeyer et al., 2007). The Alzheimer's Association and the National Institute on Aging, developed new diagnostic guidelines for Alzheimer's disease (Albert et al., 2013), containing an updated classification of the phases of Alzheimer's disease. These guidelines provide recommendations for the diagnosis of preclinical mild cognitive impairment, mild cognitive impairment, and Alzheimer's disease dementia and stress the lack of and urgent need for reliable biomarkers that can be used for detection of mild cognitive impairment and preclinical phases of Alzheimer's disease. Therefore, this prompts the urgent need for an ideal diagnostic biomarker. A good biomarker must be sensitive and specific, can identify the disease at a considerable time before the onset of symptoms, with adequate reliability and, low-cost, minimally invasive, and easy to be applied for mass screening (Dallé et al., 2020). All this provides preliminary evidence for the inclusion of plasma-based biomarkers.

MicroRNAs (miRNA) are small non-coding RNA, typically 22–23 nucleotides, that control gene expression by binding to the 3′-untranslated region (UTR) in messenger RNA (mRNA). Through this, they suppress translation or induce degradation of the target genes (Ha and Kim, 2014). Unlike mRNAs, miRNAs are stable enough in biological fluids, including serum, plasma, and CSF (Zhang et al., 2018). Many of them target genes directly involved in AD pathophysiologies such as presenilins, beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), amyloid precursor protein (APP) (Liu et al., 2014) and brain-derived neurotrophic factor (BDNF) (Croce et al., 2013, Keifer et al., 2015). Analysis of miRNAs in body fluids is a relatively simple procedure (Kalogianni et al., 2018) and a non-invasive approach. A comparison of miRNAs to conventional protein-based biomarkers of AD shows that the level of sensitivity achieved for miRNAs due to amplification by PCR is far superior to what is currently available for proteins (Kumar et al., 2017). Besides, the cost of miRNA quantification is far lower than that of established biomarkers, such as the positron emission tomography (PET) for molecular neuroimaging and the structural magnetic resonance imaging (MRI). These miRNAs secreted into the extracellular space are in micro-vesicle encapsulated form or exosomes (Liang et al., 2014, Turchinovich et al., 2013). The demonstration of miRNA alterations between AD patients and age-matched controls is of further support to this proposition (Goodall et al., 2013). For this purpose, many miRNAs appear to be promising.

MicroRNA107 is implicated in AD-related phenotypes in early phases of the disease (Wang et al., 2010, Nelson and Wang, 2010, Wang et al., 2008). MicroRNA107 is especially dysregulated in the brain as well as in blood of patients with AD, making it an ideal candidate biomarker (Wang et al., 2008, Wang

et al., 2015a, Leidinger et al., 2013). MicroRNA107 targets BACE1, an enzyme that cleaves the amyloid precursor protein (APP), creating the neurotoxic β-amyloid peptide ($A\beta_{(1–42)}$) (Stratman et al., Haniu et al., 2000, Vassar et al., 1999). BACE1 is the initiating and putatively rate-limiting enzyme in Aβ generation. While its inhibition would block the production of Aβ and prevent the development of Aβ-associated pathologies, overexpression of this enzyme has been shown to initiate or accelerate AD pathogenesis (Fukumoto et al., 2002, Li and Südhof, 2004). Studies have shown that the dysregulation of BACE1 appears to directly contribute to the pathogenesis of AD (Dominguez et al., 2004, Durham and Shepherd, 2006, Guo and Hobbs, 2006, John, 2006).

Currently, bio-fluid derived markers like miRNAs are being explored as possible biomarkers due to their stability and RNase resistant ability for pre-clinical AD diagnosis, because it is in these initial stages that modifying therapies are likely to have the greatest chance of impact. However, the clinical diagnostic application in the use of miRNA as biomarkers has not been evaluated thus far, in a way that validates the status as evident in the brain, thus providing a bi-directional translational approach.Therefore, this study aims to investigate the feasibility of miRNA107 as a possible plasma biomarker by correlating its expression with BACE1 levels in the brain tissue.

2. Materials and methods

2.1. Experimental design and animals

The experimental protocol for this study was approved by the Ethical Review Board of the University of KwaZulu-Natal (AREC/015/018D). A total of 80 male Sprague–Dawley rats weighing between 300 and 350 g (8/9 weeks of age) obtained from the Biomedical Resource Unit of the University of KwaZulu-Natal were used in this study (Figure 1). The animals had shelter under controlled temperature (21 ± 2 ° C) and humidity (55–60%), a light-controlled room with an alternating 12-hour light to 12-hour dark cycle, with free access to food and water. The animals were subjected to pre-lesion behavioral test (Morris water maze test) and then randomly divided into a vehicle group of rats (n = 40) that were stereotaxically infused with phosphate buffered saline and an $A\beta_{(1–42)}$ group of rats (n = 40) that were infused with the neurotoxin $A\beta_{(1–42)}$ peptide. Each group was further sub-divided into four groups (Day 3 group: animals euthanized 3 days after $A\beta_{(1–42)}$ or vehicle infusion; Day 7 group: animals euthanized 7 days after $A\beta_{(1–42)}$ or vehicle infusion; Day 10 group: animals euthanized 10 days after $A\beta_{(1–42)}$ or vehicle infusion, and Day 14 group: animals euthanized 14 days after $A\beta_{(1–42)}$ or vehicle infusion) (n = 10/group). At post-lesion day 3, 7, 10 and 14, the post-lesion behavioral test was conducted and blood was collected for analysis before whole brain excision. We set our sample size according to a previous study by (Shallie et al., 2020).

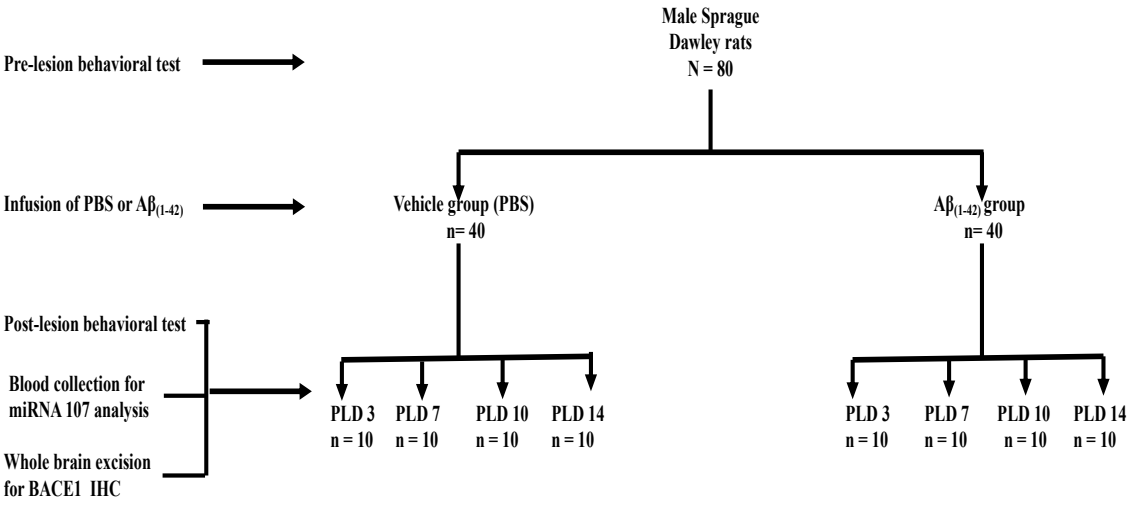

Figure 1. Experimental design. PLD- Post lesion day, PBS- Phosphate buffered saline, $A\beta_{(1–42)}$- Amyloid-beta 1-42, miRNA107- MicroRNA 107, BACE1- Beta site amyloid precursor protein cleaving enzyme 1, IHC- Immunohistochemistry.

2.2. Stereotaxic infusion of $A\beta_{(1–42)}$ lesion

The rats were anesthetized with a ketamine (100 mg/Kg, intraperitoneally) and xylazine (5 mg/Kg, intraperitoneally) solution before being placed in the stereotaxic apparatus (David Kopf instrument, Tujunga, USA) (Bagheri et al., 2011). After complete anesthesia was observed following the absence of hind paw reflex on pinching, animals were bilaterally infused using a Hamilton syringe coupled to a 25 G needle, with either 10 µL (5 µL on each side) solution of $A\beta_{(1–42)}$ peptide (DLD Scientific, South Africa) or an equal volume of phosphate-buffered saline solution (Vehicle). Infusion was made into the CA1 field of the dorsal hippocampus (dCA1) according to the following stereotaxic coordinates referenced in millimeters from bregma: Anteroposterior (AP) = −4.8 mm; mediolateral (ML) = ±3.4 mm; dorsoventral (DV) = −3.0 mm) (Paxinos and Watson, 2006). The needle was kept in for 1 min prior to the infusion and for 2 min following the infusion to maximize diffusion. The incision was thereafter sutured, cleaned, and the animals were placed under a heating lamp to prevent hypothermia during recovery. Animals were injected with Temgesic (0.05 mg/kg subcutaneously; Sigma, USA), a postoperative analgesic before being returned to their home cages.

2.3. Behavioral test

The Morris water maze (MWM) test was used to assess spatial learning and memory in this study. The procedure was performed as previously described by (Vorhees and Williams, 2006). All animals were trained in a water maze (diameter: 1 m) filled with water (23 ± 1 °C). The pool was divided into four virtual quadrants, each with a cue that was kept constant throughout the experiment to assist the rat in finding the hidden platform. During pre-lesion training, the platform was kept in the centre of the pool

and exposed one inch above the surface, so the rat knows that it's there. Each rat was placed in a quadrant other than where the hidden platform is located, facing the wall of the pool and was given 120 s to find the platform and 15 s to stay on it. Rats that did not find the platform were gently guided and placed on it during the training. An inter-trial interval of at least 2 minutes was maintained for uniformity in training for all rats. The pool was cleaned out periodically to ensure that the platform is in place and to check water temperature. After all rats completed their trials, they each performed one probe trial, in which the platform was removed from the pool. The probe trial is performed to verify the rat's understanding of the platform location, and observe the strategy that the animal follows when it discovers the platform is not there. Memory was assessed post-lesion in the probe test as the time spent in the target quadrant. When all the probe trials were complete, the animals were dried off and the pool was drained. Video recorded sessions were analysed for each rat.

2.4. Sample Preparation

Whole blood sample (3 ml) acquired aseptically from both the vehicle and Aβ(1–42) lesioned group of rats was collected into ethylenediaminetetraacetic acid (EDTA) coated tubes. The blood samples were centrifuged at a speed of 2000 x g for 10 minutes at 4 ◦C in a refrigerated centrifuge (Z326, Lasec, South Africa). Plasma samples were transferred into cryo-tubes and stored at -80°C within 2 hours from blood sample collection. Brain tissues were transcardially perfused and fixed with phosphate-buffered saline (PBS) followed by 10% Neutral buffered formalin (NBF) (Ijomone et al., 2019). The excised brain tissues were then post-fixed in 10% NBF until further analysis.

2.5. Immunohistochemistry of BACE1

Serial brain sections cut at 3 μm thickness were mounted on positively charged glass slides (leica® slides, Germany) for immunohistochemical staining using primary antibody: beta-secretase enzyme 1 (BACE1) (Cell Signalling, diluted 1:100), together with a secondary "Ultravision detection system" (ThermoScientific, USA). Brain sections were deparaffinized and hydrated in xylene and graded descending concentrations of alcohol, respectively, then incubated with a blocking solution (3% hydrogen peroxide, ThermoScientific, USA) for 15 mins. Incubation of slides was achieved in a humidified light-protected chamber at room temperature, and slides were continuously kept wet. Slides were washed twice in phosphate buffer, incubated in antigen retrieval solution (pepsin), and rewashed 4 times in phosphate buffer. The ultra V block (ThermoScientific, USA) was applied and incubated for 5 mins. The primary antibody was applied unto the sections, and each was incubated for 30 mins. Sections were then washed, and biotinylated goat antipolyvalent antibody (secondary antibody) was applied for 10 mins, rewashed, followed by streptavidin peroxidase for 10 mins and washed after. The reaction was developed with a drop of DAB Plus chromogen added to 2 ml of DAB Plus substrate (Vector Labs, USA), mixed and applied on tissues for 5–15 mins. Sections were then rinsed in water

and counterstained with Mayer's hematoxylin. A coverslip was applied using Dibutyl Phthalate Xylene (Dako, Denmark) as the mounting media. A positive reaction appeared as brown color. Negative controls were processed likewise, but the incubation with primary antibodies was omitted.

The immunostained slides were digitized using the Leica SCN400 Slide Scanner (Leica Microsystems, Wetzlar, Germany). Six to ten random non-overlapping areas of the hippocampus were snapped at X40 using the Lecia SlidePath Gateway software. Using the Rat Brain Atlas (George and Charles, 2007) as a reference, CA3 area of the hippocampus was examined. Images were imported on the NIH-sponsored ImageJ software for analysis. Immunoreactivity of BACE1 expression was quantified by intensity measurements, as previously described by (Ijomone et al., 2019). The optical density was obtained by converting the intensity numbers in the results window to an optical density (OD) with the following formula OD = log (max intensity/Mean intensity), where maximum intensity = 255 for 8-bit images.

2.6. Real-time quantitative PCR (qPCR) analysis for miRNA107

Thawed plasma samples were re-centrifuged at 3000 x g for 15 mins at room temperature before microRNA purification to reduce contamination from possible residual platelets (Binderup et al., 2016). MicroRNA purification of plasma samples was carried out using Nucleospin®miRNA Plasma (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. NanoPhotometer® NP80 (IMPLEN, A260: A280 ratio) was used to determine the quality and concentration of the total RNA, purity of 1.7–2.1 was recommended for use in the construction of cDNA. Total RNA was reverse-transcribed into cDNA using the iScript^{TM} cDNA synthesis kit and run in duplicate in a 20 µl reaction volume in a 96-well plate format, containing 500 nM of each oligonucleotide primer and SYBR Green PCR Master Mix (Bio-Rad). The primers were synthesized by Inqaba Biotech (Pretoria, South Africa), with U6 serving as the reference gene (See table 1). Primer sequences were reconstituted in RNA nuclease-free water according to the manufacturer's report and were added to a master mix comprising of SYBR green dye and nuclease-free water. We ensured that all qPCR reactions produced a single peak with the melt curve analysis. Relative expression levels were calculated after normalization against the reference gene U6 using $2^{-\Delta\Delta CT}$ method, and results were expressed as 2−(gene of interest mean Ct value − control gene mean Ct value) for the qRT-PCR experiments (Livak and Schmittgen, 2001).

Table 1. Primer sequences for RT-qPCR

Primer sequence	Forward (5'- 3')	Reverse (5'- 3')
miRNA107	GCCAAGCCCACTCAGCTGCCAGCC	GGCTGGCAGCTGAGTGGGCTTGGC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

2.7. Statistical Analysis

Data were analyzed using the software program GraphPad Prism (version 7.0, San Diego, California, USA). The Shapiro-Wilk test was used to test for normality of distribution, and where data met requirements, a parametric test was used. One-way or Two-way analysis of variance (ANOVA) was used followed by Tukey's post-hoc test where applicable. Pearson's correlation coefficient was used to assess the correlation between data sets, where "*r*" indicates the strength of the relationship. A *p*-value < 0.05 was considered significant in all analyses.

3. Results

3.1. $A\beta_{(1–42)}$ lesion of CA1 decreases time spent in target quadrant of the MWM

A significant interaction [F (3, 48) = 5.7, $p = 0.0020$] was observed between lesion and day and a significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on spatial memory [F (1, 48) = 234.2, $p < 0.0001$] with decreased time spent in target quadrant on post-lesion day 3 ($p < 0.01$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the corresponding vehicle-treated groups. A significant day effect was observed within the $A\beta_{(1–42)}$ lesioned groups [F (3, 48) = 2.242, $p = 0.0954$] differing with increasing significance from post-lesion day 3 vs. day 7 ($p = 0.0176$), day 3 vs. day 10 ($p = 0.0097$) and day 3 vs. day 14 ($p = 0.0052$) (Figure 1).

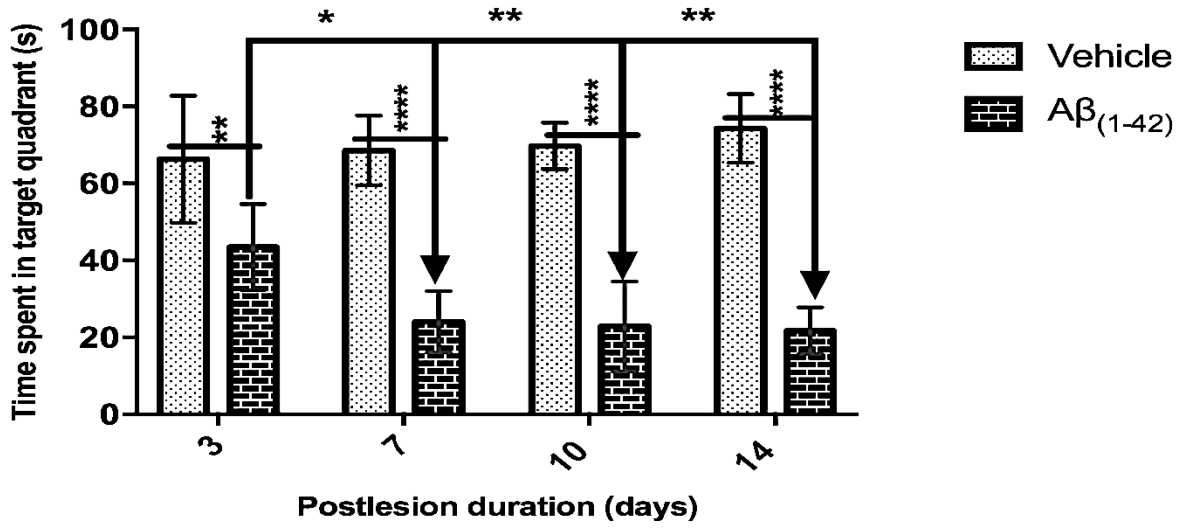

Figure 2. Time spent in the target quadrant for all groups on day 3, 7, 10 and 14 in the MWM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (two-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 10/ group).

3.2. $A\beta_{(1–42)}$ lesion of CA1 increases BACE1 expression progressively over the post-lesion days.

Significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on relative BACE1 expression was observed [$F(4, 45) = 104.3$, $p < 0.0001$], with upregulated expressions on post-lesion day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the vehicle injected group. Significant upregulated day effect was also observed within the $A\beta_{(1–42)}$ lesioned groups at post-lesioned day3 vs day 7 ($p < 0.0083$); day 3 vs day 10 ($p < 0.0001$); day 3 vs. day 14 ($p < 0.0001$); day 7 vs day 10 ($p = 0.0004$) and day 7 vs. day 14 ($p < 0.0001$); day 10 vs. day 14 ($p < 0.0001$) (Figure 2).

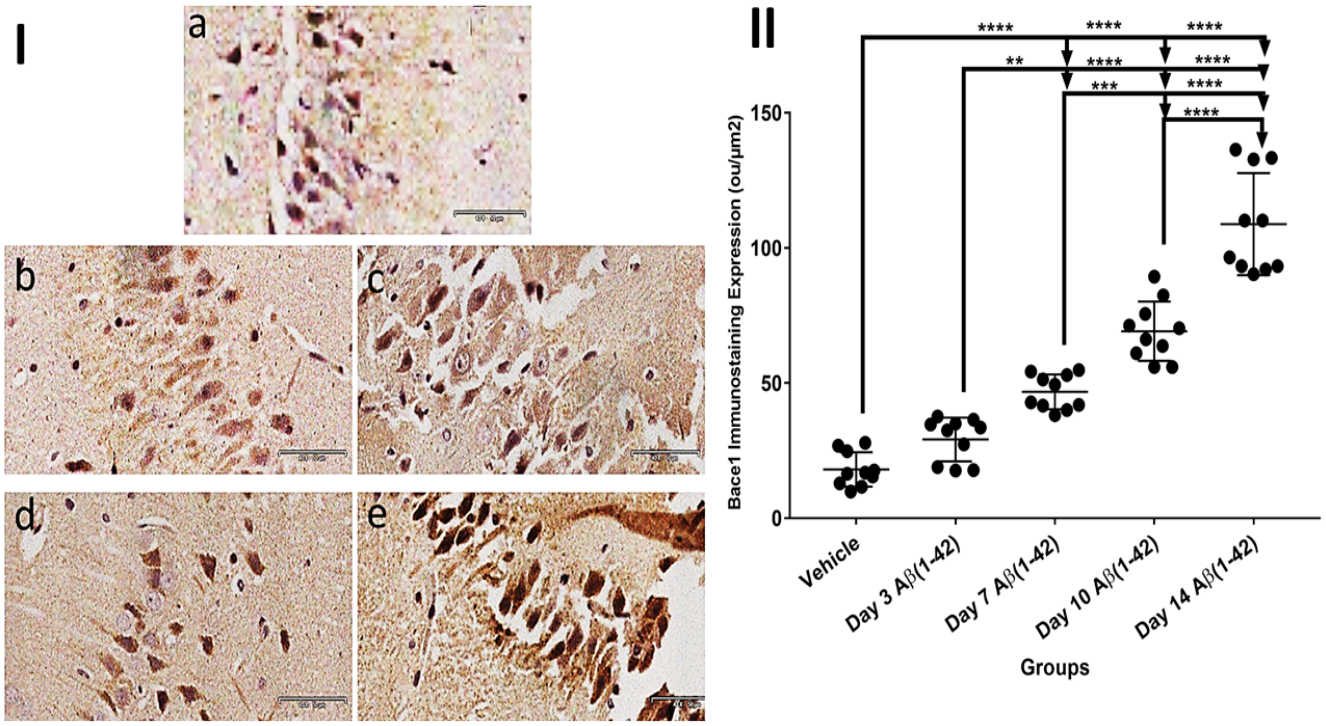

Figure 3. (I) Representative photomicrographs of BACE1 immunostaining intensity in (a) vehicle-treated rat (b) $A\beta_{(1–42)}$ lesioned rat at post-lesion day 3 (c) $A\beta_{(1–42)}$ lesioned rat at post-lesion day 7 (d) $A\beta_{(1–42)}$ lesioned rat at post-lesion day 10 (e) $A\beta_{(1–42)}$ lesioned rat at post-lesion day 14. Scale bar for all images represent 50µm. (II) Relative BACE1 immunostaining expression in the hippocampus of vehicle-treated and $A\beta_{(1–42)}$ lesioned rats across different post-lesion days * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (one-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 10/ group).

3.3. BACE1 expression negatively correlates with time spent in target quadrant in $A\beta_{(1–42)}$ lesioned rats.

Negative correlations were observed between BACE1 immunostaining expression and time spent in the target on post-lesion day 3 ($r = -0.6900$, $p = 0.0270$) (Fig. 3a); post-lesion day 7 ($r = -0.9319$, $p = 0.8680$) (Fig. 3b); post-lesion day 10 ($r = -0.8660$, $p = 0.0012$) (Fig. 3c) and post-lesion day 14 ($r = -0.8750$, $p = 0.0009$) (Fig. 3d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Figure 3).

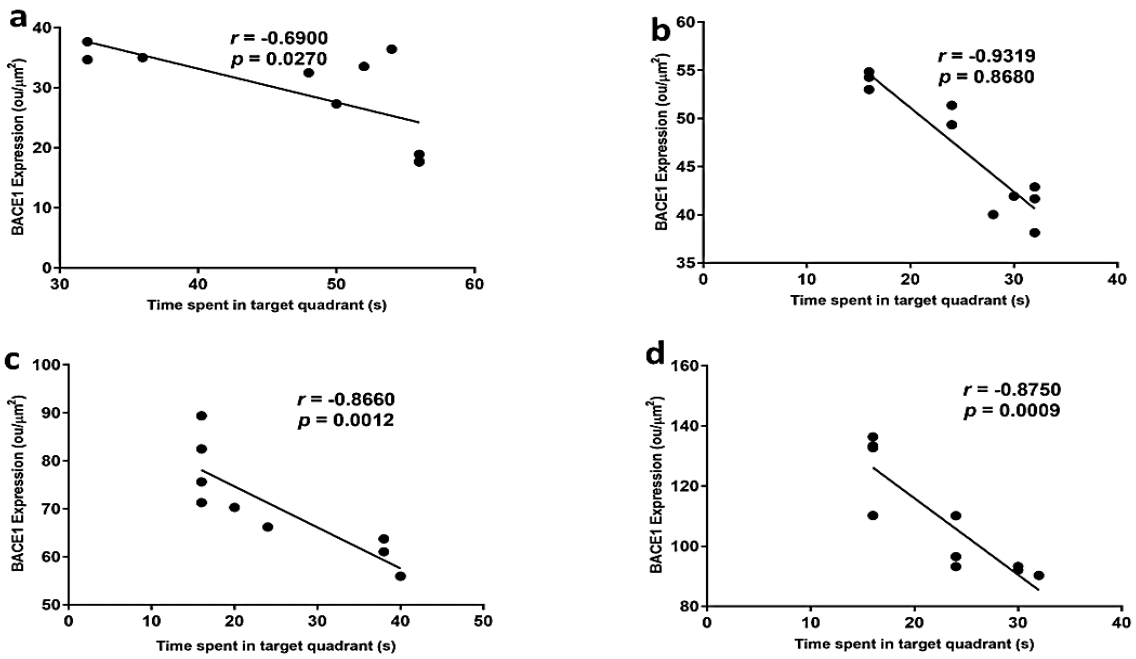

Figure 4. XY scatter plots of BACE1 expression and time spent in the target quadrant on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1–42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 10) in each group.

3.4. $A\beta_{(1–42)}$ lesion of CA1 decreases miRNA107 expression progressively over the post-lesion days.

Significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on relative miRNA107 expression in plasma was observed [F (4, 45) = 239.8, $p < 0.0001$], with downregulated expressions on post-lesion day3 ($p < 0.0001$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the vehicle injected group. Significant downregulated day effect was also observed within the $A\beta_{(1–42)}$ lesioned groups at post-lesioned day 3 vs day 7 ($p < 0.0001$); day 3 vs day 10 ($p < 0.0001$); day 3 vs. day 14 ($p < 0.0001$); day 7 vs day 10 ($p < 0.0001$) and day 7 vs. day 14 ($p < 0.0001$); day 10 vs. day 14 ($p < 0.0001$) (Figure 4).

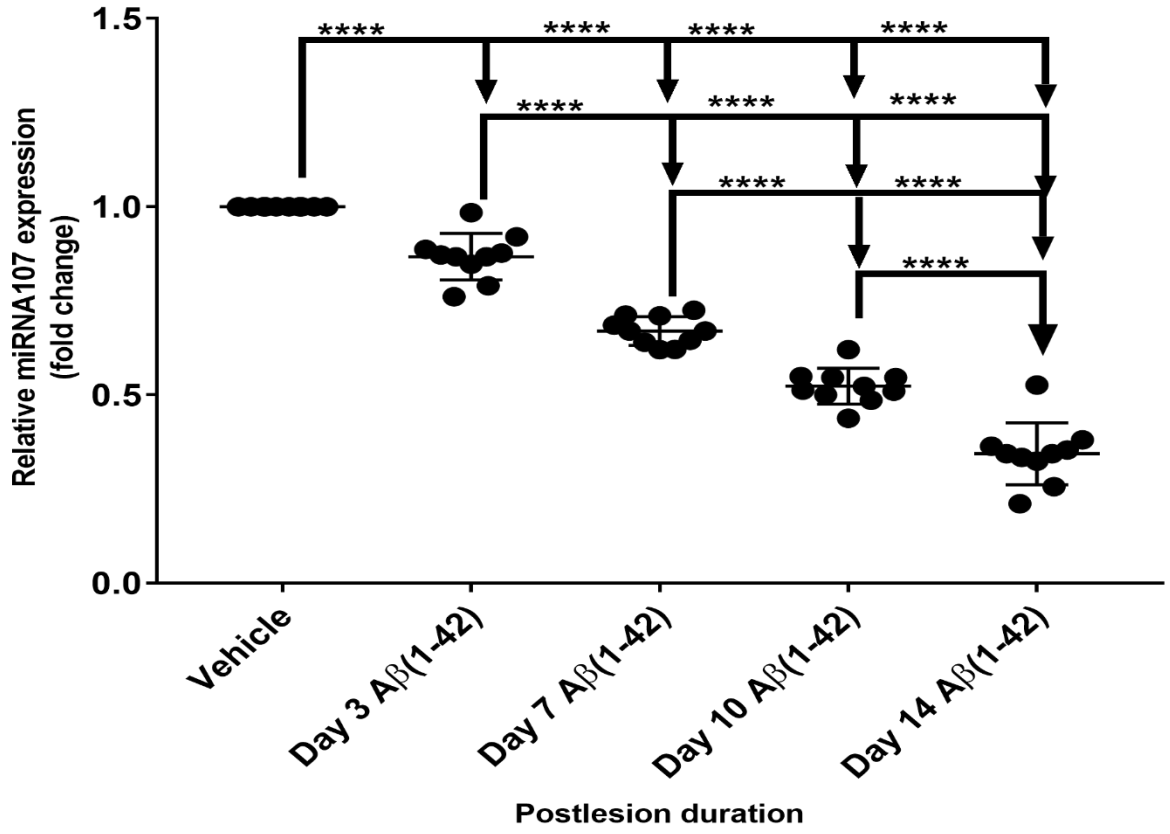

Figure 5. Relative miRNA107 expression in plasma of Aβ $_{(1–42)}$ lesioned rat model of AD in vehicle-treated and Aβ$_{(1–42)}$ lesioned rats across different post-lesion days * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (one-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 10/ group).

3.5. Downregulated expression of miRNA107 negatively correlates with BACE1 expression.

Negative correlations were observed between miRNA107 expression in plasma and BACE1 expression on post-lesion day 3 (r = -0.7680, p = 0.0095) (Fig. 5a); post-lesion day 7 (r = -0.8930, p = 0.0005) (Fig. 5b); post-lesion day 10 (r = -0.9170, p = 0.0002) (Fig. 5c) and post-lesion day 14 (r = -0.6100, p = 0.0612) (Fig. 5d) in the Aβ$_{(1–42)}$ lesioned rat model of AD (Figure 5).

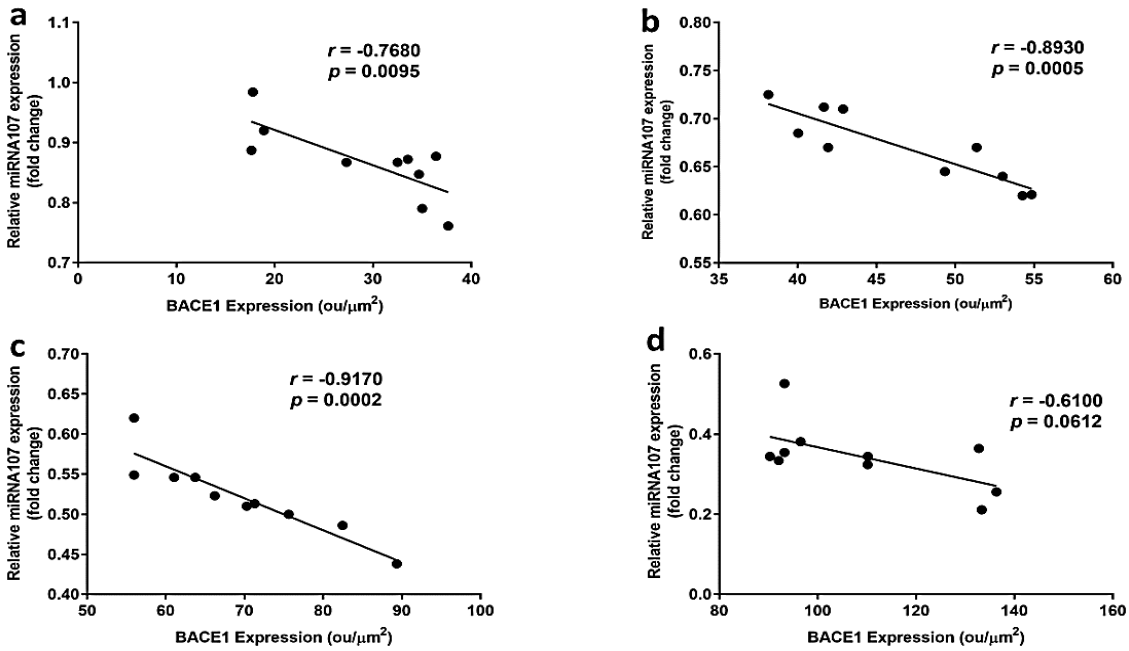

Figure 6. XY scatter plots of the relative expressions of miRNA107 and BACE1 expression on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1–42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 10) in each group.

4. Discussion

This study investigated the diagnostic feasibility of miRNA107 as a possible plasma biomarker by correlating its expression with BACE1 levels in the brain tissue. Several reports have proposed the detection of circulating miRNAs as a potentially valuable tool for early prediction of AD (Geekiyanage et al., 2012, Grasso et al., 2014, Cogswell et al., 2008, Lugli et al., 2015, Tan et al., 2014).

In our study, we evaluated the impact of $A\beta_{(1–42)}$ lesion of CA1 region of the hippocampus on spatial memory, we observed that the $A\beta_{(1–42)}$ lesioned rats spent lesser time in the target quadrant when compared to the vehicle-treated rats. This indicates a deficit in memory resulting from the intrahippocampal infusion of $A\beta_{(1-42)}$, possibly due to the expression of inflammatory mediators that exacerbate progression of the disease, ultimately leading to loss of memory. This result is supported by a study that also reported impaired spatial memory following intrahippocampal infusion of $A\beta_{(1–42)}$ (Wang et al., 2017).

We further observed an increase in the expression of BACE1 in the hippocampus as the disease progressed. This indicates that there is a continuous increase in the production of amyloidogenic AβPP processing that likely plays a role in this intractable disease. Since BACE1 is critical for Aβ biosynthesis, it is likely that factors that elevate BACE1 may lead to increased Aβ generation and promote AD. In high-order association brain regions affected by Aβ deposition, BACE1 protein levels

and activity were increased significantly in AD brain compared to non-demented control brain (Yang et al., 2003, Holsinger et al., 2002). An AD feedback loop has long been surmised, whereby $A\beta_{(1-42)}$ deposition in AD causes BACE1 (and possibly APP) levels to rise in nearby neurons. Increased Aβ production may then ensue, initiating a vicious cycle of additional amyloid deposition followed by further elevated BACE1 levels. Given the elevation of BACE1 around $A\beta_{(1-42)}$ plaque cores it seems possible that $A\beta_{(1-42)}$ somehow triggers the BACE1 increase. Additionally, it has been shown that exogenous administration of $A\beta_{(1-42)}$ causes increase in BACE1 level (Li et al., 2019).

As memory deficit constitutes the core symptom of AD, we correlated the time spent in the target quadrant of the MWM to BACE1 expression in the hippocampus and observed that an increase in BACE1 expression leads to decline in memory. This inverse relationship suggests that increased BACE1 expression may be an antecedent to other pathophysiologic changes that arise during the disease. This is possibly because BACE1 catalyses the rate limiting step in the production of Aβ, the principal component of plaque pathology in AD and the excessive production of which may possibly be a primary cause of cognitive dysfunction in AD. Besides considerable evidence indicates that Aβ production is critical to the deterioration related to Alzheimer's disease (Stockley and O'Neill, 2008).

Detection of dysregulated miRNA expression by relative qRT-PCR in the plasma of AD subjects has been previously reported (Kayano et al., 2016, Nagaraj et al., 2017, Zirnheld et al., 2016). Our study shows a time-dependent difference in the expression of miRNA 107. We observed a downregulation of miRNA in $A\beta_{(1–42)}$ lesioned rats when compared to the vehicle infused rats and the expression decreases over the post-lesion days. This downregulated expression of miRNA107 implies that the corresponding target gene (BACE1) would be expressed in higher abundance owing to the release of the inhibitory effect of miRNAs on the target genes being regulated. Our result is consistent with other studies that reported a downregulated expression of plasma miRNA107 in AD patients (Leidinger et al., 2013, Yılmaz et al., 2016). Studies have shown that exogenously administered miRNA107 mimics prevents the neurotoxicity and blood brain barrier dysfunction induced by Aβ (Liu et al., 2016, Shu et al., 2018). Although the exact mechanism of action of exogenously administered miRNA107 mimics were not elucidated in the present study. We can however infer that the negative correlation of BACE1 expression in the hippocampus with plasma miRNA107 level of $A\beta_{(1-42)}$ lesioned rats observed in our study shows that an increase in the miRNA107 expression would confer neuroprotection and also provide evidence for its feasibility as a plasma based biomarker of AD.

5. Conclusion

Our result shows that $A\beta_{(1–42)}$ lesion causes memory deficit in a time-dependent manner and this validates our model of AD, this lesion also caused dysregulation of plasma miRNA107 expression. We

also observed progressive upregulation of BACE1 expression which correlates with decreased miRNA107 expression as the disease progressed. This bi-directional interdependence between tissue-based BACE1 expression and plasma miRNA107 level makes miRNA107 a potential biomarker for the early diagnosis of Alzheimer's diseases.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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R References

1. Ulep, M.G., S.K. Saraon, and S. McLea, *Alzheimer disease.* The Journal for Nurse Practitioners, 2018. **14**(3): p. 129-135.
2. Shi, L., et al., *A decade of blood biomarkers for Alzheimer's disease research: an evolving field, improving study designs, and the challenge of replication.* Journal of Alzheimer's Disease, 2018. **62**(3): p. 1181-1198.
3. Leidinger, P., et al., *A blood based 12-miRNA signature of Alzheimer disease patients.* Genome biology, 2013. **14**(7): p. R78.
4. Brookmeyer, R., et al., *Forecasting the global burden of Alzheimer's disease.* Alzheimer's & dementia, 2007. **3**(3): p. 186-191.
5. Albert, M.S., et al., *The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Focus, 2013. **11**(1): p. 96-106.
6. Dallé, E., M.V. Mabandla, and W.M. Daniels, *Dielectric Constant and Conductivity of Blood Plasma: Possible Novel Biomarkers for Alzheimer's Disease.* Oxidative Medicine and Cellular Longevity, 2020. **2020**.
7. Ha, M. and V.N. Kim, *Regulation of microRNA biogenesis.* Nature reviews Molecular cell biology, 2014. **15**(8): p. 509-524.
8. Zhang, Z., T. Yang, and J. Xiao, *Circular RNAs: promising biomarkers for human diseases.* EBioMedicine, 2018. **34**: p. 267-274.

9. Liu, C.-G., et al., *MicroRNA-384 regulates both amyloid precursor protein and β-secretase expression and is a potential biomarker for Alzheimer's disease.* International journal of molecular medicine, 2014. **34**(1): p. 160-166.
10. Croce, N., et al., *NPY modulates miR-30a-5p and BDNF in opposite direction in an in vitro model of Alzheimer disease: a possible role in neuroprotection?* Molecular and cellular biochemistry, 2013. **376**(1-2): p. 189-195.
11. Keifer, J., Z. Zheng, and G. Ambigapathy, *A microRNA-BDNF negative feedback signaling loop in brain: implications for Alzheimer's disease.* MicroRNA, 2015. **4**(2): p. 101-108.
12. Kalogianni, D.P., et al., *Advances in microRNA analysis.* Analytical and bioanalytical chemistry, 2018. **410**(3): p. 695-713.
13. Kumar, S., et al., *MicroRNAs as peripheral biomarkers in aging and age-related diseases*, in *Progress in molecular biology and translational science*. 2017, Elsevier. p. 47-94.
14. Liang, H., et al., *The origin, function, and diagnostic potential of extracellular microRNAs in human body fluids.* Wiley Interdisciplinary Reviews: RNA, 2014. **5**(2): p. 285-300.
15. Turchinovich, A., et al., *Circulating miRNAs: cell–cell communication function?* Frontiers in genetics, 2013. **4**: p. 119.
16. Goodall, E.F., et al., *Neuronal dark matter: the emerging role of microRNAs in neurodegeneration.* Frontiers in cellular neuroscience, 2013. **7**: p. 178.
17. Wang, W.-X., et al., *miR-107 regulates granulin/progranulin with implications for traumatic brain injury and neurodegenerative disease.* The American journal of pathology, 2010. **177**(1): p. 334-345.
18. Nelson, P.T. and W.-X. Wang, *MiR-107 is reduced in Alzheimer's disease brain neocortex: validation study.* Journal of Alzheimer's Disease, 2010. **21**(1): p. 75-79.
19. Wang, W.-X., et al., *The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of β-site amyloid precursor protein-cleaving enzyme 1.* Journal of Neuroscience, 2008. **28**(5): p. 1213-1223.
20. Wang, T., et al., *The feasibility of utilizing plasma MiRNA107 and BACE1 messenger RNA gene expression for clinical diagnosis of amnestic mild cognitive impairment.* The Journal of clinical psychiatry, 2015. **76**(2): p. 135-141.
21. Stratman, N., et al., *1: Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science. 1999 Oct.*
22. Haniu, M., et al., *Characterization of Alzheimer's β-secretase protein BACE a pepsin family member with unusual properties.* Journal of Biological Chemistry, 2000. **275**(28): p. 21099-21106.

23. Vassar, R., et al., *β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE.* science, 1999. **286**(5440): p. 735-741.
24. Fukumoto, H., et al., *β-Secretase protein and activity are increased in the neocortex in Alzheimer disease.* Archives of neurology, 2002. **59**(9): p. 1381-1389.
25. Li, Q. and T.C. Südhof, *Cleavage of amyloid-β precursor protein and amyloid-β precursor-like protein by BACE 1.* Journal of Biological Chemistry, 2004. **279**(11): p. 10542-10550.
26. Dominguez, D.-I., D. Hartmann, and B. De Strooper, *BACE1 and presenilin: two unusual aspartyl proteases involved in Alzheimer's disease.* Neurodegenerative Diseases, 2004. **1**(4-5): p. 168-174.
27. Durham, T.B. and T.A. Shepherd, *Progress toward the discovery and development of efficacious BACE inhibitors.* Current opinion in drug discovery & development, 2006. **9**(6): p. 776-791.
28. Guo, T. and D.W. Hobbs, *Development of BACE1 inhibitors for Alzheimer's disease.* Current medicinal chemistry, 2006. **13**(15): p. 1811-1829.
29. John, V., *Human β-secretase (BACE) and BACE inhibitors: progress report.* Current topics in medicinal chemistry, 2006. **6**(6): p. 569-578.
30. Shallie, O.F., E. Dalle, and M.V. Mabandla, *Memory decline correlates with increased plasma cytokines in amyloid-beta (1–42) rat model of Alzheimer's disease.* Neurobiology of Learning and Memory, 2020. **169**: p. 107187.
31. Bagheri, M., et al., *Genistein ameliorates learning and memory deficits in amyloid β (1–40) rat model of Alzheimer's disease.* Neurobiology of Learning and Memory, 2011. **95**(3): p. 270-276.
32. Paxinos, G. and C. Watson, *The rat brain in stereotaxic coordinates: hard cover edition*. 2006: Elsevier.
33. Vorhees, C.V. and M.T. Williams, *Morris water maze: procedures for assessing spatial and related forms of learning and memory.* Nature protocols, 2006. **1**(2): p. 848.
34. Ijomone, O.K., P.D. Shallie, and T. Naicker, *Nω-nitro-l-arginine methyl model of pre-eclampsia elicits differential IBA1 and EAAT1 expressions in brain.* Journal of chemical neuroanatomy, 2019. **100**: p. 101660.
35. George, P. and W. Charles, *The rat brain in stereotaxic coordinates.* Qingchuan Zhuge translate, 2007. **32**.
36. Binderup, H.G., et al., *Pre-storage centrifugation conditions have significant impact on measured microRNA levels in biobanked EDTA plasma samples.* Biochemistry and biophysics reports, 2016. **7**: p. 195-200.
37. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method.* methods, 2001. **25**(4): p. 402-408.

38. Geekiyanage, H., et al., *Blood serum miRNA: non-invasive biomarkers for Alzheimer's disease.* Experimental neurology, 2012. **235**(2): p. 491-496.
39. Grasso, M., et al., *Circulating miRNAs as biomarkers for neurodegenerative disorders.* Molecules, 2014. **19**(5): p. 6891-6910.
40. Cogswell, J.P., et al., *Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways.* Journal of Alzheimer's disease, 2008. **14**(1): p. 27-41.
41. Lugli, G., et al., *Plasma exosomal miRNAs in persons with and without Alzheimer disease: altered expression and prospects for biomarkers.* PloS one, 2015. **10**(10).
42. Tan, L., et al., *Genome-wide serum microRNA expression profiling identifies serum biomarkers for Alzheimer's disease.* Journal of Alzheimer's Disease, 2014. **40**(4): p. 1017-1027.
43. Yang, L.-B., et al., *Elevated β-secretase expression and enzymatic activity detected in sporadic Alzheimer disease.* Nature medicine, 2003. **9**(1): p. 3-4.
44. Holsinger, R.D., et al., *Increased expression of the amyloid precursor β-secretase in Alzheimer's disease.* Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 2002. **51**(6): p. 783-786.
45. Li, F., et al., *The effect of BACE1-AS on β-amyloid generation by regulating BACE1 mRNA expression.* BMC molecular biology, 2019. **20**(1): p. 23.
46. Stockley, J. and C. O'Neill, *Understanding BACE1: essential protease for amyloid-β production in Alzheimer's disease.* Cellular and molecular life sciences, 2008. **65**(20): p. 3265.
47. Kayano, M., et al., *Plasma microRNA biomarker detection for mild cognitive impairment using differential correlation analysis.* Biomarker research, 2016. **4**(1): p. 22.
48. Nagaraj, S., et al., *Profile of 6 microRNA in blood plasma distinguish early stage Alzheimer's disease patients from non-demented subjects.* Oncotarget, 2017. **8**(10): p. 16122.
49. Zirnheld, A.L., et al., *Distinguishing mild cognitive impairment from Alzheimer's disease by increased expression of key circulating microRNAs.* Curr Neurobiol, 2016. **7**: p. 38-50.
50. Yılmaz, Ş.G., et al., *Can peripheral MicroRNA expression data serve as epigenomic (upstream) biomarkers of Alzheimer's disease?* Omics: a journal of integrative biology, 2016. **20**(8): p. 456-461.
51. Liu, W., et al., *MicroRNA-107 prevents amyloid-beta induced blood-brain barrier disruption and endothelial cell dysfunction by targeting Endophilin-1.* Experimental cell research, 2016. **343**(2): p. 248-257.
52. Shu, B., et al., *MicroRNA-107 prevents amyloid-β-induced neurotoxicity and memory impairment in mice.* International journal of molecular medicine, 2018. **41**(3): p. 1665-1672.

CHAPTER 6
Synthesis and Conclusion

6.1 Synthesis

Alzheimer's disease (AD) is a multifactorial disorder with memory impairment as its most common presenting symptom and is currently incurable. It is said that the prevalence of Alzheimer's disease is as high as 75 million people worldwide and is predicted to increase to 135 million people by 2050. Cumulating evidence suggests that the amyloid-beta theory, currently thought to be the predominant mechanism underlying AD, needs re-evaluation, considering all treatments and new drug trials based upon this theory have been unsuccessful. The delay in identifying a definitive cure is probably due to the scant knowledge of the cellular and molecular mechanisms implicated in its pathogenesis. However, the role of neuroinflammation has been affirmed. Neuroinflammation is the brain's response to irritations caused by various cues, including toxic metabolites such as Aβ(1-42). Neuroinflammation is generally chronic due to sustained activation of the brain's immune cells, including microglia and astrocytes. The brain is typically shielded from harmful toxicants by the blood-brain barrier, a specialised structure composed of astrocytes and endothelial cells. However, this barrier may become compromised, allowing for communication between peripheral immune cells, neurons, and glial cells, thereby perpetuating the immune response. Although the immune response aims to protect the brain, sustained glial cell activation can become toxic, resulting in widespread neuroinflammation. Therefore, a better understanding of the fundamental role of neuroinflammation in Alzheimer's disease can be an excellent pointer for developing viable therapeutic targets and possible identification of biomarkers.

To respond to these challenges, we designed the study to assess the role of neuroinflammation and identify possible biomarkers in an Aβ(1-42) rat model of Alzheimer's disease over a progressive period. The specific neuroinflammatory roles were evaluated 3, 7, 10, and 14 days post-Aβ(1-42) infusion, and our findings are summarised in Figure 1. Our model's validity was confirmed by evaluating memory-like behaviour pre- and post-lesion with Aβ(1-42) using tools such as the Morris water maze and the fear conditioning test. The Morris water maze was used to test for spatial learning and memory while the fear conditioning test assessed associative fear learning and memory, a form of Pavlovian learning that involved making associations between stimuli and their aversive consequences. Our Alzheimer's disease model showed deficits in memory using both forms of learning and memory tests, hence, validating the model.

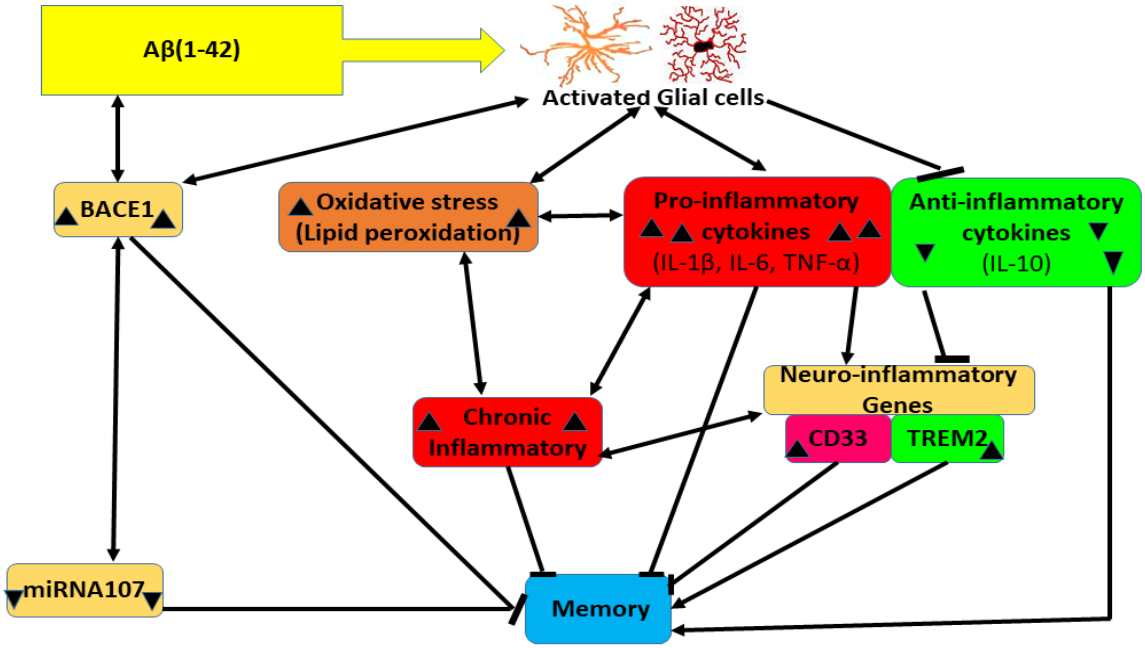

Figure 1: Role of neuroinflammation in a beta-amyloid model of Alzheimer's disease. Amyloid-beta infusion excites BACE1 feedback expression leading to glial cell activation, oxidative stress, and pro-inflammatory cytokines release. The release of cytokines exaggerates oxidative stress, induce chronic inflammation, and stimulate neuroinflammatory genes (CD33 and TREM2) with the resultant memory decline. In contrast, glial activation inhibits anti-inflammatory cytokine (IL-10) to enhance memory and regulate neuroinflammatory genes, which in turn triggers chronic inflammation and memory decline. An increase in BACE1 expression inhibits miRNA107 leading to memory decline. Arrows represent excitatory connections; Bars represent inhibitory connections; BACE1 (beta-site amyloid precursor cleaving enzyme 1); Aβ(1-42) (amyloid-beta 42); miRNA107 (MicroRNA107); CD33 (Cluster of differentiation 33); TREM2 (Triggering receptor expressed on myeloid cells 2); IL-1β (Interleukin 1β); TNF-α (Tumour Necrosis Factor α); IL-6 Interleukin 6; IL-4 Interleukin 4; IL-10 Interleukin 10. Triangle shapes represent upregulation, and inverted triangles represent downregulation.

The cellular players involved in the neuroinflammatory process associated with Alzheimer's disease are the microglia and astrocytes found closely associated with the amyloid plaques. We examined the level of reactivity of these glial cells in the hippocampus in a progressive post-lesion interval and observed an increase in their reactivity level using the immunofluorescence technique. Although both glial cells were activated at all time points and correlated positively in this study, we observed an overall increase in microglia activity compared to astrocyte activity. Evidence shows that microglia are implicated in the brain's first-line immunity and may be responsible for their increased activity. Microglia cells have also been implicated in exaggerating neuroinflammation by secreting oxidative substances to wade off the Aβ(1-42). These oxidative substances which induce cellular stress and exacerbate the immune response are referred to as oxidative stressors. We observed a positive relationship between microglia marker (ionized calcium-binding adaptor molecule 1) and a marker for oxidative stress (lipid peroxidation) in plasma, indicating an interdependence between the two factors. The observed

interaction may facilitate the understanding of Alzheimer's disease pathogenesis, resulting in a strategy directed at controlling neuroinflammation and oxidative stress, in developing therapeutics for AD.

The confirmation that our AD model showed progressively increased activation of cells involved in the immune response and its relationship to oxidative stress led to further evaluation of the cytokines' plasma concentration in our animal model. Cytokines are produced by sustained activation of the cellular players of inflammation. These substances may damage the blood-brain barrier and increase permeability through activation and destruction of tight junctions of microvascular endothelial cells. Our study showed an increase in concentration of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and a decrease in anti-inflammatory cytokine (IL-10) concentration in plasma. The implication of cytokines in controlling memory recall led to our evaluation of their possible relationship with learning and memory. We observed that the increase in the concentration of pro-inflammatory cytokines was related to a decrease in memory recall, while an increase in the anti-inflammatory cytokines was related to an increase in recall. These results demonstrated an association between memory and plasma cytokine concentration and shed light on the importance of these inflammatory markers and the need to evaluate genes regulating their synthesis.

Quantification of the level of gene expression provides valuable information as reliance on messenger RNA measurements can be traced to the relative ease of availability of messenger RNA compared to protein data. Moreover, the messenger RNA expression levels can provide information on gene functionality with the implicit assumption that differentially expressed messenger RNAs impact their respective experimental conditions via protein differences. Hence, the mRNA expression levels of microglia genes such as CD33 and TREM2 regulating neuroinflammation were evaluated. CD33 is likened to the "on switch", while TREM2 is the "off switch" for neuroinflammation. While both genes were upregulated in our AD model compared to the control, we observed a decrease in the expression of CD33 throughout the experimental period. This was accompanied by a corresponding increase in trem2, suggesting that this was counteracting the CD33 effect. Contextual fear memory assessed following CA1 lesion decreased progressively throughout the observation, indicating that the dorsal CA1 region of rats has strong spatial properties, with well-defined place fields capable of encoding a context. Increased expression of the CD33 gene, which is the driver gene for inflammation in the brain in our AD model, showed an inverse relationship with the contextual freezing time, which implies that the gene's increased expression is related to a decline in memory. Therefore, sustained neuroinflammatory response resulting from Aβ(1–42) lesion of CA1 region of the hippocampus may impact both neural circuit activity and expression of microglial genes regulating neuroinflammation, indicating a dynamic interaction between the immune and nervous systems. As a contribution to knowledge, we suggest that this integrative approach may be used to investigate the role of

neuroinflammation in memory processes and, importantly, to identify the possibility of stalling the brain's immune response to restore cell network activity and cognitive function.

The observed impacts of neuroinflammation in our Aβ(1–42) AD model led to the need to identify a biomarker, bearing in mind that it had to be easily accessible, cost-effective, and, most importantly, reflecting the status of disease progression in the brain. We investigated the potential feasibility of microRNA107 as a plasma biomarker by correlating its expression with BACE1 levels in the brain. The identification of microRNA as a possible biomarker stems from its ability to control gene expression, through which it suppresses translation or induces degradation of target genes. MicroRNA107 targets genes directly involved in AD pathophysiologies such as BACE1. BACE1 is the initiating and putatively rate-limiting enzyme in Aβ generation. Although its inhibition would block the production of Aβ and prevent the development of Aβ-associated pathologies, overexpression of BACE1 enzyme has been shown to initiate or accelerate Alzheimer's disease pathogenesis. In our study, we evaluated the plasma concentration of microRNA107, which is known to regulate the expression of BACE1. Firstly, the expression of BACE1 was analysed in hippocampal tissue, we observed an upregulated progressive expression of this enzyme. MicroRNA107 evaluation in plasma showed downregulated expression over the progressive post-lesion day intervals, which correlated negatively to BACE1 expression. This bi-directional interdependence between BACE1 and microRNA107 makes microRNA107 a potential biomarker for the early diagnosis of Alzheimer's disease.

6.2 Conclusion

It is incontrovertible that neuroinflammation holds a pivotal role in AD pathology. Our findings implicate cellular and molecular mechanisms in neuroinflammation, as shown by prolonged and progressive activation of glial cells, resulting in a bidirectional interplay between neuroinflammation and oxidative stress. These interconnections resulted in the concomitant release of brain cytokines as a secondary response to AD's basis, which impacted both neural circuit activity and expression of microglial genes regulating neuroinflammation, indicating dynamic crosstalk between the immune and nervous systems. These interactions will facilitate the understanding of AD's pathogenesis and hold the potential for an integrative approach to validate the role of neuroinflammation. These interactions also pave the way for establishing innovative early diagnostic biomarkers for therapeutic design and policies geared toward reducing this devastating disease's socio-economic burden.

6.3 Future Recommendations

Our results highlight some emerging mechanisms that provide deeper insights into AD pathogenesis and may serve as novel diagnostic and therapeutic targets for AD. However, there is a need for further study to:

- Include female rats, which were excluded to prevent experimental bias due to hormonal changes. Nevertheless, this exclusion did not affect our results; however, the long-held assumption that female rodents' oestrous cycle renders them more variable than male rodents requires a reappraisal. Sex differences are, in any case, incompletely explained by the actions of sex hormones. Therefore, future studies should incorporate both sexes in the experimental design and possibly evaluate the different sex responses.
- Include a different age bracket since the rats used in this study were 8 to 9 weeks old at the study's commencement. Although it may appear that these subjects were around mid-adulthood, we considered the fact that normal aging of the brain is described by hippocampal volume loss and increased demyelination, which contributes to learning and memory deficits as well as the possible motor decline leading to functional limitation. Therefore, we sought our research subjects within the mid-adulthood age because all these factors could cause experimental bias.
- Investigate other brain regions, particularly considering that soluble oligomeric Aβ(1–42) can diffuse into all parts of the brain.
- Investigate other AD models that replicate the spectra of AD to clarify the mechanisms of the interactions between neuroinflammation and oxidative stress. This will enhance the development of blood-based AD biomarkers, which are of utmost importance in the early diagnosis of AD.

Appendix I

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

04 May 2018

Mrs Oluwadamilola Faith Shallie (217082125)
School of Laboratory Medicine & Medical Sciences
Westville Campus

Dear Mrs Shallie,

Protocol reference number: AREC/015/018D
Project title: Evaluation of possible plasma and neuroinflammatory Biomarkers in β-amyloid rat model of Alzheimer's disease

Full Approval – Research Application

With regards to your revised application received on 12 April 2018 and 24 April 2018. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 04 May 2019.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

[redacted]

.....

Professor Shahidul Islam, PhD
Chair: Animal Research Ethics Committee

/ms

Cc Supervisor: Professor Musa Mabandla
Cc Academic Leader Research: Dr Michelle Gordon
Cc Registrar: Mr Simon Mokoena
Cc NSPCA: Ms Anita Engelbrecht
Cc BRU – Dr Linda Bester

Animal Research Ethics Committee (AREC)
Ms Mariette Snyman (Administrator)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 8350 **Facsimile:** +27 (0) 31 260 4609 **Email:** animalethics@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx

Appendix II

SAVC
SOUTH AFRICAN VETERINARY COUNCIL

ANNEXURE: OLUWADAMILOLA FAITH SHALLIE
CONDITIONS OF AUTHORISATION IN TERMS OF SECTION 23 (1) (c) OF THE VETERINARY AND PARA-VETERINARY PROFESSIONS ACT, ACT 19 OF 1982

Oluwadamilola Faith Shallie with Passport number A08238451 (Nigeria) is authorised subject to the following conditions:-

1. Authorisation is granted for the duration of studies and research project to enable you to perform the procedures as per the application with authorisation number **AR18/16971.**

2. The authorisation is limited to research at the **Biomedical Resourse Unit, University of Kwazulu-Natal.**

3. Your supervisor **must submit a 6-monthly report** on the procedures performed under authorisation.

4. You render the services under supervision of a registered veterinarian.

5. You are bound by the Veterinary and Para-Veterinary Professions Act 19 of 1982, Rules and Regulations promulgated in terms thereof.

6. You are authorised to perform the following procedures in research on **rats**:
   - 6.1 Animal handling;
   - 6.2 Intraperitoneal injections;
   - 6.3 Intramuscular Injections;
   - 6.4 Sub-cutaneous Injections;
   - 6.5 Oral gavage; and
   - 6.6 Stereotaxic surgery.

7. An annual maintenance fee equivalent to the maintenance of a para-veterinary professional is due and payable during the period of authorisation.

[redacted]

5 September 2018
Date

Appendix III

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

LMMS RESEARCH SYMPOSIUM 2019

CERTIFICATE OF PRESENTATION

Awarded to

OLUWADAMILOLA SHALLIE

University of KwaZulu-Natal

For presenting the oral:

Memory Decline Correlates with Increased Plasma Cytokines in Amyloid Beta (1-42) Rat Model of Alzheimer's Disease

At the

Annual Laboratory Medicine and Medical Sciences Research Symposium 2019

Which took place in Westville, Durban, South Africa

On the 6th of September 2019

Yours sincerely,

[redacted]

Dr De Gama

Academic Leader Research SLMMS

Appendix IV

Neurobiology of Learning and Memory 169 (2020) 107187

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Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Memory decline correlates with increased plasma cytokines in amyloid-beta (1–42) rat model of Alzheimer's disease

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Discipline of Human Physiology, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa

ARTICLE INFO

Keywords:
Alzheimer's disease
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Glial cells
Inflammatory cytokines
Memory
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ABSTRACT

Dysregulation of inflammatory markers like cytokines is implicated in the pathophysiology of Alzheimer's disease (AD). Altered level of these cytokines show that pathogenesis of AD is beyond dysfunction of neurons resulting from amyloid beta accumulation but involves neuroinflammatory mechanisms elicited by the neuroimmune cell. In this study, we investigated the effect of amyloid-beta (1–42) ($A\beta_{(1-42)}$) on memory and how inflammatory markers respond to this model of AD. Male Sprague-Dawley rats were used for this study. The animals were randomly divided into four groups euthanized on day 3, 7, 10 and 14 post-lesion with amyloid-beta (5 μg/5 μl) while corresponding control groups were stereotaxically injected with a vehicle (5 μl of 0.01 M phosphate- buffered saline). The Morris water maze (MWM) test to access learning and memory was conducted pre and post-lesion and blood was collected through cardiac puncture on day 3, 7, 10 and 14 post lesion. Multiplex immunoassay was performed to determine the plasma levels of IL-1β, IL-6, IL-10 and TNF-α. Our results showed impaired spatial memory and elevated plasma levels of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) with a concomitantly lowered level of the anti-inflammatory cytokine (IL-10) in the $A\beta_{(1-42)}$ lesioned rats when compared to the vehicle groups. This study showed a negative correlation between the decline in performance of the spatial memory task and plasma levels of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and positive correlation with the anti-inflammatory cytokine IL-10. In conclusion, this study most importantly demonstrated an association between progressive decline in spatial memory and increased plasma cytokine level induced by the infusion of $A\beta_{(1-42)}$.

1. Introduction

Alzheimer's disease (AD) is an escalating neurodegenerative disease that represents a major factor for the prevalence of dementia in the aged population worldwide (Mohajeri, Troesch, & Weber, 2015). Dementia currently affects an estimated number of 50 million people worldwide, and this population has been projected to rise to 152 million by 2050, thereby making it an indubitable public health mandate (Organization, 2018). Cumulative evidence indicates that Alzheimer's disease is caused by the toxicity resulting from the buildup of amyloid beta (Eckman & Eckman, 2007; Hardy & Selkoe, 2002). However, neuroinflammation is presently being proposed as a possible mechanism for neuronal death observed in the disease progression (Ahmad, Fatima, & Mondal, 2018; McKenzie et al., 2017). This is supported by the presence of abundantly activated glial cells observed in postmortem evaluation of AD brains and by the recurrent failure of drugs that only target amyloid plaques in the disease (Hopperton, Mohammad, Trepanier, Giuliano, & Bazinet, 2018; Kametani & Hasegawa, 2018). The hippocampus is the main seat of memory and one of the earliest parts of the brain affected in this disease (Bondi et al., 2008; R. G. Morris & Baddeley, 1988; Webster, Bachstetter, Nelson, Schmitt, & Van Eldik, 2014). Therefore, an impaired memory that deteriorates with disease progression is a core clinical manifestation in AD (Perry, Watson, & Hodges, 2000; Stopford, Thompson, Neary, Richardson, & Snowden, 2012). Neuropathological characterization of AD is based on the occurrence of intracellular neurofibrillary tangles and extracellular plaques, while the main component of this plaque is amyloid beta-protein (Domingues, AB da Cruz e Silva, & Henriques, 2017). Amyloid-beta 1–42 ($A\beta_{1-42}$) is the most toxic form of this peptide, it is derived from β-amyloid precursor protein (βAPP), and triggers AD pathogenesis by quickening age-related memory decline (Ferreira, Lourenco, Oliveira, & De Felice, 2015; Sasmita, Kuruvilla, & Ling, 2018).

Cytokines are inflammatory markers that contribute significantly to the pathophysiology of AD (Alam et al., 2016). They are synthesized in response to inflammation by microglia, the resident neuroimmune cells

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Available online 12 February 2020
1074-7427/

tasked with maintaining homeostatic balance in the brain (Norris & Kipnis, 2019; Ransohoff & Brown, 2012). Deposition of amyloid-beta 1–42 ($A\beta_{1-42}$) in the brain activates the microglia, which in turn incites the surrounding brain tissue and triggers the expression of inflammatory mediators including cytokines such as interleukin (IL)-1β, IL-6, IL-10 and tumor necrosis factor-α (TNF-α). These secreted cytokines in turn act to either exacerbate or attenuate the inflammatory state and progression of the disease (Block & Hong, 2005; Glass, Saijo, Winner, Marchetto, & Gage, 2010; Shastri, Bonifati, & Kishore, 2013). Their action is dependent on one another, as no particular cytokine can mediate inflammation alone (Donzis & Tronson, 2014; Platzer, Meisel, Vogt, Platzer, & Volk, 1995; Steensberg, Fischer, Keller, Møller, & Pedersen, 2003). Our aim was therefore to investigate the effect of $A\beta_{(1-42)}$ on memory and to assess cytokine response to this model of AD, also, we further examined the response of these markers in association with the state of memory in $A\beta_{(1-42)}$ lesioned rat model of AD.

2. Materials and methods

2.1. Animals

All procedures were reviewed and approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (AREC/015/018D). Fifty-six (56) male Sprague-Dawley rats weighing between 300 and 350 g (8/9 weeks of age) were obtained from the Biomedical Research Unit of the University of KwaZulu- Natal and kept under standard laboratory conditions with food (Rodent Ripe Pellets, Meadow, South Africa) and water freely available. The animals were randomly divided into four groups (Day 3 group: animals euthanized 3 days after lesioning; Day 7 group: animals euthanized 7 days after lesioning; Day 10 group: animals euthanized 10 days after lesioning, and Day 14 group: animals euthanized 14 days after lesioning) (n = 14/group). Each group was further sub-divided into a vehicle group of rats (n = 7) that were injected with phosphate buffered saline and an $A\beta_{(1-42)}$ group (n = 7) that were injected with the neurotoxin $A\beta_{(1-42)}$ peptide (Lonappan, Dallé, Afullo, & Daniels, 2017) (Figure 1). The animals were weighed prior to all experiments and were brought to the experimentation room 1 h before commencing behavioral tests.

2.2. Drugs and reagents

$A\beta_{(1-42)}$ peptide was purchased from DLD Scientific (Durban North, South Africa). Ketamine, xylazine, and temgesic were obtained from Sigma (ST. Louis MO, USA). Bio-Plex Pro™ Rat Cytokine assay kit (catalogue number: 10014905) was purchased from Bio-Rad Laboratories (CA, USA).

2.3. Behavioral test

The Morris water maze (MWM) test commonly used to assess spatial learning and memory in rodents was used in this study (Cassim, Qulu, & Mabandla, 2015; Garthe & Kempermann, 2013; Morris, 1984). The procedure was performed as previously described by (Cassim et al., 2015). Animals were trained in a water maze (diameter: 1 m) filled with water (23 ± 1 °C). The pool was divided into four virtual quadrants, each with a cue to assist the rat in finding the hidden platform, the platform was submerged in the middle of one of the four quadrants. Each rat was placed in a quadrant other than where the hidden platform is located, facing the wall of the pool and was given 120 s to find the platform and 15 s to stay on it. Animals that did not find the platform were gently guided and placed on it during the learning test. Learning was assessed pre-lesion, it measures the time taken for the animal to locate the hidden platform while the time spent in the quadrant of the hidden platform is regarded as the ability to remember (memory), which was assessed post-lesion.

2.4. Alzheimer's disease rat model ($A\beta_{(1-42)}$ lesion)

Rats were anesthetized with ketamine (100 mg/Kg, intraperitoneally) and xylazine (5 mg/Kg, intraperitoneally) solution before being placed in the stereotaxic apparatus (David Kopf instrument, Tujunga, USA) (Bagheri, Joghataei, Mohseni, & Roghani, 2011). After complete anaesthesia was observed following the absence of hind paw reflex on pinching, animals were bilaterally injected using a Hamilton syringe coupled to a 25 G needle, with either 10 µl (5 µl on each side) solution of $A\beta_{(1-42)}$) peptide or an equal volume of phosphate buffered saline solution (Vehicle) into the CA1 field of the dorsal hippocampus (dCA1) according to the following stereotaxic co-ordinates referenced in millimeters from bregma: Anteroposterior (AP) = −4.8 mm; mediolateral (ML) = ± 3.4 mm; dorsoventral (DV) = −3.0 mm) (Paxinos & Watson, 2006; Watson & Paxinos, 1986). The needle was kept in the dCA1 for 1 min prior to the injection and for 2 min following the injection to maximize diffusion. The incision was thereafter sutured, cleaned, and the animals were placed under a heating lamp to prevent hypothermia during recovery. Animals were injected with Temgesic (0.05 mg/kg subcutaneously) a post-operative analgesic before being returned to their home cages. Post-lesion behavioral test was conducted before the animals were euthanized on days 3, 7, 10 and 14, according to previous study in our laboratory (Lonappan et al., 2017).

2.5. Decapitation and neurochemistry

All rats were euthanized on post-lesion days 3, 7, 10 and 14 by

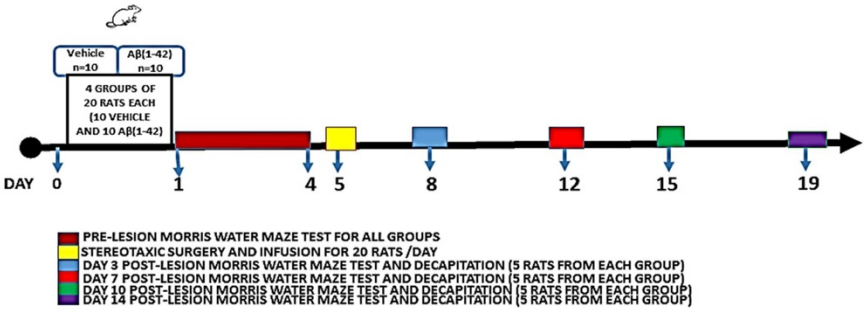

Figure 1. Experimental timeline. Rats received bilateral intra-dCA1 infusion of $A\beta_{(1-42)}$ or vehicle solution after pre-lesion MWM test. On 3, 7, 10 and 14 days post-lesion, separate cohorts of rats belonging to $A\beta_{(1-42)}$ or vehicle-infused groups were tested for spatial memory function and euthanized 1 h after each probe trial depending on the post-lesion group.

halothane overdose. Blood (4 ml) acquired aseptically through cardiac puncture from both the vehicle and $A\beta_{(1-42)}$ lesioned rats were collected into ethylenediaminetetraacetic acid (EDTA) coated tubes, which were then centrifuged at 1000g for 15 min at 4 °C in a refrigerated centrifuge (Z326, Lasec, South Africa). In order to completely remove platelets and precipitates, the acquired plasma sample was centrifuged again at 10,000g for 10 min at 4 °C. Plasma were transferred into Eppendorf vials, quickly snap frozen in liquid nitrogen and stored at – 80 °C until analysis.

2.6. Multiplex assay

Plasma samples stored at −80 °C were thawed and used for the measurement of the cytokine levels (IL-1β, IL-6, IL-10, and TNF-α). Multiplex immunoassay was performed using a Bio-Plex Pro™ Rat Cytokine assay kit (catalog number: 10014905) in accordance with the manufacturer's instructions. Using a 96-well flat bottom assay plate, coupled magnetic beads (50 µl) were added into each well of the assay plate followed by washing twice using Bio-plex wash buffer (100 µl). Four-fold serial dilutions of both the standard and sample (50 µl) were then prepared and dispensed into designated wells followed by washing thrice using Bio-plex wash buffer (100 µl). Incubation on shaker at 850 ± 50 rpm at room temperature for 1 h allowed for the interaction of the antigen with the coupled magnetic beads. Biotinylated detection antibodies (25 µl) (IL-1β, IL-6, IL-10, and TNF-α) were also added to the plates with subsequent incubation on shaker at 850 ± 50 rpm at room temperature for 30 min and washed thrice using Bio-plex wash buffer (100 µl). The addition of streptavidin–phycoerythrin (SAPE) (50 µl) incubated on shaker at 850 ± 50 rpm at room temperature for 10 min to complete the reaction was also concluded with washing thrice using Bio-plex wash buffer (100 µl). Plates were suspended in assay buffer (125 µl) on a shaker at 850 ± 50 rpm at room temperature for 30 s and read on a Bio-Plex® MAGPIX™ Multiplex system (Bio-Rad Laboratories Inc., USA). Data from the multiplex analysis was obtained using the Bio-Plex Manager™ version 4.1 software.

2.7. Statistical analysis

GraphPad Prism (version 7, Software Inc.) was used to analyze the data. Descriptive statistics for continuous data were reported as mean ± standard error of mean (SEM). Shapiro-Wilk normality test was used to assess distribution of data. Two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons were used to assess statistical significance within the study population. Pearson's correlation coefficient was used to assess the correlation between spatial memory and interleukin level, where "r" indicates the strength of the relationship. A value of $p < 0.05$ was considered statistically significant in all analyses.

3. Results

3.1. Spatial learning and memory

There was a significant day effect on spatial learning [$F(3, 208) = 337.9$, $p < 0.0001$] indicated by a decrease in time to locate the hidden platform (escape latency) over the training days for all groups (See Figure 2a). We also observed a significant group effect [$F(3, 208) = 6.404$, $p = 0.0004$] with specific significance between day 3 vs. day 10 ($p = 0.036$) and between day 7 vs. day 10 ($p = 0.0037$). Significant day effect within groups were observed: In Day 3 group: On day 1 vs. day 3 ($p < 0.0001$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 3 ($p = 0.002$); on day 2 vs. day 4 ($p < 0.0001$) and on day 3 vs. day 4 ($p = 0.0012$). In Day 7 group: On day 1 vs. day 3 ($p = 0.0031$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 3 ($p = 0.021$); on day 2 vs. day 4 ($p < 0.0001$) and on day 3 vs. day 4 ($p < 0.0001$). In Day 10 group: On day 1 vs. day 3 ($p = 0.0005$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 4 ($p = 0.0003$) and on day 3 vs. day 4 ($p = 0.0031$). In Day 14 group: On day 1 vs. day 3 ($p = 0.0015$); on day 1 vs. day 4 ($p < 0.0001$); on day 2 vs. day 3 ($p = 0.0006$); on day 2 vs. day 4 ($p < 0.0001$) and on day 3 vs. day 4 ($p = 0.0234$) (Figure 2a).

Figure 2b shows a significant interaction [$F(3, 48) = 5.7$, $p = 0.0020$] between lesion and day and a significant lesion effect of intrahippocampal injection of $A\beta_{(1-42)}$ on spatial memory [$F(1, 48) = 234.2$, $p < 0.0001$] with decreased time spent in the quadrant of the hidden platform on post-lesion day 3 ($p < 0.01$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the corresponding vehicle injected groups. A significant day effect was observed within the $A\beta_{(1-42)}$ lesioned groups [$F(3, 48) = 2.2$, $p = 0.0954$] differing with increasing significance from post-lesioned day 3 vs. day 7 ($p = 0.0176$), day 3 vs. day 10 ($p = 0.0097$) and day 3 vs. day 14 ($p = 0.0052$) (Figure 2b).

Figure 2c shows a significant lesion effect of intrahippocampal injection of $A\beta_{(1-42)}$ on time taken to locate the quadrant of the hidden platform [$F(1, 48) = 684.7$, $p < 0.0001$] between the vehicle and their corresponding $A\beta_{(1-42)}$ lesioned groups. A significant day effect was also observed within the $A\beta_{(1-42)}$ lesioned groups [$F(3, 48) = 27.42$, $p = 0.0001$]. With specific significance between ($Day3_{A\beta(1-42)}$ vs. $Day7_{A\beta(1-42)}$, $p < 0.0001$), ($Day3_{A\beta(1-42)}$ vs. $Day10_{A\beta(1-42)}$, $p = 0.0001$), ($Day3_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$), ($Day7_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p = 0.0001$) and ($Day3_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p = 0.0328$) (Figure 2).

Table 1 shows the swimming speed and duration of time spent in opposite quadrant, expressed in cm/sec ± SEM and sec ± SEM respectively.

3.2. Cytokine levels in the plasma

Pro-inflammatory cytokines level in the plasma are illustrated in Figure 3a-c.

We observed a significant interaction [$F(3, 48) = 13.38$, $p < 0.0001$] between lesion and day. A lesion effect was found for pro-inflammatory cytokine IL-1β, as this cytokine increased in plasma of $A\beta_{(1-42)}$ lesioned groups [$F(1, 48) = 75.17$, $p < 0.0001$] when compared to the corresponding vehicle groups, with specific significant increase ($p < 0.0001$) on post-lesion day 3 and 7 groups. A day effect was found as IL-1β levels decreased progressively in post-lesion day 3 to day 14 of $A\beta_{(1-42)}$ lesioned groups [$F(3, 48) = 17.86$, $p < 0.0001$], with specific day decline between day 3 versus 10 ($p < 0.0001$), day 3 versus 14 ($p < 0.0001$), day 7 versus 10 ($p = 0.0009$) and day 7 versus 14 ($p < 0.0001$) (See Figure 3a). Similarly, a significant interaction [$F(3, 48) = 22.28$, $p < 0.0001$] was found between lesion and day for IL-6 levels in plasma. Lesion effect was found in plasma IL-6 levels as $A\beta_{(1-42)}$ lesioned groups [$F(1, 48) = 29.43$, $p < 0.0001$] increased when compared to the vehicle groups, with specific significant ($p < 0.0001$) increase in post-lesion $A\beta_{(1-42)}$ day 14 compared to its control. A significant day effect [$F(3, 48) = 27.97$, $p < 0.0001$] was also found with specific significant ($p < 0.0001$) differences between post-lesion $A\beta_{(1-42)}$ days 3, 7, 10 versus day 14 (See Figure 3b). A significant interaction was observed [$F(3, 48) = 7.32$, $p < 0.0004$] between lesion and day for TNF-α levels. We also found a significant lesion effect in plasma TNF-α levels as the $A\beta_{(1-42)}$ lesioned groups [$F(1, 48) = 38.1$, $p < 0.0001$] increased when compared to the vehicle groups, with specific significant ($p < 0.0001$ & $p = 0.0093$) increase in post-lesion $A\beta_{(1-42)}$ day 3 compared to its control and day 10 compared to its control respectively. A significant day effect [$F(3, 48) = 3.39$, $p = 0.0253$] was also observed (See Figure 3c).

Anti-inflammatory cytokine level in plasma is illustrated in Figure 3d.

We observed a significant interaction [$F(3, 48) = 57.63$, $p < 0.0001$] between lesion and day for IL-10 levels in plasma. A significant lesion effect was observed in plasma IL-10 levels as $A\beta_{(1-42)}$

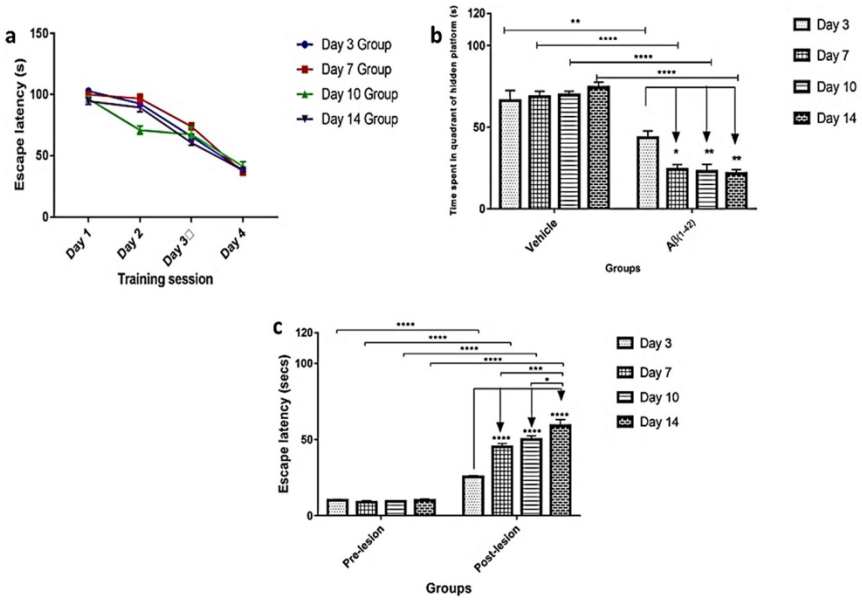

Figure 2. (a) Time taken to locate the hidden platform for all rats of day 3, 7, 10 and 14 groups pre-lesion in the MWM. Values are expressed as Mean ± SEM (n = 14/group). (b) Time spent in the quadrant of the hidden platform for all groups at post-lesion days 3, 7, 10 and 14 in the MWM. **($Day3_{vehicle}$ vs. $Day3_{Aβ(1–42)}$, $p < 0.01$), ****($Day7_{vehicle}$ vs. $Day7_{Aβ(1–42)}$, $p < 0.0001$), ****($Day10_{vehicle}$ vs. $Day10_{Aβ(1–42)}$, $p < 0.0001$), ****($Day14_{vehicle}$ vs. $Day14_{Aβ(1–42)}$, $p < 0.0001$), *($Day3_{Aβ(1–42)}$ vs. $Day7_{Aβ(1–42)}$, $p = 0.0176$), **($Day3_{Aβ(1–42)}$ vs. $Day10_{Aβ(1–42)}$, $p = 0.0097$), **($Day3_{Aβ(1–42)}$ vs. $Day14_{Aβ(1–42)}$, $p = 0.0052$). (c) Time taken to locate the quadrant of the hidden platform for all groups between pre-lesion and post-lesion days 3, 7, 10 and 14 in the MWM is significant at **** ($p < 0.0001$). ****($Day3_{Aβ(1–42)}$ vs. $Day7_{Aβ(1–42)}$, $p < 0.0001$), ****($Day3_{Aβ(1–42)}$ vs. $Day10_{Aβ(1–42)}$, $p = 0.0001$), ****($Day3_{Aβ(1–42)}$ vs. $Day14_{Aβ(1–42)}$, $p < 0.0001$), ***($Day7_{Aβ(1–42)}$ vs. $Day14_{Aβ(1–42)}$, $p = 0.0001$), **($Day3_{Aβ(1–42)}$ vs. $Day14_{Aβ(1–42)}$, $p = 0.0328$). Statistical analysis made by two-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.

Table 1
Speed to reach the target quadrant and the time spent in the quadrant opposite to the target quadrant. Data represents the average swimming speed (cm/sec ± SEM) and the average time spent (sec ± SEM) in the quadrant opposite to the target quadrant in the Morris water maze during the probe tests conducted 3 days, 7 days, 10 day and 14 days post infusion.

		Speed (cm/sec) ± SEM	Duration in the opposite Quadrant (sec) ± SEM
Day 3 group	$Aβ_{(1–42)}$	18.82 ± 1.21	36.5 ± 4.33
	Vehicle	17.69 ± 0.60	40.8 ± 3.75
Day 7 group	$Aβ_{(1–42)}$	17.97 ± 0.98	19.4 ± 2.65
	Vehicle	17.69 ± 0.60	38.9 ± 3.07
Day 10 group	$Aβ_{(1–42)}$	18.32 ± 0.76	21.3 ± 1.21
	Vehicle	17.69 ± 0.60	42.6 ± 3.15
Day 14 group	$Aβ_{(1–42)}$	19.03 ± 0.87	34.4 ± 4.21
	Vehicle	19.54 ± 1.03	44.5 ± 3.80

lesioned groups [F (1, 48) = 141.8, $p < 0.0001$] decreased when compared to the vehicle groups, with significant decrease ($p < 0.0001$) observed in post-lesion $Aβ_{(1–42)}$ days 7 and 14 groups when compared to their respective controls. A significant day effect [F (3, 48) = 172.7, $p < 0.0001$] showed progressive increase of IL-10 on days 3–14 post-lesion in both vehicle and $Aβ_{(1–42)}$ injected groups, with a significant ($p < 0.0001$) increase in Day 3 versus Day 7, Day 10, and Day 14; as well as Day 7 versus Day 14; and Day 10 versus Day 14 in the vehicle injected rats; and Day 3 versus 10; Day 3 versus 14; Day 7 versus 10 and Day 7 versus 14 in the $Aβ_{(1–42)}$ lesioned rats. (See Figure 3d).

3.3. Correlation between memory decline and plasma cytokine levels in $Aβ_{(1–42)}$ lesioned rats

The results presented in Figure 4a–d depicts the correlation between memory decline expressed as time spent in the quadrant of the hidden platform of the Morris water maze and the plasma levels of cytokines in $Aβ_{(1–42)}$ lesioned rats. This statistical analysis was performed in order to determine whether there was a relationship between the progression of the disease with respect to memory decline and the fluctuations in the plasma cytokine levels.

A strong negative correlation was observed between the time spent in the quadrant of the hidden platform and plasma IL-1β level ($r = -0.6772$, $p < 0.0001$) in the $Aβ_{(1–42)}$ lesioned rat model of AD (Figure 4a). Negative significant correlation was also observed between the time spent in the quadrant of the hidden platform and plasma IL-6 levels ($r = -0.5027$, $p = 0.0064$), in the $Aβ_{(1–42)}$ lesioned rat model of AD (Figure 4b). Furthermore, there was a strong significant negative correlation between the time spent in the quadrant of the hidden platform and plasma TNF-α level ($r = -0.646$, $p = 0.0002$) in the $Aβ_{(1–42)}$ lesioned rat model of AD (Figure 4c). However, we observed positive significant correlation between the time spent in the quadrant of the hidden platform and plasma levels of IL-10 ($r = 0.7780$, $p = 0.0001$) in the $Aβ_{(1–42)}$ lesioned rat model of AD (Figure 4d).

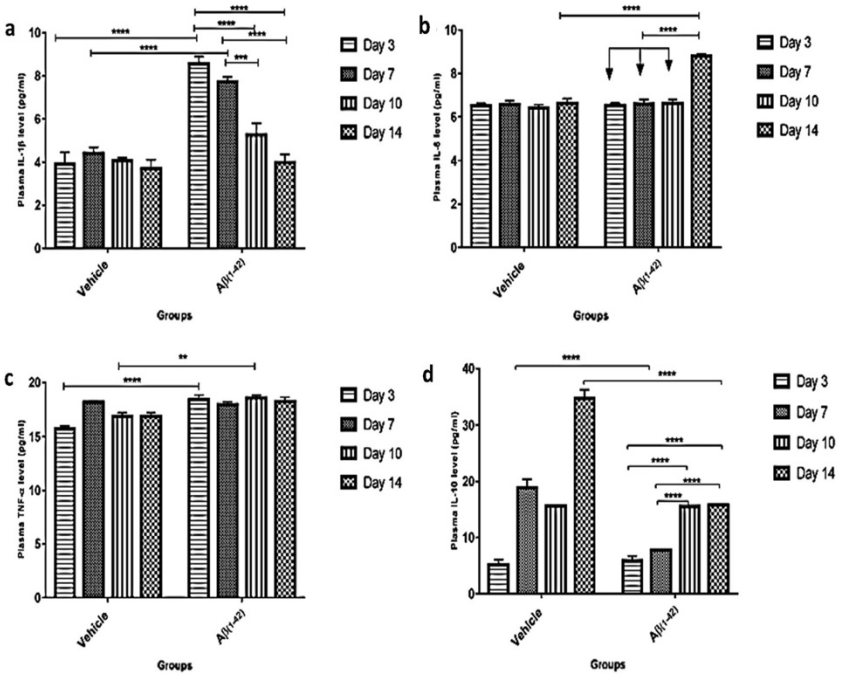

Figure 3. (a) Plasma IL-1β levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($Day3_{vehicle}$ vs $Day3_{A\beta(1-42)}$, $p < 0.0001$), **** ($Day7_{vehicle}$ vs $Day7_{A\beta(1-42)}$, $p < 0.0001$), ****($Day3_{A\beta(1-42)}$ vs $Day10_{A\beta(1-42)}$, $p < 0.0001$), ****($Day3_{A\beta(1-42)}$ vs $Day14_{A\beta(1-42)}$, $p = 0.0014$), ***($Day7_{A\beta(1-42)}$ vs $Day10_{A\beta(1-42)}$, $p = 0.0009$), ****($Day7_{A\beta(1-42)}$ vs $Day14_{A\beta(1-42)}$, $p < 0.0001$). (b) Plasma IL-6 levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($Day14_{vehicle}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$), ****($Day3_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$), ****($Day7_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$), ***($Day10_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$). (c) Plasma TNF-α levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($D3_{vehicle}$ vs $D3_{A\beta(1-42)}$, $p < 0.0001$), **($D10_{vehicle}$vs $D10_{A\beta(1-42)}$, $p = 0.0093$). (d) Plasma IL-10 levels in vehicle injected and $A\beta_{(1-42)}$ lesioned rats. ****($Day7_{vehicle}$ vs. $Day7_{A\beta(1-42)}$, $p < 0.0001$), ****($Day14_{vehicle}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$), ****($Day7_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$), ***($Day10_{A\beta(1-42)}$ vs. $Day14_{A\beta(1-42)}$, $p < 0.0001$). Values are expressed as Mean ± SEM (n = 7/group). Statistical analysis made by two-way ANOVA followed by Tukey's post-hoc test for multiple comparison.

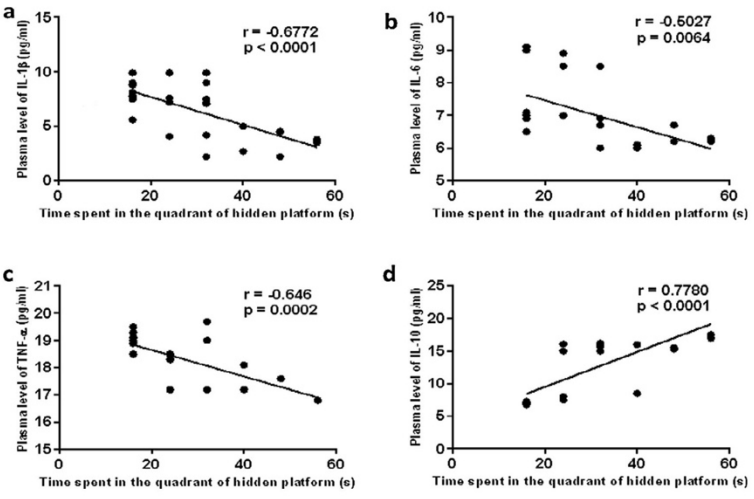

Figure 4. (a) XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-1β for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ lesioned rat model of AD. (b) XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-6 for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ rat model of AD. (c) XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of TNF-α for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ rat model of AD. (d) XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-10 for post-lesion day 3 to post-lesion day 14 groups of the $A\beta_{(1-42)}$ rat model of AD. The degree and nature of the correlation between the time spent in the quadrant of the hidden platform and the plasma parameter are given by the value of r (Pearson's correlation coefficient). A value of $p < 0.05$ was considered as statistically significant for the number of rats (n = 28) in each group.

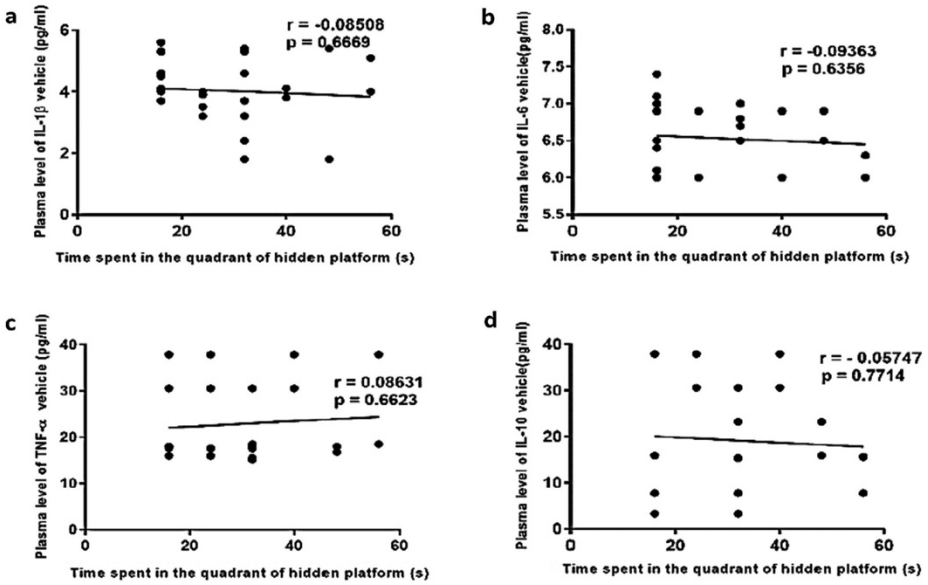

Figure 5. (a) XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-1β for post-lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. (b) XY scatter plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-6 for post- lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. (c) XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of TNF-α for post-lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. (d) XY scatters plots of the time spent in the quadrant of the hidden platform and plasma levels of IL-10 for post-lesion day 3 to post-lesion day 14 groups in the vehicle-infused (Control) rats. The degree and nature of the correlation between the time spent in the quadrant of the hidden platform and the plasma parameter are given by the value of r (Pearson's correlation coefficient). A value of $p < 0.05$ was considered as statistically significant for the number of rats ($n = 28$) in each group.

3.4. Correlation between memory decline and plasma cytokine levels in the vehicle-infused (Control) rats

The results presented in Figure 5 depicts the correlation between memory decline expressed as time spent in the quadrant of the hidden platform of the Morris water maze and the plasma levels of cytokines in the vehicle-infused (Control) rats. This statistical analysis was performed in order to determine whether the relationship observed in $A\beta_{(1-42)}$ lesioned rats with respect to memory decline and the fluctuations in the plasma cytokine levels was not due to the stress associated with the stereotaxic surgery.

Weak correlations were observed between the time spent in the quadrant of the hidden platform and: (5a) plasma IL-1β level ($r = -0.08508$, $p < 0.6669$) in the vehicle-infused (Control) rats; (Figure 5b) plasma IL-6 levels ($r = -0.09363$, $p = 0.6356$) in the vehicle-infused (Control) rats; (Figure 5c) plasma TNF-α level ($r = -0.08631$, $p = 0.6623$) in the vehicle-infused (Control) rats; (Figure 5d) plasma levels of IL-10 ($r = -0.05747$, $p = 0.7714$) in the vehicle-infused (Control) rats.

4. Discussion

This study aimed to mimic Alzheimer's disease progression following $A\beta_{(1-42)}$ infusion into the hippocampal region of rat. $A\beta_{(1-42)}$ infusion into the hippocampal area of the brain has been shown to elicit cognitive deficits as a result of its deleterious role in memory processing (Christensen, Marcussen, Wörtwein, Knudsen, & Aznar, 2008; Wang, Zhang, Li, & Zhang, 2017). In our study we firstly investigated the effect of $A\beta_{(1-42)}$ on memory and secondly evaluated how $A\beta_{(1-42)}$ influences plasma levels of pro- and anti-inflammatory markers. We further concluded by highlighting the relationship between the decline in spatial memory and plasma levels of these inflammatory markers.

In the MWM test, we observed that all rats learnt to locate the hidden platform as indicated by a decreased escape latency over the training period of days, this shows that spatial learning was not affected pre-lesion. We however observed a lesion effect following infusion of $A\beta_{(1-42)}$, as the rats spent lesser time in the quadrant of the hidden platform indicating a deficit in memory. The lesion effect was prolonged as the post-lesion day 14 group of rats also showed memory deficits. Our findings are in agreement with studies that showed that intrahippocampal $A\beta_{(1-42)}$ infusion results to impaired spatial memory (Christensen et al., 2008; Wang et al., 2017). Cognitive impairment was observed in our study following a single bilateral intrahippocampal injection of $A\beta_{(1-42)}$, resulting into a time-dependent impairment, thereby establishing our model. We also observed differences in time spent in quadrant of hidden platform between the vehicle and $A\beta_{(1-42)}$ lesioned rats in the post-lesion Day 3, 7, 10 and 14 groups. Impairment of spatial memory in animals tested at delayed time-points following $A\beta_{(1-42)}$ injection into the dorsal hippocampus has been reported by some studies (Christensen et al., 2008; Hruska & Dohanich, 2007).

Markers of neuroinflammation such as IL-1β, IL-6, TNF-α and IL-10 have been shown to have direct effects on cognition and memory (Gibertini, Newton, Friedman, & Klein, 1995; Khemka et al., 2014; Swardfager et al., 2010). In order to illuminate the interaction between these cytokines and how the resultant synergistic effect modulates memory, we engaged the use of multiplex assay and found that $A\beta_{(1-42)}$ infusion led to IL-1β, IL-6 and TNF-α elevation in the plasma of the post-lesion day 3, 7, 10 and 14 group of rats when compared to the respective vehicle group of rats. The elevated plasma levels of these

pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) observed in this study, is an indication of the unguarded inflammatory response in the progression of the disease. This is perhaps due to the induced expression of pro-inflammatory genes, which could result into loss of neurons as well as the clustering of phosphorylated tau (Von Bernhardi, Tichauer, & Eugenín, 2010).

We propose that the initial upsurge in plasma level of IL-1β may be due to its role in initiating immune response to neuro-inflammatory cascade and this initial stimulus for elevation is likely the result of microglia activation to the lesioning effect of $A\beta_{(1-42)}$ (Simard, Soulet, Gowing, Julien, & Rivest, 2006). The plasma level of IL-6 showed a clear upsurge as observed in the post-lesion day 14 group suggestive of its role as a critical cytokine controlling the transition from innate to acquired immunity, which is imperative for dealing properly with injured or infected CNS tissue (Swartz et al., 2001). Studies also suggest that the detrimental influence of IL-6 on memory processes is potentiated over a progressive period of time in animals, this may therefore account for the late elevation of this cytokine as observed in this study. This age-dependent effect is demonstrated by showing progressive memory decline in mice that had increased levels of IL-6, while a gradual memory improvement is observed in mice with reduced levels of IL-6 (Braida et al., 2004; Heyser, Masliah, Samimi, Campbell, & Gold, 1997). TNF-α levels in the plasma of $A\beta_{(1-42)}$ lesioned rats was also elevated, causing a decline in memory, although this effect is less robust than the effects of IL-1β and IL-6. This statement is true of our findings as there was a transient increase in the TNF-α levels in the lesioned rats compared to the vehicle injected rats. The process through which TNF-α mediates decline in long term potentiation (LTP) is quite alike to the process involved in the deleterious effect of IL-1β on LTP. Just like IL-1β, TNF-α prompts the activation of p38 mitogen-activated protein (MAP) kinase in the dentate gyrus (Butler, O'connor, & Moynagh, 2004). The observed significance in the day 3 $A\beta_{(1-42)}$ lesioned rats when compared to the days 7, 10 and 14 groups can be associated with the ability of p38 mitogen -activated protein (MAP) kinase to only moderates primarily the initial inhibitory effect of TNF-α on LTP. Another suggestive cause for the observed fluctuations in plasma TNF-α level is the TNF receptor deprivation and attenuation of its expression mediated by IL-10 in a bid to reduce cellular response. Our results are in agreement with studies by Wang, Tan, Yu, and Tan (2015) that demonstrated that peripheral inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, traverse the blood brain barrier to prompt neuro-inflammatory reactions under pathologic conditions. However, the observed decline in plasma level of IL-1β across the post-lesion day 3, 7, 10 and 14 groups is suggestive of the counteracting effect of IL-10 to limit inflammation by reducing the synthesis of IL-1β, subduing expression of the receptor for IL-1β and by hindering activation of the associated receptor in the brain (Strle, Zhou, Shen, Broussard, Johnson, Freund, & Kelley, 2001).

Concomitantly, we found that the plasma level of anti-inflammatory cytokine IL-10 decreased in the lesioned rats from post-lesion day 7 to the post-lesion day 14 groups. IL-10 is an anti-inflammatory cytokine that brings to bear surfeit immunomodulatory functions during an inflammatory response. The degree of pathology in the brain determines IL-10 expression, with the aim of promoting survival of neurons and glial cells, and dampening of inflammatory responses through signalling pathways (Garcia et al., 2017; Strle et al., 2001). IL-10 limits inflammation by reducing the expression of cytokine receptors and inhibiting activation of receptors (Garcia et al., 2017; Strle et al., 2001). However, the significant increase in the plasma levels of IL-10 in the vehicle-infused groups could be due to cellular stress in this case suggestive of induced by traumatic brain injury (TBI) resulting from the stereotaxic surgery. Previous studies have described overexpression of IL-10 in association with cellular stress (Le Moine et al., 1996). Our results showed a progressive time-based increased in the level of IL-10 in the vehicle groups from day 3 to day 7 followed by decrease in the day 10 group, with the uptrend continuing in day 14 group. The increase recorded in the vehicle-infused groups may be associated with increase expression of IL-10 mRNA immediately following TBI, which correlated with increase in IL-10 protein as reported by (Kamm, VanderKolk, Lawrence, Jonker, & Davis, 2006). These findings suggest that the surge in IL-10 levels may be due to an increase in local IL-10 synthesis rather than systemic IL-10 entering through a compromised blood–brain barrier (BBB). Early reports found that plasma IL-10 levels peak within the first 3 hrs (Hensler et al., 2002, 2000), while a later study showed the peak is between 5 and 6 days post-injury (Helmy, Antoniades, Guilfoyle, Carpenter, & Hutchinson, 2012). Other studies state that IL-10 levels may remain elevated for up to 22 days (Csuka et al., 1999; Maier et al., 2001) or even up to 6 months (Caplan et al., 2015). Cases of a second peak in concentration have also been reported, this may explain the slight decrease noticed on day 10 giving rise to the second peak on day 14 (Csuka et al., 1999; T Hensler et al., 2000). However, there is inconsistency in the literature as to whether IL-10 levels are more increased in the CSF or serum following TBI (Csuka et al., 1999; Maier et al., 2001), rendering it difficult to determine the source of increased IL-10 levels. It has been reported that IL-10 can abrogate memory deficit observed in models of Alzheimer's disease (Kawahara et al., 2012; Kiyota et al., 2012) probably via down-regulating the elevation of the pro-inflammatory cytokines.

Overall, the correlative test showed that increase in the peripheral levels of IL-1β, IL-6 and TNF-α in $A\beta_{(1-42)}$ lesioned rats was associated with a decrease in the time spent in the quadrant of the hidden platform. On the other hand, decrease in anti-inflammatory cytokine IL-10 was associated to a decrease in the time spent in the quadrant of the hidden platform and vice-versa. From what has already been stated from our results, we can infer that plasma cytokine levels reflect the state of the central nervous system. This goes further to indicate that there is a suggestive breakdown of the blood brain barrier, hence the pathway of communication between the central nervous system and peripheral immune system (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Wilson, Finch, & Cohen, 2002).

Based on previous findings by (Faucher, Mons, Micheau, Louis, & Beracochea, 2016), we can infer that the progressive spatial memory decline observed in our study is not due to the occurrence of amyloid plaques but caused by the molecular alterations elicited following the infusion of $A\beta_{(1-42)}$. However, stress is also a consequence of disease and the resulting psychological outcome may lead to exacerbated cellular stress as a result of oxidative damage and inflammation (Donovan et al., 2018; Hayashi, 2015; Miller & Sadeh, 2014). The study by Santos, Beckman, and Ferreira (2016) showed that microglial cells could be sensitized by stress to generate heightened responses to a second stimulus. This indicates that $A\beta_{(1-42)}$ induced stress is a probable cause for the increased plasma cytokine level observed in this study.

This suggestive evidence depicted by the altered peripheral cytokine level in this study clearly shows a role for IL-1β, IL-6 and TNF-α in the modulation of memory, despite the variations across groups for each cytokine. As reflected in our results, these cytokines most likely exert an indirect synergistic effect on memory, rather than acting independently as postulated by (Donzis & Tronson, 2014). For instance, the observed increase in IL-1β is not in isolation as increase in IL-6 and TNF-α are also observed along with a decrease in IL-10.

The limitation of this study, which is mostly related to its design, includes the use of only male rats, as female rats were excluded to prevent experimental bias that could result from hormonal changes. Nevertheless, this exclusion does not affect the validity of our results; however, the long-held assumption that the estrous cycle of female rodents renders them more variable than male rodents requires a re-appraisal. Sex differences are, in any case, incompletely explained by actions of sex hormones.

5. Conclusion

This study is consistent with a growing body of literature that

implicates inflammatory cytokines in AD pathology. The present study demonstrated that peripheral pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α are over-expressed in $A\beta_{(1-42)}$ lesioned rat, indicating a profound involvement of neuroinflammation in initiating and exacerbating AD pathology. Our results also suggest that the release of brain cytokines is a secondary response to the hallmarks of AD, occurring over a progressive phase of the disease. This study most importantly demonstrated that memory decline may be associated with cytokine dysregulation.

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Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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References

Ahmad, M. H., Fatima, M., & Mondal, A. C. (2018). Influence of microglia and astrocyte activation in the neuroinflammatory pathogenesis of Alzheimer's disease: Rational insights for the therapeutic approaches. *Journal of Clinical Neuroscience*.

Alam, Q., Zubair Alam, M., Mushtaq, G., Damanhouri, A., Rasool, G., Amjad Kamal, M., & Haque, A. (2016). Inflammatory process in Alzheimer's and Parkinson's diseases: Central role of cytokines. *Current Pharmaceutical Design, 22*(5), 541–548.

Bagheri, M., Joghataei, M.-T., Mohseni, S., & Roghani, M. (2011). Genistein ameliorates learning and memory deficits in amyloid β (1–40) rat model of Alzheimer's disease. *Neurobiology of Learning and Memory, 95*(3), 270–276.

Block, M. L., & Hong, J.-S. (2005). Microglia and inflammation-mediated neurodegeneration: Multiple triggers with a common mechanism. *Progress in Neurobiology, 76*(2), 77–98.

Bondi, M. W., Jak, A. J., Delano-Wood, L., Jacobson, M. W., Delis, D. C., & Salmon, D. P. (2008). Neuropsychological contributions to the early identification of Alzheimer's disease. *Neuropsychology Review, 18*(1), 73–90.

Braida, D., Sacerdote, P., Panerai, A. E., Bianchi, M., Aloisi, A. M., Iosuè, S., & Sala, M. (2004). Cognitive function in young and adult IL (interleukin)-6 deficient mice. *Behavioural Brain Research, 153*(2), 423–429.

Butler, M., O'connor, J., & Moynagh, P. N. (2004). Dissection of tumor-necrosis factor-α inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early—but not late—phase LTP. *Neuroscience, 124*(2), 319–326.

Caplan, B., Bogner, J., Brenner, L., Kumar, R. G., Boles, J. A., & Wagner, A. K. (2015). Chronic inflammation after severe traumatic brain injury: Characterization and associations with outcome at 6 and 12 months postinjury. *Journal of Head Trauma Rehabilitation, 30*(6), 369–381.

Cassim, S., Qulu, L., & Mabandla, M. V. (2015). Prenatal stress and early life febrile convulsions compromise hippocampal genes MeCP2/REST function in mid-adolescent life of Sprague-Dawley rats. *Neurobiology of Learning and Memory, 125*, 195–201.

Christensen, R., Marcussen, A. B., Wörtwein, G., Knudsen, G., & Aznar, S. (2008). Aβ (1–42) injection causes memory impairment, lowered cortical and serum BDNF levels, and decreased hippocampal 5-HT2A levels. *Experimental Neurology, 210*(1), 164–171.

Csuka, E., Morganti-Kossmann, M. C., Lenzlinger, P. M., Joller, H., Trentz, O., & Kossmann, T. (1999). IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: Relationship to IL-6, TNF-α, TGF-β1 and blood–brain barrier function. *Journal of Neuroimmunology, 101*(2), 211–221.

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: When the immune system subjugates the brain. *Nature Reviews Neuroscience, 9*(1), 46.

Domingues, C., AB da Cruz e Silva, O., & Henriques, A. (2017). Impact of cytokines and chemokines on Alzheimer's disease neuropathological hallmarks. *Current Alzheimer Research, 14*(8), 870–882.

Donovan, N. J., Locascio, J. J., Marshall, G. A., Gatchel, J., Hanseeuw, B. J., Rentz, D. M., ... Study, H. A. B. (2018). Longitudinal association of amyloid beta and anxious-depressive symptoms in cognitively normal older adults. *American Journal of Psychiatry, 175*(6), 530–537.

Donzis, E. J., & Tronson, N. C. (2014). Modulation of learning and memory by cytokines: Signaling mechanisms and long term consequences. *Neurobiology of Learning and Memory, 115*, 68–77.

Eckman, C. B., & Eckman, E. A. (2007). An update on the amyloid hypothesis. *Neurologic Clinics, 25*(3), 669–682.

Faucher, P., Mons, N., Micheau, J., Louis, C., & Beracochea, D. J. (2016). Hippocampal injections of oligomeric amyloid β-peptide (1–42) induce selective working memory deficits and long-lasting alterations of ERK signaling pathway. *Frontiers in Aging Neuroscience, 7*, 245.

Ferreira, S. T., Lourenco, M. V., Oliveira, M. M., & De Felice, F. G. (2015). Soluble amyloid-b oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. *Frontiers in Cellular Neuroscience, 9*, 191.

Garcia, J. M., Stillings, S. A., Leclerc, J. L., Phillips, H., Edwards, N. J., Robicsek, S. A., ... Doré, S. (2017). Role of interleukin-10 in acute brain injuries. *Frontiers in Neurology, 8*, 244.

Garthe, A., & Kempermann, G. (2013). An old test for new neurons: Refining the Morris water maze to study the functional relevance of adult hippocampal neurogenesis. *Frontiers in Neuroscience, 7*, 63.

Gibertini, M., Newton, C., Friedman, H., & Klein, T. W. (1995). Spatial learning impairment in mice infected with Legionella pneumophila or administered exogenous interleukin-1-β. *Brain, Behavior, and Immunity, 9*(2), 113–128.

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., & Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell, 140*(6), 918–934.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science, 297*(5580), 353–356.

Hayashi, T. (2015). Conversion of psychological stress into cellular stress response: Roles of the sigma-1 receptor in the process. *Psychiatry and Clinical Neurosciences, 69*(4), 179–191.

Helmy, A., Antoniades, C. A., Guilfoyle, M. R., Carpenter, K. L., & Hutchinson, P. J. (2012). Principal component analysis of the cytokine and chemokine response to human traumatic brain injury. *PLoS ONE, 7*(6), e39677.

Hensler, T., Sauerland, S., Bouillon, B., Raum, M., Rixen, D., Helling, H.-J., ... Neugebauer, E. A. (2002). Association between injury pattern of patients with multiple injuries and circulating levels of soluble tumor necrosis factor receptors, interleukin-6 and interleukin-10, and polymorphonuclear neutrophil elastase. *Journal of Trauma and Acute Care Surgery, 52*(5), 962–970.

Hensler, T., Sauerland, S., Riess, P., Hess, S., Helling, H., Andermahr, J., ... Neugebauer, E. (2000). The effect of additional brain injury on systemic interleukin (IL)-10 and IL-13 levels in trauma patients. *Inflammation Research, 49*(10), 524–528.

Heyser, C. J., Masliah, E., Samimi, A., Campbell, I. L., & Gold, L. H. (1997). Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin 6 in the brain. *Proceedings of the National Academy of Sciences, 94*(4), 1500–1505.

Hopperton, K., Mohammad, D., Trepanier, M., Giuliano, V., & Bazinet, R. (2018). Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: A systematic review. *Molecular Psychiatry, 23*(2), 177.

Hruska, Z., & Dohanich, G. P. (2007). The effects of chronic estradiol treatment on working memory deficits induced by combined infusion of β-amyloid (1–42) and ibotenic acid. *Hormones and Behavior, 52*(3), 297–306.

Kametani, F., & Hasegawa, M. (2018). Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Frontiers in Neuroscience, 12*, 25.

Kamm, K., VanderKolk, W., Lawrence, C., Jonker, M., & Davis, A. T. (2006). The effect of traumatic brain injury upon the concentration and expression of interleukin-1β and interleukin-10 in the rat. *Journal of Trauma and Acute Care Surgery, 60*(1), 152–157.

Kawahara, K., Suenobu, M., Yoshida, A., Koga, K., Hyodo, A., Ohtsuka, H., ... Nakayama, H. (2012). Intracerebral microinjection of interleukin-4/interleukin-13 reduces β-amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice. *Neuroscience, 207*, 243–260.

Khemka, V. K., Ganguly, A., Bagchi, D., Ghosh, A., Bir, A., Biswas, A., ... Chakrabarti, S. (2014). Raised serum proinflammatory cytokines in Alzheimer's disease with depression. *Aging and Disease, 5*(3), 170.

Kiyota, T., Ingraham, K. L., Swan, R. J., Jacobsen, M. T., Andrews, S. J., & Ikezu, T. (2012). AAV serotype 2/1-mediated gene delivery of anti-inflammatory interleukin-10 enhances neurogenesis and cognitive function in APP+ PS1 mice. *Gene Therapy, 19*(7), 724.

Le Moine, O., Stordeur, P., Schandené, L., Marchant, A., De Groote, D., Goldman, M., & Devière, J. (1996). Adenosine enhances IL-10 secretion by human monocytes. *The Journal of Immunology, 156*(11), 4408–4414.

Lonappan, A., Dallé, E. R. H. E., Afullo, T. J. O., & Daniels, W. M. U. (2017). Analysis of behaviour pattern in Sprague-Dawley rats using microwave techniques. *Journal of Electromagnetic Analysis and Applications, 9*(11), 155.

Maier, B., Schwerdtfeger, K., Mautes, A., Holanda, M., Müller, M., Steudel, W. I., & Marzi, I. (2001). Differential release of interleukines 6, 8, and 10 in cerebrospinal fluid and plasma after traumatic brain injury. *Shock (Augusta, GA), 15*(6), 421–426.

McKenzie, J. A., Spielman, L. J., Pointer, C. B., Lowry, J. R., Bajwa, E., Lee, C. W., & Klegeris, A. (2017). Neuroinflammation as a common mechanism associated with the modifiable risk factors for Alzheimer's and Parkinson's diseases. *Current Aging Science, 10*(3), 158–176.

Miller, M. W., & Sadeh, N. (2014). Traumatic stress, oxidative stress and post-traumatic stress disorder: Neurodegeneration and the accelerated-aging hypothesis. *Molecular Psychiatry, 19*(11), 1156–1162.

Mohajeri, M. H., Troesch, B., & Weber, P. (2015). Inadequate supply of vitamins and DHA in the elderly: Implications for brain aging and Alzheimer-type dementia. *Nutrition, 31*(2), 261–275.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods, 11*(1), 47–60.

Morris, R. G., & Baddeley, A. D. (1988). Primary and working memory functioning in

Alzheimer-type dementia. *Journal of Clinical and Experimental Neuropsychology, 10*(2), 279–296.
Norris, G. T., & Kipnis, J. (2019). Immune cells and CNS physiology: Microglia and beyond. *Journal of Experimental Medicine, 216*(1), 60–70.
Organization, W. H. (2018). Dementia fact sheet. 2017. In.
Paxinos, G., & Watson, C. (2006). *The rat brain in stereotaxic coordinates: Hard cover*. Elsevier.
Perry, R. J., Watson, P., & Hodges, J. R. (2000). The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: Relationship to episodic and semantic memory impairment. *Neuropsychologia, 38*(3), 252–271.
Platzer, C., Meisel, C., Vogt, K., Platzer, M., & Volk, H.-D. (1995). Up-regulation of monocytic IL-10 by tumor necrosis factor-α and cAMP elevating drugs. *International Immunology, 7*(4), 517–523.
Ransohoff, R. M., & Brown, M. A. (2012). Innate immunity in the central nervous system. *The Journal of Clinical Investigation, 122*(4), 1164–1171.
Santos, L. E., Beckman, D., & Ferreira, S. T. (2016). Microglial dysfunction connects depression and Alzheimer's disease. *Brain, Behavior, and Immunity, 55*, 151–165.
Sasmita, A. O., Kuruvilla, J., & Ling, A. P. K. (2018). Harnessing neuroplasticity: Modern approaches and clinical future. *International Journal of Neuroscience, 128*(11), 1061–1077.
Shastri, A., Bonifati, D. M., & Kishore, U. (2013). Innate immunity and neuroinflammation. *Mediators of inflammation, 2013*.
Simard, A. R., Soulet, D., Gowing, G., Julien, J.-P., & Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron, 49*(4), 489–502.
Steensberg, A., Fischer, C. P., Keller, C., Møller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology and Metabolism, 285*(2), E433–E437.
Stopford, C. L., Thompson, J. C., Neary, D., Richardson, A. M., & Snowden, J. S. (2012). Working memory, attention, and executive function in Alzheimer's disease and frontotemporal dementia. *Cortex, 48*(4), 429–446.
Strle, K., Zhou, J.-H., Shen, W.-H., Broussard, S. R., Johnson, R. W., Freund, G. G., ... Kelley, K. W. (2001). Interleukin-10 in the brain. *Critical Reviews™ in Immunology, 21*(5).
Swardfager, W., Lanctôt, K., Rothenburg, L., Wong, A., Cappell, J., & Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biological Psychiatry, 68*(10), 930–941.
Swartz, K. R., Liu, F., Sewell, D., Schochet, T., Campbell, I., Sandor, M., & Fabry, Z. (2001). Interleukin-6 promotes post-traumatic healing in the central nervous system. *Brain Research, 896*(1–2), 86–95.
Von Bernhardi, R., Tichauer, J. E., & Eugenín, J. (2010). Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. *Journal of Neurochemistry, 112*(5), 1099–1114.
Wang, W.-Y., Tan, M.-S., Yu, J.-T., & Tan, L. (2015). Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Annals of Translational Medicine, 3*(10).
Wang, R., Zhang, Y., Li, J., & Zhang, C. (2017). Resveratrol ameliorates spatial learning memory impairment induced by Aβ1–42 in rats. *Neuroscience, 344*, 39–47.
Watson, C., & Paxinos, G. (1986). *The rat brain in stereotaxic coordinates*. San Diego, CA: Academic Press.
Webster, S. J., Bachstetter, A. D., Nelson, P. T., Schmitt, F. A., & Van Eldik, L. J. (2014). Using mice to model Alzheimer's dementia: An overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. *Frontiers in Genetics, 5*, 88.
Wilson, C. J., Finch, C. E., & Cohen, H. J. (2002). Cytokines and cognition—the case for a head-to-toe inflammatory paradigm. *Journal of the American Geriatrics Society, 50*(12), 2041–2056.

Appendix V

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Amyloid-beta (1-42) lesion of CA1 rat dorsal hippocampus reduces contextual fear memory and increases expression of microglial genes regulating neuroinflammation

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ABSTRACT

Emerging evidence indicates that the pathogenesis of Alzheimer's disease (AD) is not confined to neuronal disruptions but robustly communicates with the brain's immune system. Genome-wide analysis suggests that several genes, which increase the risk for AD, encode for factors that regulate the glial clearance of misfolded proteins and the inflammatory reaction. This study reappraises the amyloid hypothesis by focusing on the impact of neuroinflammation in a beta-amyloid model of AD, how this possibly exacerbates the disease's progression, and the correlation between genes regulating neuroinflammation (CD33 and TREM2) with post-training recall. Male Sprague-Dawley rats were used for this study, randomly divided into a vehicle group of rats (n = 40) that were infused with phosphate-buffered saline (PBS) and an $A\beta_{(1-42)}$ group (n = 40) that were infused with the neurotoxin $A\beta_{(1-42)}$ peptide. Fear conditioning test (FCT) to assess fear memory was conducted pre and post-lesion. The polymerase chain reaction was performed to determine the expression levels of CD33 and TREM2 genes. Our results show that $A\beta_{(1-42)}$ lesion of the rat CA1 hippocampal subregion significantly reduces contextual fear memory, and this reduction was exacerbated as the post-lesion days increased. We also observed an increase in the expression levels of CD33 and TREM2 genes in the $A\beta_{(1-42)}$ lesioned groups compared to their corresponding vehicle groups. Taken together, the behavioral and gene expression data provide inferential evidence that $A\beta_{(1-42)}$ infusion impairs contextual memory by disrupting cellular pattern separation processes in the hippocampus, thus linking neuroinflammation to specific neural circuit disruption and cognitive deficit.

1. Introduction

The *cornu ammonis* 1 (CA1) is a subregion of the hippocampus, crucially involved in learning, spatial orientation, and different memory functions, including the recall of remote episodic memory and the strength of integrated memories [1,2]. It represents the primary output of the hippocampus, can thus deliver information about temporally ordered events, such as specific item–position conjunction within the sequence and specific alterations of a known sequence [3]. Apart from being the primary output of the hippocampus, the CA1 integrates information from the entorhinal cortex (EC) to the CA1; a direct projection and an indirect EC projection through cornu ammonis 3 (CA3) and dentate gyrus [4–6]. The ensued matching or mismatching of information from these two projections elicits a representation of familiar or novel items [3].

Studies have shown that CA1 is one of the most affected regions in Alzheimer's disease (AD), mainly at early stages presented as neuronal alterations [7–9]. Alzheimer's disease (AD) is the most ubiquitous cause of dementia, accounting for 50 %–70 % of all cases of dementia [10]. The neuropathology of AD includes the presence of plaques of amyloid-beta (Aβ) peptides and intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated forms of microtubule-associated tau protein in the neurons [11]. Aβ is implicated as a central pathogenic event in AD, due to impaired clearance rates in AD patients. In contrast, its production rates remain unaltered when compared with those in healthy controls [12]. Due to these changes, synaptic degeneration and death of selected populations of nerve cells take place, being accompanied by pronounced inflammation of the affected brain regions [13,14].

Genome-wide association studies implicate innate immunity in susceptibility to Alzheimer's disease (AD), with over 30 genetic loci for AD identified, many related to immune response and microglia. Among these are CD33 - Cluster of Differentiation 33 [15–17] and TREM2 -Triggering Receptor Expressed on Myeloid cells 2 [18,19] which are exclusively expressed in the brain by innate immune cells.

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CD33 encodes for Cluster of Differentiation 33, a type I transmembrane protein belonging to the class of sialic acid-binding immunoglobulin-like lectins, which mediates the cell-cell interaction and inhibit normal functions of immune cells such as phagocytosis [20]. In the brain, CD33 is mainly expressed on microglial cells, and compelling evidence indicates that CD33 facilitates Aβ-related pathology in AD by impairing microglia-mediated Aβ clearance [21,22]. Increased CD33 expression by microglia appears to result in significantly reduced amyloid-beta (Aβ) peptide phagocytosis as microglia lacking CD33 were able to phagocytose significantly greater amounts of Aβ peptide than microglia expressing CD33 at normal levels [23]. TREM2 encodes for the Triggering Receptor Expressed on Myeloid cells 2 (TREM2), a protein highly expressed on the surface of microglia in the brain [24]. It has been suggested that mutated TREM2 is less effective in lipid signaling and thus may induce microglial activation leading to neuroinflammation [25]. TREM2 has been ascribed a multitude of cellular functions, including regulation of phagocytosis, inhibition of inflammatory signaling, and promotion of cell survival [26]. Its function in phagocytosis is particularly relevant in AD as a mechanism for clearing of pathogenic proteins, such as Aβ, thereby leading to a reduction in Aβ pool. TREM2 is also important for the microglial response to amyloid pathology and impairment of TREM2 function may alter plaque morphology and increase plaque-associated toxicity [27]. Moreover, data from several animal models show that TREM2 takes part in the microglia response to Aβ plaques [28,29]. These results suggest that both TREM2 and CD33, which are involved in the innate immunological response, are important for AD development. Hence, it confirms the significant role of neuroinflammatory processes in the pathogenesis of AD.

Most studies on the effects of neuroinflammation on cognition have examined the effect on memory acquisition or consolidation. In contrast, there are relatively few reports on whether neuroinflammation affects memory retrieval. Therefore, this study reappraises the amyloid hypothesis by focusing on the impact of neuroinflammation in a beta-amyloid model of AD, how this possibly exacerbates the progression of the disease and the correlation between genes regulating neuroinflammation (CD33 and TREM2) with post-training recall.

2. Materials and methods

2.1. Animals

All protocols involved studies on male Sprague-Dawley rats and were approved by the University of KwaZulu-Natal Animal Care Ethics Committee (AREC/015/018D). The animals were housed under controlled conditions of temperature (25 ± 1 °C) and luminosity (12/12 h light/dark cycle, with lights, turned on 7:00 a.m. and turned off 7:00 p.m.). Food and water were provided *ad libitum*. The animals were randomly divided into a vehicle group of rats (n = 40) that were infused with phosphate-buffered saline (PBS) and an $A\beta_{(1-42)}$ group (n = 40) that were infused with the neurotoxin $A\beta_{(1-42)}$ peptide. Each group was further sub-divided into four groups (Day 3 group: animals euthanized 3 days after infusion; Day 7 group: animals euthanized 7 days after infusion; Day 10 group: animals euthanized 10 days after infusion, and Day 14 group: animals euthanized 14 days after infusion) (n = 10/group). Before all procedures, animals were allocated to the experimental room for habituation, and all efforts were made to minimize pain, suffering, or animal discomfort. All behavioral experiments were conducted in the mornings, beginning at 8:00 a.m.

2.2. $A\beta_{(1-42)}$ lesion of CA1 hippocampal subregion (Neuroinflammatory model of AD)

The relevance of using intrahippocampal $A\beta_{(1-42)}$ as an animal model of AD has been reviewed by [30,31], and overall procedures employed in the use of this model are detailed by [32]. After anesthesia with ketamine/xylazine cocktail at 100 mg/kg ketamine and 5 mg/kg xylazine by injecting intraperitoneally (IP), the depth of anesthesia was confirmed by the loss of toe pinch reflex. The animals were subjected to stereotaxic surgery for $A\beta_{(1-42)}$ peptide (5 μg/5 μl) or control 0.01 M phosphate-buffered saline (PBS) infusion bilaterally into dorsal hippocampus subregion CA1 from bregma (AP: −4.8 mm; ML: ± 3.4 mm and DV: −3.0). The coordinates were chosen according to a rat brain atlas [33], and microinjections were performed using a 10 μL microsyringe (Hamilton Company, Reno, NV, USA) connected to a 26-gauge steel needle into the brain sites. Temgesic was administered subcutaneously for pain relief.

2.3. Drugs and reagents

$A\beta_{(1-42)}$ peptide was purchased from DLD Scientific (Durban North, South Africa). Ketamine, xylazine, and temgesic were obtained from Sigma (ST. Louis MO, USA). Real-time PCR kits were purchased from Bio-Rad Laboratories (CA, USA).

2.4. Assessment of fear learning and memory

The behavioral paradigm used to assess fear learning and memory in this study is the contextual and cued fear conditioning test [34–36]. In this test, animals are exposed to a pairing of an auditory cue (neutral or conditioned stimulus - CS) with an electric foot shock (aversive or unconditioned stimulus - US). They respond to the fear-producing stimulus by displaying freezing behavior, measured as an index of associative fear learning and memory [37,38]. The contextual and cued Fear Conditioning test was performed as described by [39] in a 26 × 32 × 21 cm operant chamber. In this study, the chamber was equipped with a speaker and a stainless-steel rod floor through which a foot shock was administered.

On day 1, training consisted of placing the rat in a conditioning chamber, and the rats were accorded free access in the chamber for 3 min.. After that, the auditory cue (tone) was presented as a CS for 30 s, and a (1.0 mA) foot shock was given to the rats as a US during the last 2 s of the sound. The presentation of the CS-US pairing was repeated three times per session (120, 240, and 360 s after the beginning of the conditioning) to strengthen the association. The rats were left in the chamber for a length of time (90 s) after the last presentation to further establish the association between the context of the chamber and the aversive experience. Context test was measured 24 h after conditioning

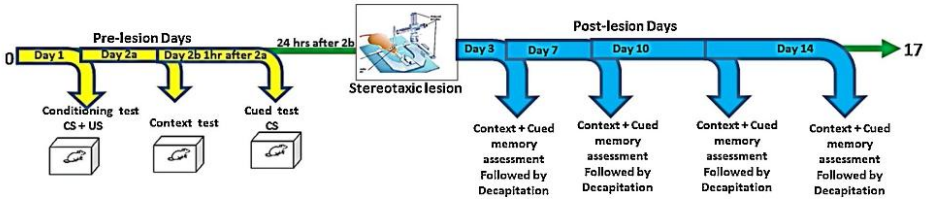

Fig. 1. Experimental timeline.

(regarded as day 2a, see Fig. 1); the rats were returned to the same conditioning chamber and allowed to explore the chamber for 3 min. freely without CS and US presentations. They were scored for freezing behavior to measure contextually conditioned fear. The cued test was conducted on the same day (regarded as day 2b, see Fig. 1) as the context test. In this test, rats were placed into another testing chamber with different properties, providing a new context that was not related to the conditioning chamber for another 3 min. At the end of the initial 3 min, the auditory cue that was presented at the time of conditioning was given to rats for 3 min in the novel context environment. In this protocol, the cued test was performed a few hours after the context test. Rats were given free access in the chamber for 360 s. During this first 3 min, neither a CS nor US was presented, and after that, a CS (a 55 dB white noise) was presented for the last 3 min. Rats (n = 10/ group) were trained and tested for two consecutive days before lesioning and post-training recall was assessed for all groups (see Fig. 1).

2.5. Euthanization and neurochemical analysis

Animals were euthanized via deep anaesthesia using the halothane chamber. The excised brain tissues were immediately placed in frozen 0.9 % saline slush before the hippocampus was carefully dissected out and quickly frozen in a bio-freezer at −80 °C.

2.6. Real-time quantitative PCR (qPCR) analysis for CD33 and TREM2 mRNA levels

Hippocampal tissue was suspended in lysis buffer at a ratio of 1:6, the samples were homogenized using a Tissue Sonicator (CML-4, Fisher, USA), and total RNA isolation was carried out as per manufacturer's protocol (ZR RNA MiniPrep™, USA). A NanoDrop Spectrophotometer (A260: A280 ratio), was used to determine the quality and concentration of the total RNA, purity of 1.7–2.1 recommended for use in the construction of cDNA was maintained. Total RNA was reverse-transcribed into cDNA using the iScript™cDNA synthesis kit and run in duplicate in a 20 µl reaction volume in a 96-well plate format, containing 500 nM of each oligonucleotide primer and SYBR Green PCR Master Mix (Bio-Rad). The primers (Table 1) were synthesized by Inqaba Biotech (Pretoria, South Africa), with Gapdh serving as the reference gene. Primer sequences were reconstituted in RNA nuclease-free water according to the manufacturer's report and were added to a master mix comprising of SYBR green dye and nuclease-free water. PCR was performed using Lightcycler 96 consisting of denaturation at 95 °C for 10 s, and then 20 s of primer annealing at 60 °C (for TREM2), 60 °C (for CD33) and 60 °C (for Gapdh), followed by final elongation at 72 °C for 20 s. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to normalize the expression of the target [40]. We ensured that all qPCR reactions produced a single peak with the melt curve analysis. Relative expression levels were calculated after normalization against the reference gene GAPDH using $2-\Delta\Delta CT$ method, and results were expressed as 2−(gene of interest mean Ct value–control gene mean Ct value) for the qRT-PCR experiments [41]. Our qPCR analyses followed the most recommended criteria for minimal information for publication of quantitative real-time PCR experiments (MIQE) [42].

2.7. Statistical analysis

The data were analysed using the software program GraphPad Prism (version 7.0, San Diego, California, USA). The Shapiro-Wilk test was used to test for normality of distribution and where data met requirements, parametric test was used. The main factors in each analysis included lesion and day. Two-way analysis of variance (ANOVA) was used. A p-value < 0.05 was considered significant in all analysis. Significant main effects were followed by Tukey's post hoc test.

3. Results

3.1. $A\beta_{(1-42)}$ lesion of CA1 reduces contextual fear memory

Contextual fear memory was assessed pre-lesion and post-lesion with $A\beta_{(1-42)}$ on days 3, 7, 10 and 14. A significant interaction ($F(3,72) = 5.894$, $p = 0.0016$) was observed between lesion and day, the post-lesioned $A\beta_{(1-42)}$ group of rats showed reduced freezing behavior relative to the pre-lesioned group when placed in the context, as measured by the mean percent of time freezing across all three minutes (main effect of lesion : ($F(1,72) = 82.22$, $p < 0.0001$). Freezing also varied significantly as a function of day across the post-lesion days in the context (main effect of day: ($F(3,72) = 8.806$, $p < 0.0001$) (Fig. 2).

3.2. $A\beta_{(1-42)}$ lesion of CA1 unalters cued fear memory

Cued fear memory was assessed on the same day as the contextual fear memory, pre-lesion and post-lesion with $A\beta_{(1-42)}$ on days 3,7,10 and 14. No significant difference in freezing was detected between the $A\beta_{(1-42)}$ post-lesioned group and the pre-lesion group of rats. (main effect of lesion: $F(1,72) = 0.02609$, $p = 0.8724$); main effect of day: $F(3,72) = 0.7217$, $p = 0.5439$), nor an interaction ($F(3,72) = 0.2348$, $p = 0.8717$) was detected, therefore *post hoc* tests within each group were not performed (Fig. 3).

3.3. Hippocampus modulates contextual but not cued memory in $A\beta_{(1-42)}$ rat model of AD

A significant ($F(3,72) = 9.143$, $p < 0.0001$) interaction was observed between the vehicle-treated and $A\beta_{(1-42)}$ lesioned rats in the contextual but not in the cued tests. In addition, we observed a main effect of lesion ($F(1,72) = 10.15$, $p < 0.0001$) was observed between the vehicle-treated group and the post-lesion $A\beta_{(1-42)}$ group in the context test but not in the cued test, furthermore, a significant day effect ($F(3,72) = 105.5$, $p < 0.0001$) between the vehicle-treated and the post-lesion $A\beta_{(1-42)}$ group in the context test was observed (Fig. 4).

3.4. Upregulated mRNA CD33 and TREM2 expression

Significant lesion effect of intrahippocampal injection of $A\beta_{(1-42)}$ on relative CD33 gene expression was observed [$F(4, 45) = 90.79$, $p < 0.0001$], with upregulated expressions on post-lesion day 3 ($p < 0.0001$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the vehicle injected group. Significant downregulated day effect was observed within the $A\beta_{(1-42)}$ lesioned groups at post-lesioned day 7 vs day 14 ($p = 0.0483$) and day 3 vs. day

Table 1
Nucleotide sequence of primers used for real-time PCR.

Gene	Forward	Reverse
TREM2	5′-AAGATGCTGGAGACCTCTGG-3′	5′-GGATGCTGGCTGTAAGAAGC-3′
CD33	5′-ATGAGAGAGCTGGTCCTGGT-3′	5′-CCCATGTGCACTGACAGCTT-3′
GAPDH	5′-TGTGAACGGATTTGGCCGTA-3′	5′-ATGAAGGGGTCGTTGATGGC-3′

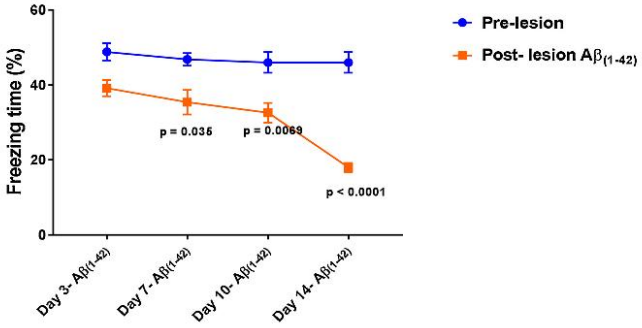

Fig. 2. Mean percent freezing time in pre and post-lesion $A\beta_{(1–42)}$ contextual fear test.

14 ($p = 0.0083$) (Fig. 5A). Significant lesion effect of intrahippocampal injection of $A\beta_{(1–42)}$ on relative TREM2 gene expression [$F(4, 45) = 39.5$, $p < 0.0025$] with upregulated expressions on post-lesion day 3 ($p = 0.0002$), day 7 ($p < 0.0001$), day 10 ($p < 0.0001$) and day 14 ($p < 0.0001$) when compared to the vehicle injected group was observed. Significant upregulated day effect was also observed within the $A\beta_{(1–42)}$ lesioned groups at post-lesioned day 3 vs day 10 ($p < 0.0001$); day 3 vs. day 14 ($p < 0.0001$); day 7 vs day 10 ($p = 0.0004$) and day 7 vs. day 14 ($p < 0.0001$) (Fig. 5B).

3.5. Correlative expressions of CD33 and TREM2 genes

Strong negative correlations were observed between the relative expressions of CD33 and TREM2 genes on post-lesion day 3 ($r = -0.9664$, $p < 0.0001$) (Fig. 6a); post-lesion day 7 ($r = -0.8777$, $p = 0.0008$) (Fig. 6b); post-lesion day 10 ($r = -0.9060$, $p = 0.0003$) (Fig. 6c) and post-lesion day 14 ($r = -0.8649$, $p = 0.0012$) (Fig. 6d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Fig. 6).

3.6. Correlation between CD33 mRNA expression with contextual freezing time

Strong negative correlations were observed between the relative expressions of CD33 mRNA and contextual freezing time on post-lesion day 3 ($r = -0.9285$, $p < 0.0001$) (Fig. 7a); post-lesion day 7 ($r = -0.9047$, $p < 0.0003$) (Fig. 7b); post-lesion day 10 ($r = -0.9328$, $p < 0.0001$) (Fig. 7c) and post-lesion day 14 ($r = -0.9339$, $p < 0.0001$) (Fig. 7d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Fig. 7).

3.7. Correlation between TREM2 mRNA expression with contextual freezing time

Strong positive correlation was observed between the relative expression TREM2 mRNA and contextual freezing time on post-lesion day 3 ($r = 0.9829$, $p < 0.0001$) (Fig. 8a); post-lesion day 7 ($r = 0.9093$, $p < 0.0003$) (Fig. 8b); post-lesion day 10 ($r = 0.8550$, $p < 0.0016$) (Fig. 8c) and post-lesion day 14 ($r = 0.9046$, $p < 0.0003$) (Fig. 8d) in the $A\beta_{(1–42)}$ lesioned rat model of AD (Fig. 8).

4. Discussion

In this study, we assessed the impact of $A\beta_{(1–42)}$ lesion of CA1 neuronal activity, as a model of AD, on fear memory after acquisition. We further investigated the expression of some microglial genes regulating neuroinflammation, such as CD33 and TREM2 and correlated these expressions to post-training recall. Our observed data provide credible reinforcement for a compelling interaction between immune system activation and neural circuit activity. Notably, this occurred when levels of IL-1β, TNF- α and IL-6 were elevated in the dorsal hippocampus of $A\beta_{(1–42)}$ lesioned rats as opposed to the vehicle infused rats [31], a structure highly mandatory for behavioral context discrimination [43].

We observed a difference in the behavioral outcomes after CA1 lesion at all post-lesion intervals, observed as decreased freezing in context fear memory but not in cued fear memory. This decreased freezing indicates that the $A\beta_{(1–42)}$ lesioned rats have impaired memory for the context test but not the cued test. These results are indicative of the specified actions of CA1 as reported by previous studies, where context fear conditioning has been shown to require the activity of neurons in the hippocampus, specifically in CA1 region, for context

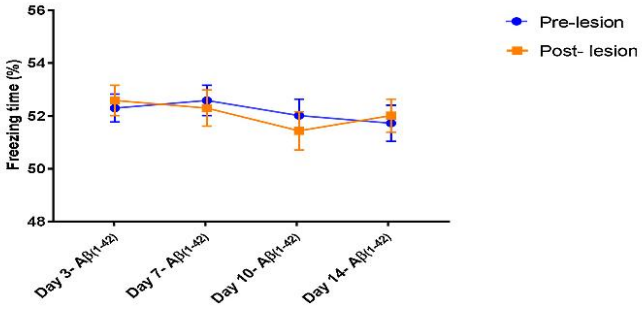

Fig. 3. Mean percent freezing time in pre and post-lesion $A\beta_{(1–42)}$ cued fear test.

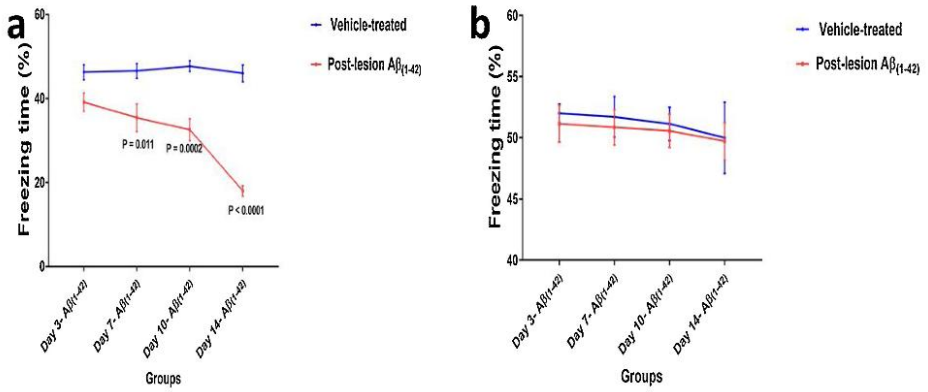

Fig. 4. Mean percent freezing time in (a) contextual and (b) cued fear test across post-lesion days 3, 7, 10 and 14 for the vehicle-treated and $A\beta_{(1-42)}$ lesioned groups.

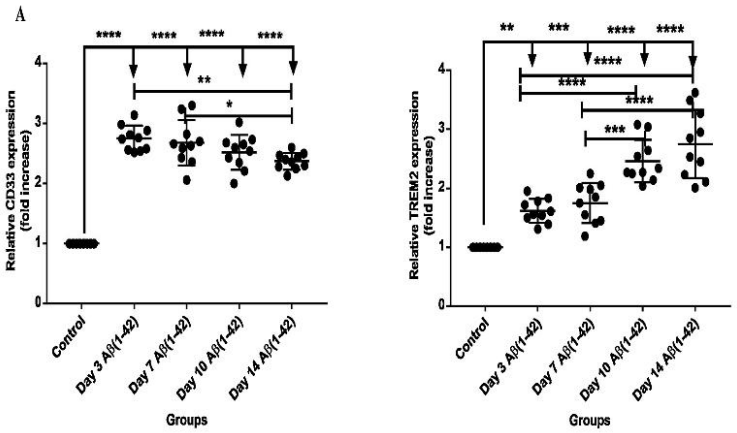

Fig. 5. (A). Relative CD33 (B) TREM2 expression in the hippocampus of $A\beta_{(1-42)}$ lesioned rats across different post-lesion days. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (two-way ANOVA followed by Tukey's post-hoc test). All data are expressed as means ± SEM. (n = 10/ group).

encoding [44–46], while cued fear conditioning requires activity from auditory regions and amygdala structures but do not usually require the hippocampus [45,47]. These latter studies are suggestive reasons for the unaltered cued fear memory in $A\beta_{(1-42)}$ lesioned rats as observed in this study, as both vehicle-infused and $A\beta_{(1-42)}$ lesioned rats exhibited similar levels of memory in the cue test. Previous studies have also shown that neurons in the dorsal CA1 region of rats have strong spatial properties, with well-defined place fields capable of encoding a context [48,49]. Hence, lesions to or inhibition of the dorsal hippocampus impair context fear memory [50,51], as observed in the dorsal CA1 of this model.

$A\beta_{(1-42)}$ lesion of the CA1 subregion of the hippocampus is known to elicit neuroinflammatory changes as a result of prolonged activation of microglia cells, causing subsequent secretion of inflammatory cytokines [31]. Our results showed that mRNA levels of TREM2 and CD33 were increased at all intervals in the hippocampus of $A\beta_{(1-42)}$ lesioned rats relative to the respective vehicle-infused rats. We observed a significant 2.8 fold increase of CD33 expression in the day 3 $A\beta_{(1-42)}$ lesioned group of rats relative to control, with a slight decrease over the subsequent post-lesion intervals, probably due to the counteracting increased expression of TREM2. The increased mRNA CD33 expression in $A\beta_{(1-42)}$ lesioned rats denotes phagocytotic inhibition, indicating that there is an impaired microglial mediated Aβ clearance. Our results of increased expression of CD33 is consistent with the previous study by [23], which showed increased mRNA levels of CD33 in AD patients. Expression of TREM2, on the other hand, showed a progressive increase across the post-lesion intervals with an initial significant 1.8 fold increase in the $A\beta_{(1-42)}$ lesioned rats relative to the control. TREM2 expression increase in the $A\beta_{(1-42)}$ lesioned rats indicates a heightened state of microgliosis, as microglial cells are over-activated to facilitate phagocytosis. Our results suggest that TREM2 mediates Aβ-induced microglial response, as supported by previous study by [52]. Although mRNA levels may not be an accurate estimate of protein level since gene expression is controlled at different stages and in many different ways. However, the ability to quantify the level at which a particular gene is expressed provides much valuable information as reliance on mRNA measurements can be traced both to the relative ease of availability of mRNA compared to protein data [53,54]. Moreover, the mRNA expression levels can be used to deduce the functionality of genes with the implicit assumption that differentially expressed mRNAs impact their respective experimental conditions via differences in proteins.

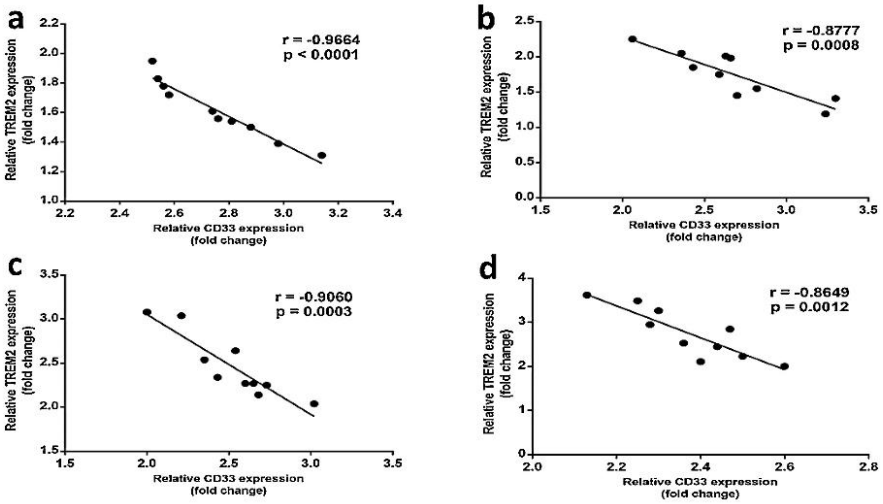

Fig. 6. XY scatter plots of the relative expressions of CD33 and TREM2 on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1-42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats ($n = 10$) in each group.

Correlative expression of both genes shows an inverse relationship, depicting an interactive platform and a possible explanation to the crosstalk between these genes, as reported by Griciuc et al., 2019. The correlative mRNA expression of the genes with contextual fear freezing time reflects the role of these genes in memory. The negative correlation of CD33 mRNA expression level with contextual freezing time indicates an impaired memory as mRNA CD33 expression increases. A study by Griciuc et al., in which the T allele of SNP rs3865444, which led to the reduction of CD33 levels in the brain, was found to be linked with decreased amyloid plaque burdens in the brain cortex of AD patients supports our finding [23]. Further corroborating this finding, Bradshaw and colleagues showed that the C allele of SNP rs3865444, which caused the elevation in CD33 levels, was associated with a higher burden of fibrillar amyloid in older asymptomatic individuals [55]. Animal models of AD have also provided more direct evidence on the association between CD33 and Aβ pathology, as amyloid precursor protein/presenilin 1 (APP/PS1) transgenic mice lacking CD33 exhibited significant lower Aβ levels as well as reduced amyloid plaque burden in the brain [23]. These observations indicated a pathogenic role of CD33 in the facilitation of Aβ pathology. More so, the deletion of CD33 in APP/PS1 transgenic mice did not alter the APP processing or the levels of pro-inflammatory cytokines in the brain, implying that CD33 contributed to Aβ pathology by interfering with Aβ clearance rather than promoting its generation [23].

The positive correlation between TREM2 mRNA expression level and contextual freezing time indicates that memory is enhanced as

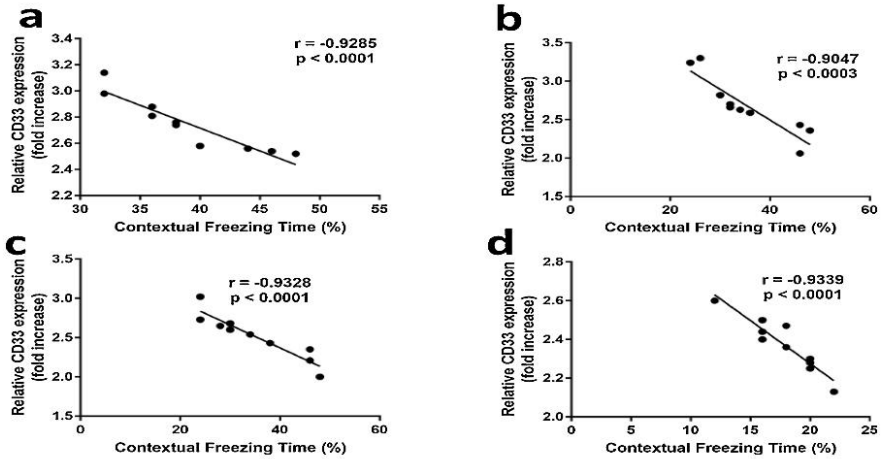

Fig. 7. XY scatter plots of the relative expressions of CD33 and contextual freezing time on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1-42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats ($n = 10$) in each group.

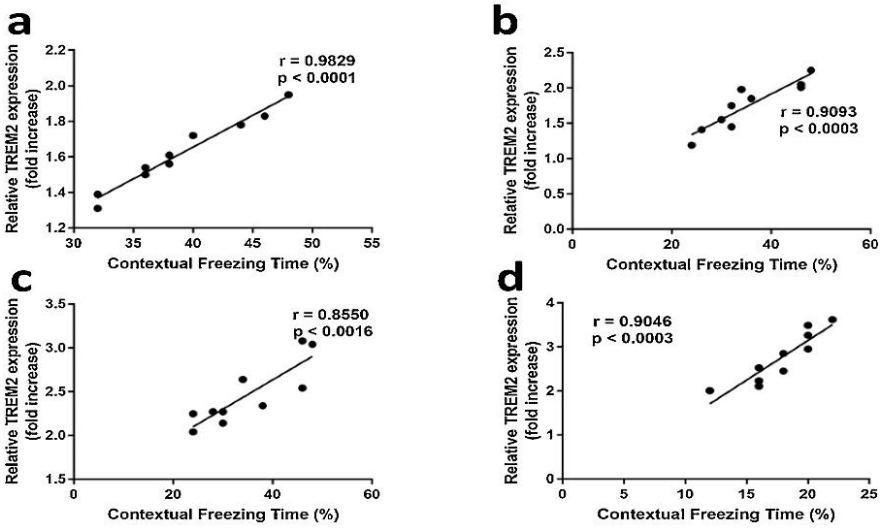

Fig. 8. XY scatter plots of the relative expressions of TREM2 and contextual freezing time on (a) post-lesion day 3 (b) post-lesion day 7 (c) post-lesion day 10 and (d) post-lesion day 14 with $A\beta_{(1-42)}$. A value of $p < 0.05$ was considered as statistically significant for the number of rats ($n = 10$) in each group.

TREM2 mRNA level increases since increased freezing time interprets to greater learning ability, as the better the memory, the more time the animal spends in freezing behavior. Studies supporting our results by Jiang et al., using APPswe/PS1dE9 mice compared to wildtype resulted in spatial learning and memory deficit when subjected to the Morris water maze test, were reversed with subsequently observed improved performance on insertion of TREM2 vector into these mice [56,57]. Lee et al., also validate the role of TREM2 in memory using the 5XFAD mice, which demonstrated impaired contextual memory as opposed to 5XFAD/TREM2 OE and APPPS1/TREM2 OE which had similar performance to the wildtype, indicating that TREM2 overexpression improves cognition in amyloid mouse models since TREM2 overexpression alone did not affect performance [58].

5. Conclusion

Our findings lend support to the knowledge that neuroinflammatory response resulting from $A\beta_{(1-42)}$ lesion CA1 impacts both neural circuit activity and expression of microglial genes regulation neuroinflammation, indicating a dynamic interaction between the immune and nervous systems. This integrative approach can be used to query the role of neuroinflammation in memory processes, and, importantly, to identify the possibility of blocking the immune response in the brain to restore cell network activity and cognitive function.

Author statement

All authors contributed equally to the manuscript.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

[1] D. Amaral, P. Lavenex, Hippocampal Neuroanatomy, (2007).
[2] T. Bartsch, J. Döhring, A. Rohr, O. Jansen, G. Deuschl, CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness, Proc. Natl. Acad. Sci. 108 (42) (2011) 17562–17567.
[3] S.A. Barrientos, V. Tiznado, Hippocampal CA1 subregion as a context decoder, J. Neurosci. 36 (25) (2016) 6602–6604.
[4] K.C. Bittner, C. Grienberger, S.P. Vaidya, A.D. Milstein, J.J. Macklin, J. Suh, et al., Conjunctive input processing drives feature selectivity in hippocampal CA1 neurons, Nat. Neurosci. 18 (8) (2015) 1133.
[5] T. Jarsky, A. Roxin, W.L. Kath, N. Spruston, Conditional dendritic spike propagation following distal synaptic activation of hippocampal CA1 pyramidal neurons, Nat. Neurosci. 8 (12) (2005) 1667.
[6] N. Van Strien, N. Cappaert, M. Witter, The anatomy of memory: an interactive overview of the parahippocampal–hippocampal network, Nat. Rev. Neurosci. 10 (4) (2009) 272.
[7] T. Gómez-Isla, R. Hollister, H. West, S. Mui, J.H. Growdon, R.C. Petersen, et al., Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease, Ann. Neurol. 41 (1) (1997) 17–24.
[8] M. Llorens-Martín, L. Blazquez-Llorca, R. Benavides-Piccione, A. Rabano, F. Hernandez, J. Avila, J. DeFelipe, Selective alterations of neurons and circuits related to early memory loss in Alzheimer's disease, Front. Neuroanat. 8 (2014) 38.
[9] X. Yang, C. Yao, T. Tian, X. Li, H. Yan, J. Wu, et al., A novel mechanism of memory loss in Alzheimer's disease mice via the degeneration of entorhinal–CA1 synapses, Mol. Psychiatry 23 (2) (2018) 199.
[10] B. Winblad, P. Amouyel, S. Andrieu, C. Ballard, C. Brayne, H. Brodaty, et al., Defeating Alzheimer's disease and other dementias: a priority for European science and society, Lancet Neurol. 15 (5) (2016) 455–532.
[11] B.T. Hyman, C.H. Phelps, T.G. Beach, E.H. Bigio, N.J. Cairns, M.C. Carrillo, et al., National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease, Alzheimer's Dement. 8 (1) (2012) 1–13.
[12] K.G. Mawuenyega, W. Sigurdson, V. Ovod, L. Munsell, T. Kasten, J.C. Morris, et al., Decreased clearance of CNS β-amyloid in Alzheimer's disease, Science 330 (6012) (2010) 1774–1774.
[13] M. Goedert, Alzheimer's and Parkinson's diseases: the prion concept in relation to assembled Aβ, tau, and α-synuclein, Science 349 (6248) (2015) 1255555.
[14] M. Martucci, S. Sarria, M. Toledo, P. Coscojuela, C. Vert, S. Siurana, et al., Cerebral

amyloid angiopathy-related inflammation: imaging findings and clinical outcome, Neuroradiology 56 (4) (2014) 283–289.
[15] L. Bertram, R.E. Tanzi, Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses, Nat. Rev. Neurosci. 9 (10) (2008) 768.
[16] P. Hollingworth, D. Harold, R. Sims, A. Gerrish, J.-C. Lambert, M.M. Carrasquillo, et al., Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease, Nat. Genet. 43 (5) (2011) 429.
[17] A.C. Naj, G. Jun, G.W. Beecham, L.-S. Wang, B.N. Vardarajan, J. Buros, et al., Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease, Nat. Genet. 43 (5) (2011) 436.
[18] R. Guerreiro, A. Wojtas, J. Bras, M. Carrasquillo, E. Rogaeva, E. Majounie, et al., TREM2 variants in Alzheimer's disease, N. Engl. J. Med. 368 (2) (2013) 117–127.
[19] T. Jonsson, H. Stefansson, S. Steinberg, I. Jonsdottir, P.V. Jonsson, J. Snaedal, et al., Variant of TREM2 associated with the risk of Alzheimer's disease, N. Engl. J. Med. 368 (2) (2013) 107–116.
[20] P.R. Crocker, J.C. Paulson, A. Varki, Siglecs and their roles in the immune system, Nat. Rev. Immunol. 7 (4) (2007) 255.
[21] E.M. Bradshaw, L.B. Chibnik, B.T. Keenan, L. Ottoboni, T. Raj, A. Tang, et al., CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology, Nat. Neurosci. 16 (7) (2013) 848.
[22] K. Malpass, Alzheimer disease: functional dissection of CD33 locus implicates innate immune response in Alzheimer disease pathology, Nat. Rev. Neurol. 9 (7) (2013) 360.
[23] A. Griciuc, A. Serrano-Pozo, A.R. Parrado, A.N. Lesinski, C.N. Asselin, K. Mullin, et al., Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta, Neuron 78 (4) (2013) 631–643.
[24] H. Neumann, K. Takahashi, Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis, J. Neuroimmunol. 184 (1–2) (2007) 92–99.
[25] F.L. Yeh, Y. Wang, I. Tom, L.C. Gonzalez, M. Sheng, TREM2 binds to apolipoproteins, including APOE and CLU/APOJ, and thereby facilitates uptake of amyloid-beta by microglia, Neuron 91 (2) (2016) 328–340.
[26] M. Colonna, TREMs in the immune system and beyond, Nat. Rev. Immunol. 3 (6) (2003) 445–453.
[27] J.D. Ulrich, T.K. Ulland, M. Colonna, D.M. Holtzman, Elucidating the role of TREM2 in Alzheimer's disease, Neuron 94 (2) (2017) 237–248.
[28] T.K. Ulland, M. Colonna, TREM2—a key player in microglial biology and Alzheimer disease, Nat. Rev. Neurol. 14 (11) (2018) 667–675.
[29] J.D. Ulrich, M.B. Finn, Y. Wang, A. Shen, T.E. Mahan, H. Jiang, et al., Altered microglial response to Aβ plaques in APPPS1-21 mice heterozygous for TREM2, Mol. Neurodegener. 9 (1) (2014) 20.
[30] J.G. McLarnon, J.K. Ryu, Relevance of Aβ 1-42 intrahippocampal injection as an animal model of inflamed Alzheimer's disease brain, Curr. Alzheimer Res. 5 (5) (2008) 475–480.
[31] O.F. Shallie, E. Dalle, M.V. Mabandla, Memory decline correlates with increased plasma cytokines in amyloid-beta (1–42) rat model of Alzheimer's disease, Neurobiol. Learn. Mem. 169 (2020) 107187.
[32] Y.Y. Jean, J. Baleriola, M. Fà, U. Hengst, C.M. Troy, Stereotaxic infusion of oligomeric amyloid-beta into the mouse hippocampus, JoVE (Journal of Visualized Experiments) (100) (2015) e52805.
[33] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition, Elsevier, 2006.
[34] J.N. Crawley, What's Wrong With My Mouse?: Behavioral Phenotyping of Transgenic and Knockout Mice, John Wiley & Sons, 2007.
[35] M.S. Fanselow, A.M. Poulos, The neuroscience of mammalian associative learning, Annu. Rev. Psychol. 56 (2005) 207–234.
[36] J.E. LeDoux, Emotion circuits in the brain, Annu. Rev. Neurosci. 23 (1) (2000) 155–184.
[37] M.S. Fanselow, Contextual fear, gestalt memories, and the hippocampus, Behav. Brain Res. 110 (1–2) (2000) 73–81.
[38] S. Maren, Neurobiology of Pavlovian fear conditioning, Annu. Rev. Neurosci. 24 (1) (2001) 897–931.
[39] H. Shoji, K. Takao, S. Hattori, T. Miyakawa, Contextual and cued fear conditioning test using a video analyzing system in mice, JoVE (Journal of Visualized Experiments) (85) (2014) e50871.
[40] C. Guo, S. Liu, M.-Z. Sun, Novel insight into the role of GAPDH playing in tumor, Clin. Transl. Oncol. 15 (3) (2013) 167–172.
[41] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, Methods 25 (4) (2001) 402–408.
[42] S.A. Bustin, V. Benes, J.A. Garson, J. Hellemans, J. Huggett, M. Kubista, et al., The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments, Clin. Chem. 55 (4) (2009) 611–622.
[43] J. Czerniawski, J.F. Guzowski, Acute neuroinflammation impairs context discrimination memory and disrupts pattern separation processes in hippocampus, J. Neurosci. 34 (37) (2014) 12470–12480.
[44] I. Goshen, M. Brodsky, R. Prakash, J. Wallace, V. Gradinaru, C. Ramakrishnan, K. Deisseroth, Dynamics of retrieval strategies for remote memories, Cell 147 (3) (2011) 678–689.
[45] J. Muller, K.P. Corodimas, Z. Fridel, J.E. LeDoux, Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli, Behav. Neurosci. 111 (4) (1997) 683.
[46] M. Zelikowsky, S. Hersman, M.K. Chawla, C.A. Barnes, M.S. Fanselow, Neuronal ensembles in amygdala, hippocampus, and prefrontal cortex track differential components of contextual fear, J. Neurosci. 34 (25) (2014) 8462–8466.
[47] R. Phillips, J. LeDoux, Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, Behav. Neurosci. 106 (2) (1992) 274.
[48] M.W. Jung, S.I. Wiener, B.L. McNaughton, Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat, J. Neurosci. 14 (12) (1994) 7347–7356.
[49] E.I. Moser, E. Kropff, M.-B. Moser, Place cells, grid cells, and the brain's spatial representation system, Annu. Rev. Neurosci. 31 (2008) 69–89.
[50] M.R. Hunsaker, R.P. Kesner, Dissociations across the dorsal–ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear, Neurobiol. Learn. Mem. 89 (1) (2008) 61–69.
[51] B.J. Wiltgen, M.J. Sanders, S.G. Anagnostaras, J.R. Sage, M.S. Fanselow, Context fear learning in the absence of the hippocampus, J. Neurosci. 26 (20) (2006) 5484–5491.
[52] Y. Zhao, X. Wu, X. Li, L.-L. Jiang, X. Gui, Y. Liu, et al., TREM2 is a receptor for β-amyloid that mediates microglial function, Neuron 97 (5) (2018) 1023-1031. e1027.
[53] T. Maier, M. Güell, L. Serrano, Correlation of mRNA and protein in complex biological samples, FEBS Lett. 583 (24) (2009) 3966–3973.
[54] C. Vogel, E.M. Marcotte, Insights into the regulation of protein abundance from proteomic and transcriptomic analyses, Nat. Rev. Genet. 13 (4) (2012) 227–232.
[55] E.M. Bradshaw, L.B. Chibnik, B.T. Keenan, L. Ottoboni, T. Raj, A. Tang, et al., CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology, Nat. Neurosci. 16 (7) (2013) 848–850.
[56] T. Jiang, L. Tan, X.-C. Zhu, Q.-Q. Zhang, L. Cao, M.-S. Tan, et al., Upregulation of TREM2 ameliorates neuropathology and rescues spatial cognitive impairment in a transgenic mouse model of Alzheimer's disease, Neuropsychopharmacology 39 (13) (2014) 2949–2962.
[57] T. Jiang, Y.-D. Zhang, Q. Chen, Q. Gao, X.-C. Zhu, J.-S. Zhou, et al., TREM2 modifies microglial phenotype and provides neuroprotection in P301S tau transgenic mice, Neuropharmacology 105 (2016) 196–206.
[58] C.D. Lee, A. Daggett, X. Gu, L.-L. Jiang, P. Langfelder, X. Li, et al., Elevated TREM2 gene dosage reprograms microglia responsivity and ameliorates pathological phenotypes in Alzheimer's disease models, Neuron 97 (5) (2018) 1032-1048. e1035.