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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2014

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Dobos, N. (2014). Neuroinflammation in depression [S.l.]: s.n.

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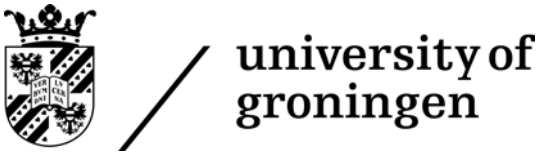
NEUROINFLAMMATION IN DEPRESSION

Nikoletta Dobos

The research presented in this thesis was carried out at the Department of Molecular Neurobiology of the University of Groningen in collaboration with the Department of Nuclear Medicine & Molecular Imaging of the University Medical Center Groningen, The Netherlands. The research was financially supported by the International Foundation for Alzheimer Research (ISAO), grant no.06511, the Gratama Foundation and the EU-grant FP6 NeuroproMiSe LSHM-CT-2005-018637.

The printing of this thesis was financially supported by the Groningen Graduate School of Sciences of the Faculty of Health Sciences.



Cover design: Nikoletta Dobos, Eszter Csákvári, Marcelo F. Masman

Layout: Nikoletta Dobos, Roland Patai

Printed by: Ipskamp Drukkers

ISBN: 978-90-367-6934-1
ISBN: 978-90-367-6933-4 (electronic version)

RIJKSUNIVERSITEIT GRONINGEN

NEUROINFLAMMATION IN DEPRESSION

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. E. Sterken,

in het openbaar te verdedigen op

woensdag 30 april 2014
om 11:00 uur

door

Nikoletta Dobos

geboren op 15 januari 1981
te Debrecen, Hongarije

Promotores:	Prof. dr. P.G.M. Luiten Prof. dr. U.L.M. Eisel Prof. dr. J.A. den Boer Dr. E.F.J. de Vries
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Dorinának

PREFACE

"In these several sessions we heard exciting new data which indicate the critical role of induced IDO activity in several important areas. The activation of dendritic cells (macrophages, astrocytes, etc.) results in IDO induction with the depletion of tryptophan levels locally or systemically. This seems to be the mechanism by which interferon inhibits the growth of certain bacteria, intracellular parasites, and viruses. Most exciting were the reports that tryptophan depletion also inhibits T lymphocyte replication which results in immunosuppression and tolerogenicity. This has far reaching implications in many fields of medicine, including fetal rejection, and organ transplant survival. However, in some cases the effects of the IDO activity may be the result of elevated kynurenine pathway metabolites rather than the depletion of tryptophan. For example, many studies have described the excitatory and toxic effects of quinolinic acid on neuronal activity. Furthermore, the depletion of tryptophan leads to decreased levels of serotonin which may produce a wide range of effects. In closing, I would like to ask us all to recognize Professor Osamu Hayaishi for his brilliant, pioneering work in elucidating the tryptophan metabolism, particularly for his important finding of indoleamine dioxygenase (IDO) and for induction by interferon-c. These findings now allow us to reinterpret many older empirical observations of the tryptophan metabolism in animals and man, which has now opened up a whole new era of better understanding the role of tryptophan in the fields of immunology, AIDS, organ transplant, autoimmune diseases, cancer, and mental functions. The next few years will, I am sure, see unbelievable advances in these areas of biology and medicine."

Prof. Raymond Brown's closing remarks in the 10th meeting of the International Study Group for Tryptophan Research (ISTRY) held at Padova in Italy in 2002 (Brown 2003; Takikawa 2005)

CHAPTER 1

General introduction

This chapter is based on the publication:

Neuroinflammation in Alzheimer's disease and major depression

Comment on Biol Psychiatry, 2010, Mar 15;67(6):550-7.

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Biological Psychiatry. 2010 Mar 15;67(6):503-4.

1. INDOLEAMINE 2,3-DIOXYGENASE (IDO)

The history of indoleamine 2,3-dioxygenase (IDO), the key enzyme of the kynurenine pathway and tryptophan catabolism began in 1967 when Professor Hayaishi and his colleagues described an enzyme, which converted D-tryptophan to D-kynurenine (Higuchi and Hayaishi 1967; Yamamoto and Hayaishi 1967). Since then, especially in the last decade, extensive research focuses on the role of IDO in many approaches.
Tryptophan is an abundant essential amino acid, a key molecule for proteins and also for the neurotransmitter, serotonin. Approximately 95% of the tryptophan is metabolized via the kynurenine pathway, only about 1% is converted into serotonin. Along the kynurenine pathway, several biologically active compounds are generated until it ends in complete oxidation of tryptophan or in the production of nicotinamide adenine dinucleotide (NAD) (Takikawa 2005).

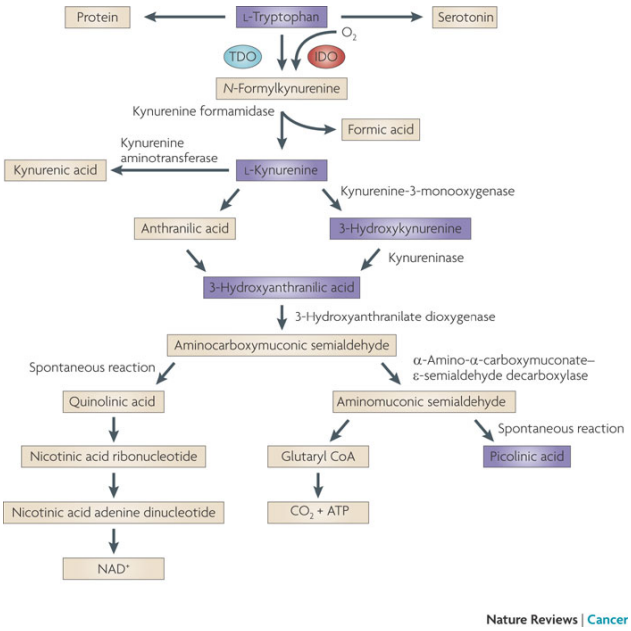

Figure 1. The kynurenine pathway. Adapted from Löb et al. 2009. (Lob, Konigsrainer et al. 2009)

The first and the rate-limiting step of the kynurenine pathway is catabolized by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO). TDO is explicitly expressed in the liver, whereas IDO is an ubiquitously expressed enzyme, it can be found in the lung, intestines, spleen, kidney, brain, and interestingly, the highest expression was measured in the placenta (Yamazaki, Kuroiwa et al. 1985; Takikawa 2005).

Among others, pro-inflammatory cytokines, e.g. Tumor Necrosis Factor alpha (TNFα), interferon gamma (IFNγ), or interleukin 6 (IL-6) can induce IDO expression and/or activity (Leonard 2007; Dantzer, O'Connor et al. 2008).
IDO has been described to be involved and play a crucial role in the defense mechanism against bacterial and viral infections. Antimicrobial, antiviral activity is explained by interferon gamma (IFNγ) induced, IDO mediated tryptohan depletion in the host cells, which results in the inhibition of the growth of the pathogens (Pfefferkorn 1984; Shemer and Sarov 1985; Byrne, Lehmann et al. 1986). IDO has been implicated in immunsuppression as well. IDO is responsible for immunological paradoxes, like maternal tolerance and fetal survival, tumor growth or persistent infections, like Acquired Immune Deficiency Syndrome (AIDS) (Mellor, Munn et al. 2003). High placental IDO levels inhibit T-cell mediated immune responses, therefore fetus-rejection is prevented (Munn, Zhou et al. 1998; Suzuki, Tone et al. 2001). It has been shown that tumor cells express IDO, which leads to local tryptophan depletion and subsequent T-cell inhibition and tumor persistance (Uyttenhove, Pilotte et al. 2003; Godin-Ethier, Hanafi et al. 2011). Human immunodeficiency virus (HIV) patients show increased IFNγ and therefore increased IDO levels as well. Chronic induction of IDO in HIV patients results in chronic T-cell inhibition and probably in immunsuppression and immunodeficiency (Brown, Ozaki et al. 1991).
In addition, IDO is also induced under several neuroinflammatory conditions, including Alzheimer's disease (AD) (Guillemin, Brew et al. 2005).

2. NEUROINFLAMMATION

Although inflammation per se is a protective reaction of the body against intruding parasites, bacteria, or viruses, inflammation is also a major component of chronic degenerative diseases. In fact, investigating immune responses in brain diseases is a relatively young science, mainly because the brain was considered an "immune privileged site." Today, we know that almost all components of the immune system are also present within the brain (Mrass and Weninger 2006). Microglia are the macrophages of the brain and comprise 10-12% of the total cell number of the brain (Kannan, Balakrishnan et al. 2009). Within the brain, microglia strongly interact with astrocytes, neurons and blood vessels. After injury or stress, microglia get activated, their morphology is changed and they start to secrete proinflammatory cytokines. Proinflammatory cytokines, such as interleukin (IL-1), TNFα and IFNγ coordinate the local and systemic inflammatory response to pathogens (Banks 2005).

The innate immune response is paramount in maintaining tissue homeostasis. Therefore, not all immune responses should be considered per se as damaging. This is especially clear for the cytokine TNFα and its receptors. Upon local challenges such as ischemia (in stroke) or amyloid precipitations (in Alzheimer's disease), TNFα and its receptors become strongly expressed. TNFα not necessarily damages brain tissue via activating TNF Receptor 1 (TNFR1). Stimulation of TNF Receptor 2 (TNFR2) by TNFα antagonizes TNFR1 death signals by inducing a neuroprotective signaling cascade that requires the activation of protein kinase B/Akt and Nuclear Factor kappa B (NF-κB) (Marchetti, Klein et al. 2004).

3. TNFα SIGNALING PATHWAY

The cytokine, TNFα was discovered about 30 years ago, as a tumor necrotizing serum production (Kiger, Khalil et al. 1980). Kiger et al. observed in Bacillus-Calmette-Guerin (BCG)-pretreated mice a delayed fibrosarcoma development without toxic side effects. Since then, the role of TNFα and its receptors have an enormous scientific and clinical interest and TNFα-related research has been expanded. TNFα is a 26 kDa type II transmembrane protein, which is cleaved by the metalloprotease TNFα-converting enzyme, that results in the 17 kDa monomer, and after trimerization it becomes active as a soluble 51 kDa protein (Black, Rauch et al. 1997; Naude, den Boer et al. 2011). TNFα exerts its biological functions via the two TNF-receptors: TNFR1 and TNFR2.

Under normal physiological conditions TNFR1 is ubiquitously expressed on almost every cell types, and gets activated rapidly, whereas TNFR2 is expressed typically at low levels in immune cells and its activation takes longer (Loetscher, Pan et al. 1990; Schall, Lewis et al. 1990; Tartaglia and Goeddel 1992; Naude, den Boer et al. 2011). TNFR1 can be activated by both, membrane-bound and soluble TNFα (Grell, Douni et al. 1995) and mainly involved in apoptosis (Eisel 2006). The TNFR1 contains a death domain, which becomes - after ligand binding to the receptor and dissociation of the silencer of death domains (SODD) – available for the adapter protein TNF receptor-associated death domain (TRADD) (Tartaglia, Ayres et al. 1993; Jiang, Woronicz et al. 1999; McCoy and Tansey 2008; Naude, den Boer et al. 2011). TRADD binding subsequently recruits other proteins that are involved in downstream signaling, for example, TNF receptor associated factor-2 (TRAF2) or receptor interacting protein (RIP) (McCoy and Tansey 2008; Naude, den Boer et al. 2011). Of note, RIP can be also recruited to TNFR1 independent of TRADD (Chen, Chio et al. 2008). RIP-dependent activation of NF-κB signaling initiates then pro-survival signaling, cellular proliferation, or cytokine production (McCoy and Tansey 2008). This complex recruits the cellular inhibitor of apoptosis proteins 1 and 2 (cIAP 1,2) which leads to the activation of ERK, JNK, p38 MAP kinase, and ceramide/sphingomyelinase pathways (Winston, Lange-Carter et al. 1995; Shu, Takeuchi et al. 1996; Lee, Huang et al. 2003). The kinetics of the JNK activation determines the effect of TNFα. A rapid, acute JNK activation is cytoprotective, whereas a sustained JNK activation results in a caspase-dependent apoptosis (McCoy and Tansey 2008). Furthermore, internalization of TNFR1 after activation results in the dissociation of the TRADD/TRAF2/RIP complex and association of Fas-associated death domain (FADD), recruitment of pro-caspase 8. Caspase-8 is responsible for extrinsic as well as intrinsic apoptosis pathway (McCoy and Tansey 2008). In conclusion, TNFR1 mediated signaling pathway can contribute to several outcomes, e.g. proliferation, cell survival or apoptosis.

Additionally, the involvement of TNFR2 makes the signaling pathway more complex. TNFR2 is expressed in certain types of cells, e.g. neuronal subtypes, oligodendrocytes, microglia and astrocytes, endothelial cells, and certain T-cell subpopulations, including lymphocytes (CD4+ and CD8+ T cells), cardiac myocytes, thymocytes and human mesenchymal stem cells (Choi, Lee et al. 2005; Faustman and Davis 2010; Naude, den Boer et al. 2011) and gets activated by the membrane-bound form of TNFα (McCoy and Tansey 2008). The restricted expression of TNFR2 is coupled with restricted biological functions.

TNFR2 lacks the death domain, and induces long-term activation of NF-κB, resulting in pro-inflammatory and pro-survival signaling pathways (McCoy and Tansey 2008; Naude, den Boer et al. 2011). TNFR2 can also activate the phosphatidylinositol 3-kinase/protein kinase B/serine-threonine kinase pathway dependent signaling to promote neuron survival (Marchetti, Klein et al. 2004).

Overall, TNFR2 mediated pathways are believed to be protective, by initiating pro-inflammatory and pro-survival signaling pathways.
TNF signaling has been shown to play a very important role within the central nervous system (CNS). TNFα is crucial for instance in microglia and astrocyte activation, in the regulation of the blood brain barrier permeability, in glutamatergic transmission or in synaptic plasticity (Selmaj, Farooq et al. 1990; Merrill 1991; Beattie, Stellwagen et al. 2002; Pickering, Cumiskey et al. 2005). Elevated TNFα levels have been found in several neurological and psychiatric disorders, e.g. ischemia (Liu, Clark et al. 1994), traumatic brain injury (Goodman, Robertson et al. 1990), multiple sclerosis (Hofman, Hinton et al. 1989), Alzheimer's disease (Fillit, Ding et al. 1991), and Parkinson's disease (Nagatsu, Mogi et al. 2000) or major depression (Tuglu, Kara et al. 2003) as well. Therefore, the idea of targeting TNFα and its receptors to treat disorders or at least modify disease development turned on the spotlight. Several studies have been done to elucidate or ameliorate diseases by inhibiting TNFα or selectively its receptors (Tobinick 2007; Griffin 2008; Tobinick and Gross 2008; Tobinick 2009; Frankola, Greig et al. 2011). Interestingly, it was recently shown, in a mouse model of AD that imipramine, a tricyclic antidepressant (Chavant, Deguil et al. 2010) can prevent cognitive decline and amyloid beta accumulation by TNFα inhibition. Of note, TNFα is a potent inducer of IDO (Dobos, de Vries et al. 2011).

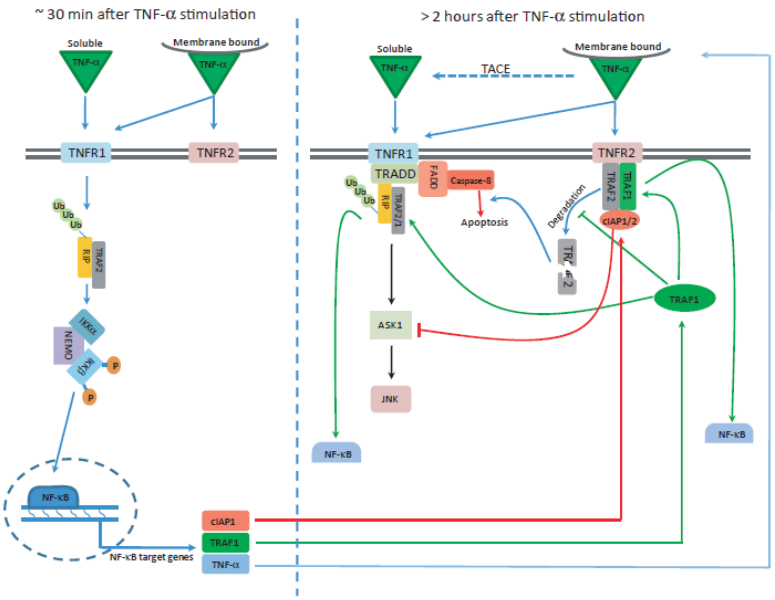

Figure 2. TNFR crosstalk. Adapted from Naude et al. 2011. (Naude, den Boer et al. 2011)

The involvement of TNFα and its receptors in cognitive and physical functions during the aging process will be further discussed in Chapter 3.

4. NEUROINFLAMMATION AND MAJOR DEPRESSION

Depression, in the clinic called major depressive disorder (MD), clinical depression, unipolar depression, is the most common mood disorder worldwide. Lifetime prevalence of depression in the general population is 10-25% for women and 5-12% for men. The diagnosis of the disease is not easy; there are few other diseases, which might show completely opposite symptoms, like depression. For instance, weight loss or weight gain, insomnia or hypersomnia, both might represent a symptom of major depression. However, in general, MD can be characterized by depressed or irritable mood, lethargy, diminished interest or pleasure in all, psychomotor agitation or retardation, fatigue or loss of energy, feelings of helplessness, worthlessness or guilt, concentration problems, indecisiveness, recurrent thoughts of death or suicide. The diagnosis of major depression can be made, when five or more of these symptoms last for at least a 2-week period of time, and depressed mood or anhedonia is a mandatory symptom (Association 2000).

There have been several hypotheses (the monoamine hypothesis, the glucocorticoid hypothesis, neuroplasticity hypothesis, cytokine hypothesis) proposed for major depression, however, to date, none of them can meet all criteria to explain the pathogenesis of this disorder. There is little doubt that MD should be defined as a multifactorial disease in which various mechanisms are involved, dependent on individual differences and vulnerability, and disease history. In this thesis, the cytokine hypothesis of depression will be the focus of study.

Since 1991, when Smith proposed the macrophage theory of depression (cytokine hypothesis), the knowledge on the relations between inflammation and depression has enormously expanded (Smith 1991). The basic idea of the cytokine-immune model of depression came from the fact, that upon the activation of the immune system, cytokines are produced which alter brain function and behavior. In the last two decades, many researchers provided direct evidence for increased cytokine levels in major depression to confirm the theory (Capuron, Ravaud et al. 2001; Banks 2005; Goeb, Even et al. 2006; Su, Huang et al. 2010). Both experimental (O'Connor, Andre et al. 2009) as well as clinical studies point to a role of IL-2, IFNα, or IFNβ in major depressive disorders (Capuron, Ravaud et al. 2001; Goeb, Even et al. 2006). Furthermore, meta analysis have been done and showed a positive correlation between elevated TNFα, TNF-β1, IL-1, IL-6, C-reactive protein (CRP) and the symptoms of major depression (Howren, Lamkin et al. 2009; Dowlati, Herrmann et al. 2010).

Cytokine-induced inflammation might be responsible also for altered serotonin synthesis. Reduction of the availability of serotonin is believed to be one of the causes of major depression. Reduction of tryptophan levels by cytokine-upregulated IDO activity affects serotonin (5-HT) synthesis, which is implicated in a variety of psychiatric disorders, but also affects cerebral plasticity, because 5-HT can increase production of neurotrophic peptides, such as brain derived neurotrophic factor (BDNF). Finally, the end product of the

kynurenine pathway, is a neurotoxic activator of the N-methyl-D-aspartate receptor, called quinolinic acid (QUIN). This factor can also contribute to excitotoxic effects in neurodegenerative diseases. These observations suggest that the tone of cerebral 5-HT should be kept within a narrow range, because deranged 5-HT levels compromise various brain functions. There is mounting evidence that IDO is a prominent player in the relation between chronic inflammation and depression, as we know from the effects on IDO of toxins like lipopolysaccharide (LPS) or pro-inflammatory cytokines, such as TNFα and IFNγ.

5. IMAGING NEUROINFLAMMATION

Aging is not only a simple physical phenomenon, but also a more complex social, cultural and economical issue. Currently, there is no cure for neurodegenerative diseases, like Alzheimer's disease. The biggest problem is with such diseases, that the diagnosis is very difficult. Symptoms overlapping with other diseases, one disorder is accompanied by the other one. Another problem is, like it has been shown in Alzheimer's disease, that the pathological changes, for instance amyloid beta accumulation and neuronal loss are present some decades before the clinical symptoms appear (Perrin, Fagan et al. 2009). Therefore, extensive research has been focusing on the development of novel non-invasive techniques and methods to investigate pathological structures and functions within the central nervous system or to diagnose neurodegenerative diseases at a very early stage, preferably at the time, when it does not show any clinical signs yet.
One of the solutions could be the use of neuroimaging techniques, like positron emission tomography (PET), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), near-infrared fluorescence imaging (NIRF), bioluminescence imaging (BLI) etc (Klohs and Rudin 2011).

Positron emission tomography is an *in vivo* nuclear imaging technique which measures the distribution of radiotracers in tissue (Klohs and Rudin 2011). Radiotracers are molecules labeled with a radionuclide which first undergoes positron emission decay resulting in the emission of a positron. The next step is called annihilation, when the positron captures an electron. Annihilation generates a pair of γ photons, emitted in opposite direction by 180°. The scintillation detector of the PET scanner detects γ photons. Two- or three-dimensional pictures are constructed by computer analysis (Venneti, Wiley et al. 2009; Klohs and Rudin 2011).
One of the main characteristics of neurodegenerative diseases is neuroinflammation. As mentioned before, during neuroinflammation, microglia, the most important immune cells of the brain get activated. Activation of microglia manifests in morphological changes as well as production of cytokines and expression of receptors, like the peripheral benzodiazepine receptors (PBRs) on the outer membrane of the mitochondria (Cagnin, Kassiou et al. 2007; Doorduin, de Vries et al. 2008).

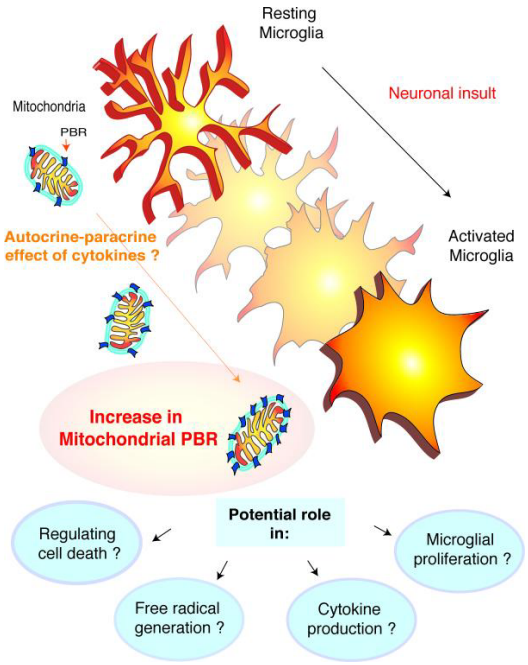

Figure 3. Microglia activation. Adapted from Venneti et al.(Venneti, Lopresti et al. 2006)

It has been shown, that microglia are the main source of PBRs in the CNS (Banati, Myers et al. 1997), but also astrocytes and infiltrating macrophages express them (Doorduin, de Vries et al. 2008). The exact fraction of PBRs expressed by activated microglia and other cells can not be determined, and this should be taken into account. However, PBRs are a good target to visualize inflammation in the brain by PET. There have been several PET tracers developed for PBR imaging. The first and for a long time the only one in the human application was the carbon-11 labeled PK11195 (1-[2-chlorophenyl]-N-methyl-N-[1-methylpropyl]-3-isoquinoline carboxamide) (Doorduin, de Vries et al. 2008). The [^{11}C]-PK11195 has been successfully used to visualize neuroinflammation by detecting activated microglia in various neurological diseases, e.g. Alzheimer's disease (Groom, Junck et al. 1995), HIV-associated dementia (Hammoud, Endres et al. 2005), Multiple sclerosis (Banati, Newcombe et al. 2000).

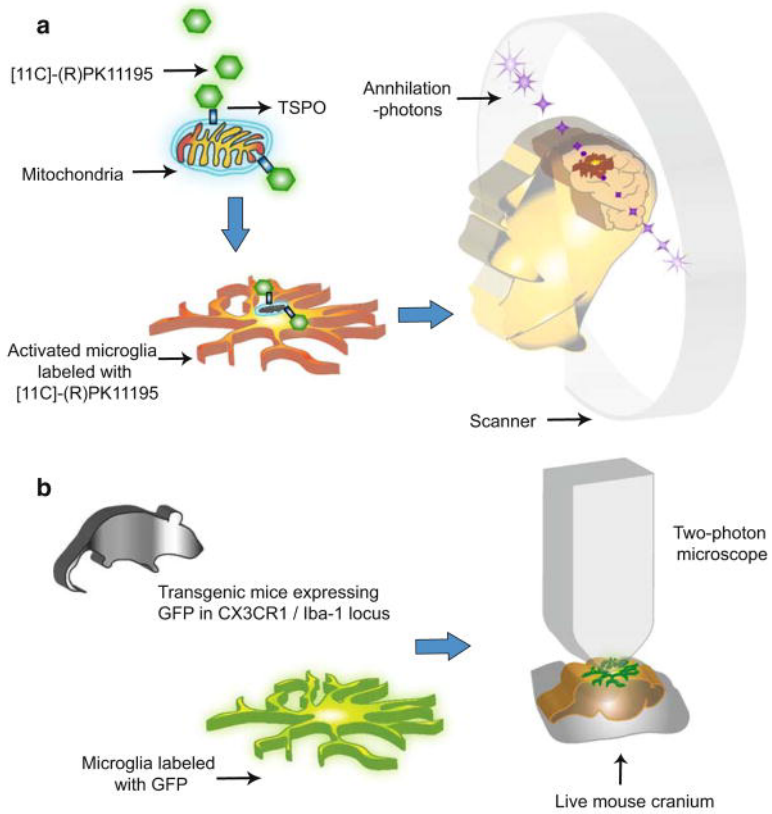

Figure 4. In vivo imaging of PBRs on the outer membrane of activated microglia. Adapted from Venneti et al. 2009.(Venneti, Wiley et al. 2009)

6. ALZHEIMER'S DISEASE

Alzheimer's disease is an irreversible progressive neurodegenerative disorder and the major cause of dementia. The disease symptoms in sporadic cases usually start after the age of 60. Time between the first expression of behavioral disease symptoms and death might be up to 10 years. Thirty-five million people worldwide are estimated to suffer from AD, and in the United States alone 5.3 million people are diagnosed with the disease. According to the World Health Organization, AD is the fifth leading killer, and presently there is no cure for the disease.

The core symptom of AD is impaired cognitive function. AD brain pathology is characterized by extracellular depositions of amyloid β, intracellular aggregates of the protein tau, and loss of cholinergic forebrain innervation. These neuropathological hallmarks of AD are mainly present in brain regions involved in cognition like cortex, hippocampus and amygdala. However, it is still uncertain whether the amyloid β plaques and neurofibrillary tangles are causative in AD. It has been shown, that burden of amyloid β plaques poorly correlates with the cognitive decline in AD (Villemagne, Pike et al. 2011). Also, mouse models of AD with abnormal plaque development, do not show the cognitive deficits (Davis and Laroche 2003). Instead, amyloid β oligomers are more toxic and responsible for

neuronal death (Larson and Lesne 2012). Besides neuronal loss and protein deposits in the brain, inflammatory processes also play an important role in AD. Astrogliosis, activation of microglia due to plaque depositions, toxic oligomers, tau-aggregations leads to widespread neuroinflammation. Microglia-driven inflammatory response includes cytokine production (TNFα, IFNγ, IL-6) and induction of inflammatory enzyme systems, like inducible nitric oxide synthase (iNOS) and the prostanoid generating cyclooxygenase-2 (COX-2), reactive oxygen species or free radicals which additionally triggers neuronal dysfunction and death (Heneka, O'Banion et al. 2010).
As mentioned before, neuroinflammation plays an important role not only in neurodegenerative diseases but in major depression as well. AD is often accompanied by symptoms of depression, anxiety, irritability and mood instability. In many cases patients have undergone long time treatments against these psychiatric symptoms before being clinically diagnosed for AD. In those cases anxiety or depression is not just a side effect of AD but rather an integral part of the typical behavioral symptoms. Interestingly, the affective status may even be of predictive value for disease development (Jost and Grossberg 1996).

7. MULTIPLE SCLEROSIS

Multiple Sclerosis (MS) is an inflammatory disorder of the central nervous system (CNS). Mostly young and middle aged adults are affected by MS (Feinstein 2000). Damaged myelin sheaths are the most important hallmark of the disease and demyelination is responsible for physical and cognitive disability.
During the disease, abnormal B and T cell response against antigens, e.g. myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG) are observed. B cell activity is located only in the CNS-cerebrospinal fluid, whereas myelin antigen-reactive T cells have been found in patients blood as well (Link 1998). Besides autoreactive B and T cells, damaged blood brain barrier, infiltration of macrophages into the CNS and activated glial cells are also detectable in MS. The general inflammation in the body leads to abnormal cytokine production, and an overall elevated cytokine profile (Link 1998; Trenova, Manova et al. 2011). There is no cure for the disease, only relapse remitting therapies can help patients with MS. Interferon beta (IFNβ) is the first-line, successful and safe therapy in MS. IFNβ is able to slow the progression of brain atrophy in MS and reduces the relapse rate by approximately 30% (Farrell and Giovannoni 2010; Rudick and Goelz 2011).
It is known that IFNβ inhibits T cell activation by down regulation of major histocompatibility complex (MHC) class II expression on antigen-presenting cells therefore IFNβ is able to alter the inflammatory response. It reduces Th1 pro-inflammatory cytokines and directs a shift towards the Th2 cytokine-profile (Markowitz 2007). The mechanism of action of IFNβ starts with its binding to the interferon α/β receptor, which consists of a signaling chain (IFNAR1) and a binding chain (IFNAR2). After creation of this complex, the intracellular part of the receptor interacts with Janus kinase 1 (JAK1) and Tyrosine kinase 2 (Tyk2). The interaction results in a phosphorylation cascade and finally activates Signal Transducer and Activator of Transcription (STATs). Activated STATs translocate into the nucleus and bind ISRE elements and subsequently induce gene transcriptions (Farrell and Giovannoni 2010).

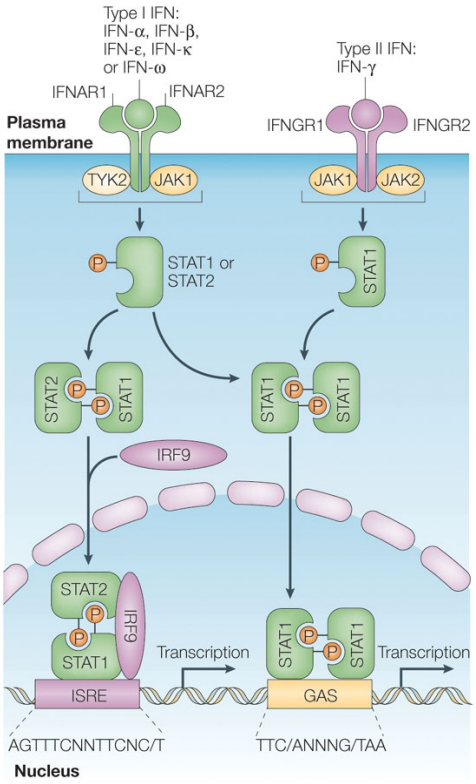

Figure 5. Mechanisms of type I and type II interferon mediated signaling. Adapted from Platanias 2005. (Platanias 2005)

The therapeutic effect of IFNβ can be direct, if IFNβ binds to its receptors and induces gene transcriptions, or indirect when gene transcription alters other cells involved in pathogenic mechanisms in MS. Although IFNβ is a safe and effective therapy, it has serious side effects, like major depression (Feinstein 2006; Fragoso, Frota et al. 2010). Interestingly, it is known, that besides pro-inflammatory cytokines, IFNβ is also a potential inducer of the depression-associated enzyme, IDO. Stimulation of ISRE by IFNβ has been shown to induce IDO expression (Suh, Zhao et al. 2007).
The link between IFNβ-induced IDO expression and behavioral changes in mice will be further discussed in Chapter 5.

8. AIM AND OUTLINE OF THE THESIS

In summary, there is growing evidence that inflammatory processes play a key role in major depression as well as in neurodegenerative diseases like AD and might be partly responsible for the depression symptoms in AD patients. The overall aim of this project was to investigate the role of neuroinflammation in depression and neurodegenerative diseases. In the present thesis we paid special attention to an ubiquitously expressed enzyme, indoleamine 2,3-dioxygenase (IDO), which is induced by inflammatory stimuli and might be responsible for the depressive symptoms in neurodegenerative diseases.

Chapter 1 provides a general insight into the field of neurodegeneration, neuroinflammation and depression. The role of IDO, the possible link between neuroinflammation and depression under neurodegenerative circumstances has been pointed out.

Chapter 2 has been published in NeuroImmune Biology – The Brain and host defense and reviews Tumor Necrosis Factor α as a neuroinflammatory mediator in Alzheimer's Disease and stroke. This book chapter focuses on molecular mechanisms and neuroinflammatory imaging.

Chapter 3 describes the role of TNFR1 and TNFR2 on behavioral changes in the aging process. Therefore, behavioral studies - to investigate cognitive and physical functions - were conducted in young (3 months) and aged (22 months) wildtype, TNFR1 knock out (TNFR1-/-) and TNFR2 knock out (TNFR2-/-) C57BL/6 mice. We showed that TNFR2 plays a key role in hippocampus-dependent memory formation and neuromuscular function. Furthermore, the results suggest, that the absence of TNFR1 or TNFR2 does not influence the aging process.

In Chapter 4, evidence for the role of IDO has been given in a mouse model of neuroinflammation induced depression. We showed, that glial activation - reflecting neuroinflammation - culminated 3 days after LPS injection. LPS injected animals displayed a significant increase of depressive-like behavior in the forced swim test (FST), a significant increase of IDO in the brainstem, and increased kynurenine/tryptophan ratio in the serum compared to vehicle injected animals. We also investigated the effect of a competitive IDO-inhibitor, 1-methyl-tryptophan (1-MT) in centrally induced neuroinflammation, and we reported, that in experimental conditions, IDO inhibition can prevent the development of depressive-like behavior.

Chapter 5 presents interferon β (IFNβ) induced IDO expression and activity with subsequent depressive-like behavior using a wildtype and an interferon α/β receptor knock out mouse strain. The results obtained in this study give evidence that IFNβ-induced IDO increase and the subsequent behavioral changes are through the interferon α/β receptors.

Finally, Chapter 6 summaries this thesis, contains general discussion and ends in future applications of the results presented here.

CHAPTER 2

Tumor necrosis factor as neuroinflammatory mediator in Alzheimer's disease and stroke. Molecular mechanisms and neuroinflammatory imaging

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NeuroImmune Biology, Volume 9, The Brain and Host Defense, Chapter 20
2010, Pages 251–267

ABSTRACT

Neuroinflammatory responses in neurodegenerative diseases have been extensively investigated over the past decade. Although still far from being fully understood, neuroinflammatory responses are now considered to be part of the physiological response repertoire of the brain to traumatic and chronic neurodegeneration and to exhibit both neurotoxic and neuroprotective functions. Recent findings in neuroinflammatory research of neurodegenerative brain diseases suggest that inflammatory responses should also be considered as opportunities to fight the disease. We discuss the possibilities of using neuroinflammatory responses for neuroimaging and diagnosis and also for developing future therapeutic strategies. Among the pro-inflammatory cytokines involved in all of the degenerative diseases of the brain tumor necrosis factor alpha (TNF) plays a role of prime importance. We discuss this role of TNF with emphasis on the mechanisms of TNF receptor-mediated intracellular processes, and the molecular pathways that underly neuroprotective signaling through TNF receptor 2. Neuroinflammatory mechanisms while associated with direct damage of or protection of nervous tissue, have multiple additional impacts on the affected brain. Other aspects of the link between neuroinflammation and neurodegeneration include activation of enzymatic processes such as indoleamine 2,3-dioxygenase, the consequences of which on neuronal function and excitability we discuss in the context of neurodegenerative disorders like Alzheimer's disease.

KEYWORDS: Neuroinflammation, TNF, Neuroimaging, PET tracers, stroke, Alzheimer's disease, Neurodegeneration

1. INTRODUCTION

Stroke and neurodegenerative diseases are among the leading causes of permanent disabilities and death in western societies. With an increasingly aged population both conditions pose a constant challenge to the individual and to society. Although in recent decades considerable advances have been made in the treatment of symptoms, for example in certain aspects of degenerative disorders like Parkinsonism, virtually no treatments are available as yet that interfere with the causal mechanisms underlying these brain diseases. This is particularly the case for treatment of cognitive failure in dementing illnesses including Alzheimer's disease and Parkinson's disease with dementia (AD; PD) as well as CVA.

With respect to the role that inflammation plays in brain diseases, our understanding of the central nervous system has evolved over the past decade from one of an immune-privileged organ, to one where inflammation is pathognomonic for some of the most prevalent neurodegenerative diseases. Inflammation, whether in the brain or periphery, is almost always a secondary response to a primary pathogen or pathophysiological process. In Alzheimer's disease, inflammation is considered as a secondary response that follows impaired processing and precipitation of amyloid oligomers, but one that likely causes additional neuronal damage and cell loss. It is now established that inflammatory mediators not only play predisposing roles in the development of atherosclerotic stroke but also in what happens to damaged post-ischemic or post-hemorrhagic tissue. This role is certainly not limited to cerebrovascular brain disease. If one compares the inflammatory responses that follow upon ischemia to the responses observed in diseases like AD or PD remarkable similarities are observed. In ischemic stroke it is accepted that in a substantial number of patients progression of neural injury continues beyond 24 hours after stroke onset (Dirnagl 2004). The mechanisms thought to be involved in lesion progression and in delayed neuronal cell death are: increasing and spreading vascular occlusions, excitotoxicity, deranged calcium homeostasis, necrosis, apoptosis and inflammation. Except for vascular occlusion, excitotoxicity, calcium accumulation, apoptosis and inflammation are also hallmarks of chronic neurodegenerative diseases (Mattson 2004).

On the other hand there is growing evidence that also for AD and PD vascular pathology and a reduced supply of oxygen and nutrients that may come into play as major risk factors that can promote neurodegenerative processes (Farkas and Luiten 2001; Kalaria 2003; de la Torre 2004; Liebetrau, Steen et al. 2004). Necrosis is believed to be the result of a short-lasting insult leading to a "non-organized" cell death. Cell necroses can be initiated by a physical insult, energy depletion or other stress factors with a fatal result characterized by release of intracellular contents, including high concentrations of glutamate, into the extracellular space. Apoptosis, or programmed cell death, on the other hand, is induced by intra- cellular mediators such as mitochondrial pathways or increases of intracellular calcium or by external cytokine ligands exemplified by members of the TNF receptor superfamily such as TNF itself, FasL or TRAIL in the absence of trophic factors and/or anti-apoptotic signals. The apoptotic program generally activates a cascade of caspases which trigger other signaling molecules and enzymes that finally lead to degradation of DNA and proteins. The cell becomes fragmented and these fragments are enveloped as small packages to be digested

by macrophages and microglia. As a result no further inflammation is triggered and no cytotoxic components come to be released into the surrounding extracellular space. The extracellular factors that induce, apoptosis are part of the innate immune response that is mediated in part by immune cells and can be considered as a tissue-mediated stress response. Protection of the whole organism by the immune system therefore includes the conversion of an unregulated necrotic into a regulated and controlled apoptotic cell death in order to prevent increased tissue damage.

From the basic neuroinflammatory mechanisms and their biochemical characteristics, the role of putative mediators that could play a key role in neurodegenerative disorders has been explored, with Alzheimer's disease serving as a prototypic disorder. Although in experimental cellular models the various biochemical aspects and processes of inflammation can be well characterized, assessment of cerebral inflammatory processes *in vivo* is still in its infancy. Whereas structural imaging shows merely late and robust anatomical consequences of an inflammatory event, functional imaging is a strong potential candidate to bridge this mechanistic gap between *in vitro* and *in vivo* knowledge. Although peripheral immune-associated cells (e.g. lymphocytes) do occasionally cross the intact blood-brain-barrier - often fulfilling a surveillance function - cerebral inflammatory reactions depend heavily on intraparenchymal elements of the brain. The only resident immune cells of the brain are the so called microglia. Microglial cells are actually macrophages and as is typical for macrophages they release - among many other immunomodulating molecules - the pro-inflammatory cytokine TNF. Microglial cells become activated in regions surrounding brain tissue lesions whether acivation is induced by AD-induced amyloid plaques, by ischemic lesions or by other pathological conditions. In fact, activation of microglia occurs independently of whether the damage to a neuron is irreversible (necrosis) or the reaction of the cells has a transient character only. In either case, activated microglial cells are a sensitive marker of affected brain regions in a wide variety of neurodegenerative diseases. In this chapter we discuss not only neurodegenerative consequences and several of the basic mechanism underlying vascular pathology and neurotoxicity, but also the prospects that microglial activation may provide a reliable marker for neuroimaging and that intelligent drugs can be directed to affected brain sites.

2. SOME BASIC ASPECTS OF CEREBRAL INFLAMMATION

The previously accepted concept of the brain as an immunologically privileged organ appears to be no longer tenable. Initially it was conceived that the brain's lack of a lymphatic drainage system (Cserr and Knopf 1992) contributed to an unusual tolerance for transplanted tissue. However, more recent insights indicate that a lymphatic-like system exists in the brain. Formerly, the immune and nervous systems were considered as making autonomous contributions to physiological homeostasis (Barker and Billingham 1977). Contemporary research has revealed that the blood-brain barrier (BBB) is, under certain conditions, less restrictive than had been thought to the migration of monocytes, lymphocytes, or natural killer cells, irrespective of antigen specificity (Mucke and Eddleston 1993). Nevertheless, inflammation threshold of the CNS is still considerably higher than that of the periphery, leading to a delay between peripheral and CNS inflammation during a general inflammatory response. For example, rapid recruitment of neutrophils into the CNS

is virtually absent, and monocytes are only recruited after a delay of several days. The reasons for this higher threshold are at least threefold. First, because only activated T lymphocytes traverse the BBB, the pool of peripherally activated T cells that enter the CNS for immune surveillance is relatively small (Hickey, Hsu et al. 1991). Yet, without peripheral T cell activation, antigens escape detection; thus, brain transplants survive despite an antigen mismatch (Head and Griffin 1985). Second, there is an active suppression of antigen expression leading to T lymphocytes not recognizing their target nor activating inflammatory mechanisms (Hart and Fabry 1995). Third, adhesion molecule expression, essential in cell to cell contacts during inflammatory cell migration, is low on cerebral endothelial cells (Lassmann, Rossler et al. 1991). CNS immune responses usually take milder courses, and it is not clear yet whether this relative deficit is explicable solely by the lack of immunological structures, or is compounded by counter-regulatory mechanisms. Recent evidence indicates that CNS immune responses are indeed downregulated, with a key role proposed for electrically active neurons (Neumann and Wekerle 1998). Both in vitro and in vivo studies have established that astrocytes and microglia (as brain resident macrophages), in addition to immune cells of peripheral origin, can initiate an inflammatory cascade within the CNS (Owens, Renno et al. 1994). Also, all components of the complement system are found in the brain and are produced by astrocytes, microglia, and, surprisingly, also by neurons.

Cytokines and chemokines are released not only from microglia, astrocytes, and lymphocytes but from neurons as well. Cytokines and growth factors function as mediators of the innate immune response and regulate stress responses, induction of proliferation and/or induction of apoptosis. Depending on their overall effect on immune cells pro- and anti-inflammatory cytokines may be distinguished. Pro-inflammatory cytokines induce an inflammatory response upon tissue damage. Whether this damage comes from ischemia or other pathological conditions, cytokine induction is a common feature in essentially all neurodegenerative diseases but with a great variation in its functional and temporal dynamic expressions. In general, inflammation can be seen as an overall reaction to tissue damage which may have a restorative character but has also been considered as detrimental because of its neurotoxic actions. Today neuroinflammation is rather seen as part of the normal physiological repertoire of the brain.

Among the pro-inflammatory cytokines tumor necrosis factor (TNF), lymphotoxin-alpha and -beta (LTα and β), interferon-γ, and the interleukins IL-1β, IL-2 and IL-6 are the most prominent (Cacquevel, Lebeurrier et al. 2004; Clarkson, Rahman et al. 2004; Sjogren, Folkesson et al. 2004). For stroke patients, circulating blood levels of TNF and IL-6 especially during the first week after the stroke appear to be predictive for outcome as shown in some recent clinical studies (Vila, Castillo et al. 2000; Intiso, Zarrelli et al. 2004; Smith, Emsley et al. 2004). Recent studies also provide evidence for strong expression of cytokines in the early stages of cognitive impairment that precede onset of full-blown dementia (Kropholler, Boellaard et al. 2007; Magaki, Mueller et al. 2007).
Glutamate- and aspartate-induced or –mediated neuronal cell death, defined as the process of excitotoxicity, is generally believed to be a key event in neurodegeneration not only in stroke but also in neurodegenerative diseases such as AD and PD (Glazner and Mattson 2000; Harkany, Abraham et al. 2000; Mattson, LaFerla et al. 2000; Mattson and Camandola 2001; Mattson and Chan 2003; Rogawski and Wenk 2003). Permanent tonic and slightly but

chronically elevated extracellular glutamate and/or intracellular calcium levels could underlie certain aspects of neuronal damage or dysfunction in the pathogenesis of diseases like AD and PD (Glazner and Mattson 2000; Harkany, Abraham et al. 2000; Mattson, LaFerla et al. 2000; Mattson and Camandola 2001; Mattson and Chan 2003; Rogawski and Wenk 2003). The excitotoxic efficacy of glutamate and aspartate is disproportionally enhanced under conditions of acute or permanent ischemia. Because of failing cation-pump activity the affected cells become overloaded with sodium, calcium and chloride ions, thereby depleting the energy generating capacity of the cell because of excessive mitochondrial calcium accumulation. Here too, immunological mediators such as TNF directly influence the survival of neuronal cells (Cooper, Kalaria et al. 2000). In this review we describe the common features of innate immune responses in neurodegenerative disease, with emphasis on TNF and TNF receptors, and discuss the beneficial and detrimental effects of neuroinflammatory responses at the level of signal transduction. We present new views on neuroprotective approaches mediated by TNF receptor systems and their downstream signaling pathways and discuss possibilities for improved diagnostics by imaging inflammatory processes such as microglial activation that may rescue neurons with, as a consequence, a rescue of behavioral brain function.

3. COMMON INFLAMMATORY FEATURES IN NORMAL BRAIN

Inflammatory mediators such as pro-inflammatory cytokines were detected in normal (non-pathological) brain tissue by many groups as reviewed by Vitkovic and coworkers (Vitkovic, Bockaert et al. 2000). Many functions like sleep regulation, fever, "sickness behavior" and even inherent neuronal plasticity functions like long term potentiation (LTP) are influenced in normal brain by cytokines (Albensi and Mattson 2000; Bluthe, Laye et al. 2000; Bluthe, Michaud et al. 2000), such as IL-1, IL-6 or TNF. Vitkovic and coworkers proposed that cytokines be viewed as neuromodulators. For example, in a very recent study it was shown that IL-1β has different signaling pathways in hippocampal neurons and astrocytes. IL-1β is able to induce stimulation of NF-κB in astrocytes generally considered as a neuroprotective mediator. In neurons, however, IL-1β activates the mitogen activated protein kinase p38 and induces CREB activation (Srinivasan, Yen et al. 2004). IL-1α was recently shown to play a role in hippocampal memory processing (Depino, Alonso et al. 2004).

Under non-pathogenic conditions TNF is believed to be involved, together with NF-κB, in neuronal functioning, such as hippocampal synaptic plasticity (Albensi and Mattson 2000) and ionotropic glutamate receptor modulation (Furukawa and Mattson 1998). Under such conditions TNF can be detected in various neuronal and glial cells, while at the same time the TNF-receptors (TNFR1 and TNFR2) are barely detectable (Fontaine, Mohand-Said et al. 2002; Neumann, Schweigreiter et al. 2002). However, upon various stress and noxious stimulations TNF and its receptors become strongly upregulated (Fontaine, Mohand-Said et al. 2002; Laabich, Li et al. 2002). Indeed, many studies have shown that basic levels of cytokines and their receptors can be found in almost all brain areas (Vitkovic, Bockaert et al. 2000). It is, therefore, difficult to determine which cytokine- and/or cytokine receptor level constitute an „inflammatory response" and under which circumstances and expression levels we should speak of neuromodulatory involvement of these cytokines.

4. INFLAMMATORY SIGNALS IN NEURODEGENERATIVE DISORDERS AND STROKE: MARKERS FOR DAMAGE OR PROTECTION?

It has long been known that in stroke clear signs of inflammation can be found very early [for reviews see (Dirnagl 2004)]. Elevated TNF levels have been repeatedly reported to occur shortly after middle cerebral artery occlusion (an animal model for stroke) or after closed head injury (Shohami, Novikov et al. 1994; Yang, Gong et al. 1999) at a time point that precedes infiltration of polymorphonuclear neutrophils. This indicates that TNF, expressed by microglia and also by neurons, may initiate infiltration of peripheral macrophages and lymphocytes. Thus it may be concluded that TNF (among other cytokines) plays a major role in initiating the immune response in stroke.

The role of TNF as an immune mediator is not limited to CVAs. It also plays a role in other neurodegenerative disorders, like Alzheimer's disease (Figure 1.), Parkinson's syndrome, Prion diseases (i.e. transmissible spongiform encephalopathies, such as Creutzfeld-Jakob disease in humans or BSE in cows) and other diseases, where a strong involvement of the immune system is an important hallmark of the pathogenic process. Production of inflammatory mediators such as IL-1β, TNF, some chemokines, like IP-10, MCP1, MIP1α or proteins such as S100β, iNOS, and GFAP is associated with neural injury in AD and PD. This has often been described as the "cytokine cycle", which leads to an ongoing inflammatory process (Ringheim and Conant 2004). Inflammation has been thought to promote development of disease pathology (Casserly and Topol 2004) rather than playing the classical role of the innate immune system as a mechanism for tissue protection. In fact, the "smoking gun" of inflammatory mediators can be seen in all kinds of neurodegenerative disorders. The level of certain cytokines in the cerebrospinal fluid of stroke patients, but also in patients suffering from AD correlates with disease outcome (Vila, Castillo et al. 2000; Intiso, Zarrelli et al. 2004; Smith, Emsley et al. 2004; Hansson, Zetterberg et al. 2006).

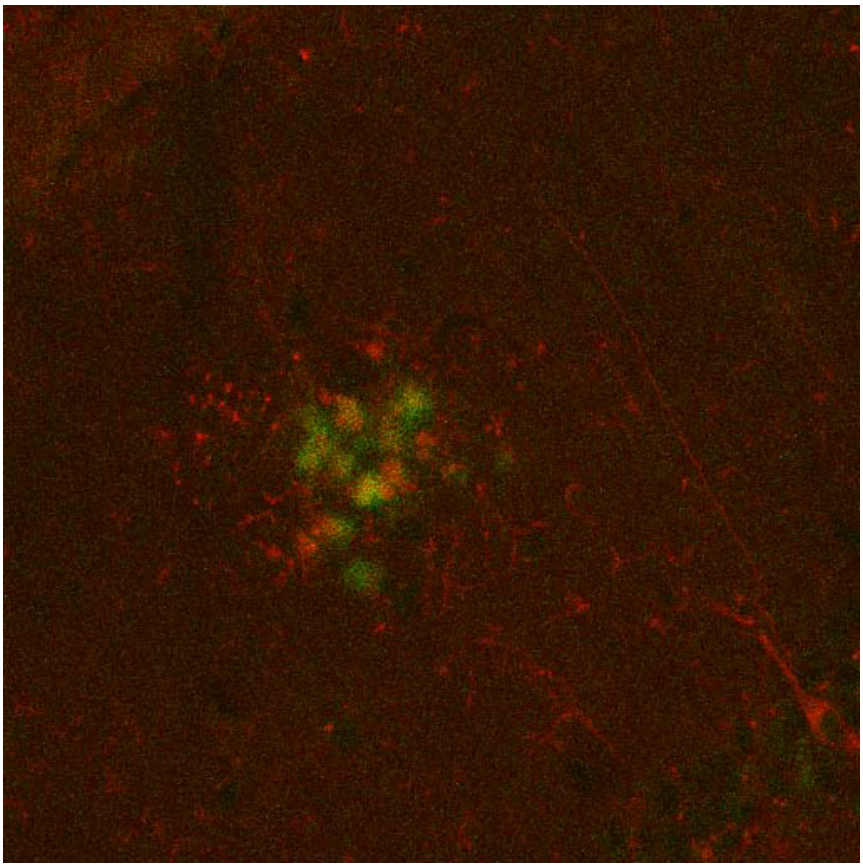

Figure 1. Dense Alzheimer plaque in the brain of a APPSL/PS-1 transgenic mouse stained with thioflavin (green) and immunostaining against TNFR2 (red) (by Granic, Nyakas, Luiten, Eisel, unpublished result).

The above could indicate that inflammation is playing an important and active part in disease development. However, the same picture would emerge were one to consider cytokine levels as dependent on the level of tissue damage. The significance of cytokine levels as markers for a neurodegenerative disease process may be interpreted in two ways. If we consider the innate immune response as a reaction to adverse stimuli and tissue damage, then cytokine and chemokine signaling should have the functions of guiding severely damaged cells into apoptosis and of activating an immune response. At the same time cells or tissue components that survive the noxious stimulus may do so as a result of cytokine-mediated survival signals. This means that resident macrophages and lymphocytes infiltrating the tissue upon damage should also be considered as protective, as suggested also by others (Wang and Feuerstein 2004). The immune response may overshoot in certain distress situations and clinicians may wish to use immunosuppressive drugs for counterbalancing. However, in stroke, glucocorticoids, which are potent anti-inflammatory drugs, have been shown to be without any therapeutic benefit and the literature is controversial on the effect of such treatment (Abraham, Harkany et al. 2000; Davis and Donnan 2004; Norris 2004;

Poungvarin 2004). Clearly, new and probably better concepts for the treatment of neurodegenerative diseases may arise once we get a better understanding of the signaling mechanisms underlying degenerative and protective immune responses.

For AD it should be emphasized that the theory of inflammation as a primary disease-aggravating hallmark, as opposed to a secondary or even a disease-ameliorating factor, remains a hypothesis. One should be aware that our current knowledge of microglia is still incomplete, speculative, and mainly based upon *in vitro* observations rather than *in vivo* studies (Rozemuller and van Muiswinkel 2000). Indeed, B or T cells and immunoglobulins (Igs) are not readily detectable in the Alzheimer dementia brain and are found only in very small amounts in relation to amyloid plaques (without IgM/IgA) (Eikelenboom and Stam 1982). Likewise, although the presence of leukocytes has been demonstrated, their role in Alzheimer dementia has not been established (Myllykangas-Luosujarvi and Isomaki 1994). As such, the evidence for an antigen-driven acquired immune response in Alzheimer dementia, with T cells eliminating amyloid and B cells producing Aβ-specific antibodies, is not as overt as in well-established neuroinflammatory diseases [e.g., multiple sclerosis,(Marx, Blasko et al. 1998)].

5. NEUROINFLAMMATORY IMAGING

Visualising neuroinflammation in neurodegenerative diseases, including Alzheimer dementia, is of interest, first for clarifying the pathophysiology, second for selecting patient subgroups that are candidates for anti-inflammatory treatment, and finally for monitoring patients during trials with such anti-inflammatory agents. Here, we review and discuss current neuroinflammatory imaging modalities, both structural and functional. Structural imaging aims to describe in detail the spatial relationship of neurodegenerative and inflammatory consequences, like mass effects, edema, vascular congestion, thrombosis, petechial hemorrhages, secondary demyelination, gliosis, and finally neuronal destruction, necrosis, or atrophy, as well as visualizing other (nonspecific) structural changes. Alternatively, functional imaging aims to assess the early and late consequences of brain-function or biochemistry during neurodegenerative processes.

5.1. Computed Tomography (CT) Imaging and Magnetic Resonance Imaging (MRI)

CT and, to a greater extent, MRI (gadolinium-enhanced) with its excellent soft-tissue contrast resolution (used mainly for the evaluation of white matter and posterior fossa) are able to detect CNS changes caused by localized inflammatory and degenerative processes (Sze and Zimmerman 1988). The degenerative processes and inflammation must already be at an advanced stage before they can be resolved by one of these imaging modalities. Sensitivity is poor at the early stages of AD (when anatomical changes are not yet detectable). But, in chronic processes, these modalities may also detect structural changes that cannot be revealed otherwise. Both CT and MRI are too insensitive to detect microglial nodules, and, for this reason, the neuroimaging appearance early in the course of neurodegenerative diseases is usually normal (Ketonen and Tuite 1992). In addition, these imaging modalities show poor correlation with histopathological findings (Kim, Tien et al. 1996). Although MRI is useful in the work-up of patients with dementia because it shows

the presence of space-occupying lesions, ventricular dilatation, cerebral atrophy, widening of sulci, or infarcts, this technique is not of particular value in the direct diagnosis of Alzheimer dementia. Promising results have been made with volumetric measurements of the (para)hippocampal and amygdala region (Scheltens 1999). Cecil et al. (Cecil and Lenkinski 1998) reviewed the newer structural or metabolic imaging tools in brain inflammation and concluded that proton MR spectroscopy is a sensitive and specific imaging tool in Creutzfeldt-Jakob disease, herpes simplex encephalitis, and AIDS, and recommended its use in longitudinal studies for predicting and monitoring the response to therapy (Cecil and Lenkinski 1998). Likewise, Bitsch et al. (Bitsch, Bruhn et al. 1999) found that the increases of choline and myo-inositol corresponded to the histopathologically verified glial proliferation and the infiltration of subcortical grey matter structures with foamy macrophages. More recently, Rovaris et al. (Rovaris, Viti et al. 2000) reported on the value of magnetization transfer imaging in measuring brain involvement in systemic immune-mediated diseases. It was found that magnetization transfer imaging provides information about brain damage with increased pathological specificity and detects subtle microscopic abnormalities in normal brain tissue, that go undetected with conventional scanning. However, in some immune-mediated diseases microscopic brain tissue damage seemed to be absent despite macroscopic MRI lesions or clinical evidence of CNS involvement (Rovaris, Viti et al. 2000).

5.2. Functional Imaging Using Radiopharmaceuticals

Nuclear medicine provides several techniques for the detection of inflammation. Studies demonstrating inflammatory lesions were reported as early as 1959, when Athens et al. (Athens, Mauer et al. 1959) labeled leukocytes by intravenous injection of diisopropylfluorophospate labeled with ^{32}P and demonstrated skin blisters in volunteers. Classically, scintigraphic imaging of inflammation has been done with ^{67}Gallium-citrate, radiolabeled leukocytes, nanocolloids, nonspecific human immunoglobulins (HIGs), and ^{18}F-deoxyglucose (FDG). Uptake mechanisms included direct binding to relevant inflammatory cells or proteins (radiolabeled leukocytes, ^{67}Gallium-citrate, HIG) over hyperemia, and binding to lactoferrin excreted *in loco* by leukocytes or to siderophores produced by microorganisms (^{67}Gallium-citrate). In addition, nonspecific local increases in blood supply, extravasation through vessels with increased permeability may give rise to expansion of the local interstitial fluid space (^{67}Gallium-citrate, nanocolloid, HIG). Finally, high glucose uptake is often seen in inflammatory cells (FDG-PET) (Corstens and van der Meer 1999), but inflammatory processes in CNS tissue cannot easily be distinguished because of the high rate of energy metabolism in otherwise unaffected tissue (even in AD). Radiolabeled leukocytes used in cerebral ischemia to detect inflammation accumulated well in massive infarcts with severe neurological impairments and little improvement (Stevens, Van de Wiele et al. 1998) but are of little use in Alzheimer dementia. This is because of the minor hemodynamic and permeability changes (little or no vasodilatation), the slow cellular turnover, and the predominant mononuclear cell infiltrate of chronic processes.

Attempts have been made to visualize inflammation with divalent cobalt radioisotopes, using positron emission tomography (PET) and single photon-emission computed tomography (SPECT). Both *in vivo* and *in vitro* experiments have shown that Ca^{2+} accumulates in the damaged nerve cell body and degenerating axons by two mechanisms: (1) a passive influx

caused by a shortage of ATP following ischemia or chronic excitotoxic overstimulation of nerve cells, resulting in the disappearance of the membrane potential, and (2) neuronal and glial uptake by divalent cation-permeable kainate-activated non-*N*-methyl-D-aspartate glutamate receptor-operated channels in the membrane (Gramsbergen, Veenma-van der Duin et al. 1988; Muller, Moller et al. 1992; Dubinsky 1993; Gibbons, Brorson et al. 1993; Hartley, Kurth et al. 1993; Linde, Laursen et al. 1996). ^{57}Co (SPECT) and ^{55}Co (PET), both as Ca^{2+}-analogs, can reflect Ca^{2+}-influx in ischemically or neurotoxically damaged cerebral tissue. In this way, both ^{57}Co SPECT and ^{55}Co PET have been shown capable of visualizing focal neurodegenerative changes, reactive gliosis, endangered brain tissue, and/or ongoing neuronal tissue decay, including inflammatory lesions in various brain diseases, for example, multiple sclerosis, trauma, tumors, and stroke (Pruss, Akeson et al. 1991; Williams, Pregenzer et al. 1992; Jansen, Willemsen et al. 1995; Jansen, van der Naalt et al. 1996; Jansen, Dierckx et al. 1997; Stevens, Van de Wiele et al. 1998; De Reuck, Stevens et al. 1999).

The limitations of ^{57}Co SPECT and ^{55}Co PET should also be mentioned here. Because of the long physical half-life (270 days) of ^{57}Co, only a limited dose can be injected which is responsible for the low count rate and the resulting low statistics. Alternatively, the PET-radionuclide ^{55}Co has been used (physical half-life 17.5 hours). Moreover, whether divalent radioactive Co visualizes specific aspects of neuronal damage or BBB integrity is still uncertain. To what extent ^{57}Co and ^{55}Co really visualize calcium-mediated processes (in vivo) and therefore reflect identical molecular uptake mechanisms has yet to be determined, although the cerebral uptake of intravenously administered radioactive ^{45}Ca and ^{60}Co in neuronal damage is highly similar (Gramsbergen, Veenma-van der Duin et al. 1988). Finally, the exact cellular site of accumulation of radioactivity is, as yet, not known. As for inflammatory imaging, however, it is interesting to note that calcium may also accumulate in activated leukocytes and that for both ^{55}Co and ^{57}Co only 12% of the total fraction is in its free form while the remainder is bound to leukocytes or plasma proteins (Haverstick and Gray 1993; Clementi, Martino et al. 1994; Jansen, Knollema et al. 1996).

Often, semiquantitative analyses are based on a regional normalisation of radioactivity with the cerebellum as reference region and thus normalisation factor. A regional rather than a global normalization (with whole brain as normalisation factor) may be preferred because a region-specific normalization is known to be more sensitive for diseases in which various regions are pathophysiologically involved, as in Alzheimer dementia (Syed, Eagger et al. 1992). Although some reports described the pathological involvement of the cerebellum in Alzheimer dementia (Joachim, Morris et al. 1989), this region was chosen as the normalisation region because it has both low pathologic susceptibility and absence or at least minimal presence of upregulated inflammatory mediators (Rozemuller, Stam et al. 1990). A previous study had already concluded that the cerebellum is the more appropriate choice of reference region in the quantification of perfusion single-photon emission computed tomography (SPECT) in primary degenerative dementia (Talbot, Lloyd et al. 1994). With regard to perfusion SPECT imaging, the cerebellum was shown to be scintigraphically uninvolved (Pickut, Dierckx et al. 1999).

5.3. Imaging of Activated Microglia in Alzheimer Dementia

PK11195 (1-[2-chlorophenyl]-*N*-[1-methyl-propyl]-3-isoquinoline carboxamide) is a specific and selective high-affinity ligand for the peripheral benzodiazepine receptor (PBR) and, in this way, can be used as a marker for neuroinflammatory lesions (Cagnin, Gerhard et al. 2002; Versijpt, Van Laere et al. 2003; Chen, Baidoo et al. 2004). The PBR is structurally and pharmacologically distinct from the central benzodiazepine receptor (associated with γ-aminobutyric acid-regulated chloride-channels) and earned its name based on its localization outside the CNS and its high affinity for several 1,4-benzodiazepines. It has neither anxiolytic nor spasmolytic activity or interactions with other receptors and has been classified as an antagonist or partial agonist (Parola, Yamamura et al. 1993). As such, Banati et al. (Banati, Myers et al. 1997) showed an increased PK11195 binding to activated microglia after facial nerve axotomy, a lesion causing a retrograde neuronal reaction without nerve cell death with a rapid proliferation and activation of microglia while keeping the BBB intact. The peak of PK11195 binding was observed 4 days after the peripheral nerve lesion, which is consistent with the well-known time course of microglial activation. Moreover, photoemulsion microautoradiography confirmed the restriction of PK11195 binding to activated (i.e., PBR-expressing) microglia, where the full transformation of microglia into parenchymal phagocytes is not necessary to reach maximal levels of PK11195 binding. It was concluded that PK11195 is a well-suited marker of microglial activation in areas of subtle brain pathology, without BBB disturbance, or the presence of macrophages (Banati, Myers et al. 1997; Chen, Baidoo et al. 2004). The PBR is found in highest concentrations in kidneys, colon membranes, heart, steroid hormone-producing cells of the adrenal cortex, ovaries, and testes, and several cell types of the immune system, such as mast cells and macrophages, a localization that is highly concordant with an immunohistochemical study on post mortem human tissue (Bribes, Carriere et al. 2004). It is also present in low concentrations throughout the brain, primarily associated with the choroid plexus, ependymal linings, and glial cells. Although the specific function of the PBR remains unknown, it is generally accepted to be involved in lipid metabolism and/or transport, heme biosynthesis, cell proliferation, or ion channel functions (Zisterer and Williams 1997). Its immunomodulatory role includes the ability to induce monocyte chemotaxis, modulate cytokine expression and superoxide generation, and stimulate antibody-producing cell formation (Zavala, Taupin et al. 1990). Interestingly, the PBR has the ability to reflect neuronal injury, neurotoxicity, and inflammatory lesions without BBB damage, by a rise in the number of binding sites in the case of activated microglia (Guilarte, Kuhlmann et al. 1995; Banati, Newcombe et al. 2000), as previously indicated autoradiographically for AD (Diorio, Welner et al. 1991; Kuhlmann and Guilarte 2000).

In vivo visualization of the human PBR has been performed with ^{11}C-radiolabeled PK11195 for PET in various diseases, like glial neoplasms, ischemic stroke, multiple sclerosis, Rasmussen's encephalitis, Alzheimer dementia, and Parkinson's disease. A signal of activated microglia was produced, which was unrelated to the influx of blood-borne macrophages (1996; Perl, Olanow et al. 1998; Di Patre, Read et al. 1999; Cummings 2000). The potential of this approach was shown in multiple sclerosis, where significant ^{11}C-PK11195 binding was detected in areas where MRI did not show any abnormalities. For instance, PK11195-

related signals were localised in deafferented grey matter regions such as the lateral geniculate body (to which the optic nerve projects) and visual cortex of patients with previous optic neuritis. ^{11}C-PK11195 PET has also been applied in early and mild dementia patients revealing an increased regional binding in the entorhinal, temporoparietal, and cingulate cortex. Moreover, serial volumetric MRI scans revealed that areas with high ^{11}C-PK11195 binding subsequently showed the highest rate of atrophy up to 12-24 months later, indicating that the presence of a local immune response in cortical areas did indeed reflect an active disease process associated with tissue loss. Comparison with FDG-PET revealed that areas with high ^{11}C-PK11195 binding were also characterized by decreased regional glucose use. In one patient with isolated memory impairment without dementia, the pattern of atrophy as seen by volumetric MRI imaging was predicted by the initial distribution of increased ^{11}C-PK11195 binding (Waldemar 1995).

Recently, PK11195 radiolabeled with iodine for SPECT has become available. ^{123}I-labeled iodo-PK11195 is a suitable agent for visualization of the PBR and indirectly for the imaging of neuroinflammatory lesions (Mann, Mohr et al. 1992). In a recent pilot study, [^{123}I]iodo-PK11195 was also applied in Alzheimer dementia, which showed a distinct difference in ligand uptake between Alzheimer dementia patients and controls, indicating the pathophysiological involvement of microglia in frontal, temporal, and parietal cortical regions that were pathognomonically compromised in patients with Alzheimer dementia (Kuhlmann and Guilarte 2000). Moreover, inverse correlations were found between regional [^{123}I]iodo-PK11195 uptake values and cognitive test results. Mean uptake values were increased in various neocortical regions pathognomonically compromised in Alzheimer dementia, and significance was particularly reached in frontal neocortical regions. Although somewhat unexpected, this is in concordance with a very recent study where an intense immunoreactivity for the immune and inflammatory mediator CD40L, expressed on microglia and involved in microglia-dependent neuron death, was found throughout the frontal cortex of AD patients (Diorio, Welner et al. 1991). Also, this frontal increase in [^{123}I]iodo-PK11195 uptake could possibly indicate the progression together with the spreading of active inflammation towards more frontal regions in patients already at an advanced stage of the disease, although the mean mini mental state examination score in that study was at a moderate level of 19. This advanced neuropathological stage is in concordance with the frontal perfusion deficits observed in the present study, deficits that typically are observed later in the course of the disease (1996). Regarding this progression towards more frontal regions, recent biopsy results also showed that the progressive neurological impairment in Alzheimer dementia patients is accompanied by a significant increase in senile plaques, neurofibrillary tangles, and microglial cell activation in the frontal cortex (Di Patre, Read et al. 1999). However, group analyses should be carefully interpreted because there is a marked heterogeneity in Alzheimer dementia patients concerning stage of the disease, progression pattern, predominant topographical lesion, and cognitive subtype, with a substantial overlap between Alzheimer dementia and other neurodegenerative conditions (Waldemar 1995; 1996; Perl, Olanow et al. 1998; Di Patre, Read et al. 1999; Cummings 2000). Such heterogeneity may contribute to the rather large range of neuropsychological scores of Alzheimer dementia patients and may also be reflected in the higher variability of [^{123}I]iodo-PK11195 uptake in Alzheimer dementia patients as compared with controls. Concerning this heterogeneity, behavioral as well as cognitive variability has

been correlated with PET and SPECT findings (Waldemar 1995). Two subgroups with distinct progression rates were already segregated by neuropsychological and cerebral metabolic profiles, in which one rapidly deteriorating group had a significantly greater impairment in executive functions attributed to the frontal lobe and a concomitant greater frontal hypo-metabolism revealed by PET scanning (Mann, Mohr et al. 1992). Age difference between AD patients and controls may explain at least some of the perfusion SPECT findings, but it cannot explain the increased [^{123}I]iodo-PK11195 uptake in Alzheimer dementia patients because age-related increases in ^{11}C PK11195 uptake have been described only in the thalamus, and no age-related effect at all was found in the present study (Banati, Newcombe et al. 2000; Cagnin, Gerhard et al. 2002). Moreover, the age discrepancy between Alzheimer dementia patients and controls probably led to an underestimation of the actual [^{123}I]iodo-PK11195 uptake as a result of the fact that atrophy was not taken into account. Atrophy is more prominent in the older AD group, particularly in the left meso-temporal region, because this area, encompassing the hippocampus, is known for its substantial atrophy in Alzheimer dementia patients (Eagger, Syed et al. 1992).
The literature reviewed here and other reports indicate that the radioligand PK11195, developed both for SPECT and PET, can be considered as a highly sensitive cellular marker for the functional monitoring of microglia *in vivo*, useful for the visualisation of chronic neurodegeneration without BBB breakdown nor other imaging findings.

6. INFLAMMATORY MOLECULES AS POSSIBLE DRUG TARGETS FOR NOVEL THERAPEUTIC STRATEGIES

Exploiting neuroinflammation for diagnostic neuroimaging can certainly help to develop therapeutic strategies that are adapted to the individual needs of patients with neurodegenerative disorders. Moreover, targeting neuroinflammatory molecules could also help to develop drug targeting strategies that act specifically at the site of brain inflammation where drug action would be needed. Therefore, it is important to understand the inflammatory responses in the brain during neurodegenerative diseases. Next we will examine the role of TNF and TNF receptor-mediated signals and their role in neurodegenerative diseases.

6.1. TNF in Neural Injury and in Neuroprotection

In 1999, Nancy J. Rothwell (Rothwell 1999) published a remarkable paper titled with the question: "Cytokines – killers in the brain?". Rothwell and many others who performed a range of studies in different disease models and including various transgenic and knockout mouse models, have in fact shown that strong upregulation of pro-inflammatory cytokines like IL-1β, TNF, interferon-γ or IL-6 and others are potent triggers of damage in the brain which can be attributed to strong inflammatory responses [for reviews see (Wang, Asensio et al. 2002) and (Eisel 2002)]. For a long time a prevalent idea was therefore to consider inflammatory responses to be a major player in the pathological process despite well-known beneficial effects [(Feuerstein, Wang et al. 1997; Rothwell 1999; Chong, Shin et al. 2002) and others]. This was especially true for cytokines like Il-1 or TNF. TNF indeed can induce programmed cell death or apoptosis via TNF receptor 1 (TNFR1) and depending on the cell type also in conjunction with TNF receptor 2 (TNFR2) (Wajant, Pfizenmaier et al. 2003). In

fact there is some evidence that TNF might contribute in part to the disease process as for example in the abnormally processed Alzheimer protein amyloid β (Aβ)-induced neuronal cytotoxicity. It was shown that TNFR1 overexpressing neurons are more sensitive to Aβ-induced toxicity when compared to TNFR1 knock-out neurons (Li, Yang et al. 2004). This study however ignored the well-known fact that overexpressed TNFRs have the tendency to participate in ligand-independent signaling. Moreover, in these studies only short term effects (within 60 minutes) were investigated. If we consider the model systems for brain-ischemia or even chronic diseases, like AD, we should be aware of the fact that we talk about mechanisms lasting from days to several years. These are real clinical situations where disease exacerbating and ameliorating effects overlap. In disease models researchers, for the sake of clarity, try to exclude as many disturbing factors as possible and therefore look for immediate responses. Usually long term responses are more difficult to investigate as too many different parameters are involved. Yet, the time course is important or even critical, especially in neurodegenerative diseases.
In 1994 the group of Mark Mattson (Cheng, Christakos et al. 1994; Bruce, Boling et al. 1996) published for the first time the observation that TNF can have neuroprotective effects on neurons treated with excitotoxic substances. Suzuki and co-workers (Suzuki, Hide et al. 2004) showed that extracellular ATP-activated $P2X_7$ microglia (microglia containing a specific subtype of ATP receptors) protect neurons against glutamate-induced toxicity primarily because they are able to release TNF. It was reported by some groups that under certain conditions TNFR1 exerts neuroprotective signaling (Carlson, Bacchi et al. 1998), while deletion of the TNFR1 and TNFR2 prevents cell death in motor neurons after facial nerve axotomy in adult mice (Raivich, Liu et al. 2002) supporting the notion that not all neuronal populations respond in a similar way to TNF signals.

Several groups in recent years have provided evidence that the protective function of TNF in many neurodegenerative disease models like ischemia or glutamate/NMDA mediated excitotoxicity needs the activation of nuclear factor-κB (NF-κB) [(Barger, Horster et al. 1995; Furukawa and Mattson 1998; Marchetti, Klein et al. 2004); for a review see (Mattson, Culmsee et al. 2000)] and PKB/Akt phosphorylation (Diem, Meyer et al. 2001; Fontaine, Mohand-Said et al. 2002; Yang, Shaw et al. 2002; Marchetti, Klein et al. 2004). Inhibition of base level NF-κB activation induces apoptosis in neurons (Chiarugi 2002). Similarly, TNFR mediated NF-κB activation is important for recovery after traumatic spinal cord injury (Kim, Xu et al. 2001).
Interestingly, the protective function of TNF/TNFR mediated signaling can be extended also to the autoimmune disease multiple sclerosis (MS), which was proven by the disastrous outcome of a clinical study using soluble TNFR as TNF scavenger (1999). A likely explanation for this negative effect of TNF scavenging may be found in the neglect of a divergent action of this cytokine in MS. This conclusion of a possibly predominant beneficial effect of TNF is supported by recent findings in a murine experimental autoimmune encephalitis (EAE) model. In this study by Kassiotis and Kollias (Kassiotis and Kollias 2001) it was shown that TNFR2 activation by TNF is a prerequisite for recovery from the disease. In a toxin mediated demyelination model, another group (Arnett, Mason et al. 2001) could also demonstrate that TNFR2 is needed for remyelination, which is an essential feature of therapeutic approaches in MS. A similar picture emerged from studies with interferon γ

knock-out animals. Although interferon γ plays a major role as mediator for a massive inflammatory response in MS, it seems that at the same time it is also necessary for the containment of inflammation (Willenborg, Fordham et al. 1996).

Pro-inflammatory cytokine function in brain diseases becomes even more complicated when different brain areas are compared. Staining for TNF or TNFR, in the mammalian brain reveals that different neuronal and non-neuronal cell types display different endogenous TNF and TNFR levels in diseased and non-diseased brain [(Fontaine, Mohand-Said et al. 2002) Eisel, Granic, Luiten and Nyakas, unpublished observations]. In the retina, for example (Fontaine, Mohand-Said et al. 2002), TNF and TNFRs are mainly expressed in the ganglion cells of the inner plexiform layer. However, no TNF expression was present in the outer nuclear layer (photoreceptor containing cells), while TNFR expression was revealed in the outer nuclear layer in cells resembling Müller glia. TNFR specific staining was however only present upon exposure of the tissue to ischemic conditions with the strongest signals at 6 hrs after ischemia but still readily detectable after 24 hrs. Photoreceptor cells of the outer nuclear layer therefore were not positive for either TNF or TNFR stainings. Interestingly, susceptibility to ischemic lesions is also reduced in photoreceptor cells, whereas those cell layers that are strongly positive for TNF and TNFRs showed the most prominent sensitivity to the ischemic conditions (Fontaine, Mohand-Said et al. 2002). From these observations alone one could conclude that there are differential cellular responses to cytokines like TNF. In fact this was recently very convincingly proven for IL-1β which in hippocampal neurons activates the mitogen activated protein (MAP) kinase pathway and CREB, and at the same time in hippocampal astrocytes activates NF-κB (Srinivasan, Yen et al. 2004). From the many studies on the effects of TNF on either survival or loss of neuronal cells and tissue, diverse views have emerged. An explanation for this seemingly contradictory outcome of the observed TNF effects may lie in the different protocols, cellular models, availability of tools but also different cellular susceptibilities due to molecular mechanisms. In the case of TNFRs for a long time it was difficult to differentiate between TNFR1 and TNFR2 mediated signaling. Distinct TNFR2 mediated signals were unknown until recently and a differentiation in signaling pathways that started with two different TNF receptors was not considered as a molecular model to explain differences in TNF action on neuronal tissues. However, since genetically manipulated mice lacking TNFR1 and TNFR2 have become available together with specific agonistic antibodies against these two TNF receptors, the contribution of distinct signal transduction pathways in neurodegeneration and neuroprotection can now be investigated *in vivo*.

A molecular mechanism for the regulation of TNFR2 signaling was shown in T cells (Pimentel-Muinos and Seed 1999). During IL-2 driven T cell proliferation, RIP, a Ser/Thr kinase required for NF-κB activation through TNFR1, is upregulated. In the presence of RIP, TNFR2 activates apoptosis, whereas in the absence of RIP, TNFR2 activates NF-κB. Right now it would be pure speculation to translate this mechanism of TNFR2 signal modulation from T cells to neurons. However, given the many similarities in signaling between T cells and neurons it would be tempting to test this hypothesis.
A very special brain region in respect to its vulnerability towards TNF is the substantia nigra (SN), the midbrain region which harbors dopaminergic neurons innervating the striatum.

Loss of these dopaminergic neurons is the cause of the typical symptoms in PD. In the 1980s taking of newly synthesized illegal drugs led to cases of severe juvenile Parkinsonian syndrome in some young Californians. The culprit substance was very soon found to be 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, better known under its shorter name MPTP [see (Langston, Forno et al. 1999)]. Since that time researchers have used this substance for induction of Parkinsonian syndromes in experimental animals. The induction of Parkinson-like symptoms by treatment of rodents with MPTP is reduced in the absence of TNF (Ferger, Leng et al. 2004). This is in accordance with another novel transgenic mouse model with inducible low and high expression of TNF in the SN. High level TNF expressing transgenic mice have reduced numbers of tyrosine hydroxylase positive cells in the SN (TH is an enzyme necessary for dopamine production) and develop Parkinson-like symptoms at 20 to 80 days of age. Low levels of TNF, on the other hand, were overall found to be neuroprotective on TH positive cells of the SN when 6-hydroxy-dopamine (6-OHDA) was used to induce lesions (Chertoff, Di Paolo et al. 2011). In dopaminergic neurons of the SN increased TNF levels induce apoptosis and seem to be involved in the pathology of MPTP-induced lesions whereas low levels of TNF appear to exert a predominantly protective action on SN cells.

The retinal ischemia model serves as a highly reproducible stroke model as the retina is part of the CNS. In this model Fontaine and co-workers (Fontaine, Mohand-Said et al. 2002) could convincingly demonstrate that the dual role of TNF can be explained by the antagonistic functions of TNFR1 and TNFR2. Using mice deficient for TNF, TNFR1 or TNFR2, respectively, they found that the size of lesions upon retinal ischemia induction was only marginally larger in mice deficient for TNF when compared to wild type mice. Here, unlike the situation with MPTP-induced lesions in the SN (as explained above), TNF is clearly not necessary for neuronal cell death. However, mice deficient for TNFR1 showed strong protection against retinal ischemia, whereas mice deficient for TNFR2 proved to be more sensitive to ischemic insult than wild type control animals (Figure 2.).

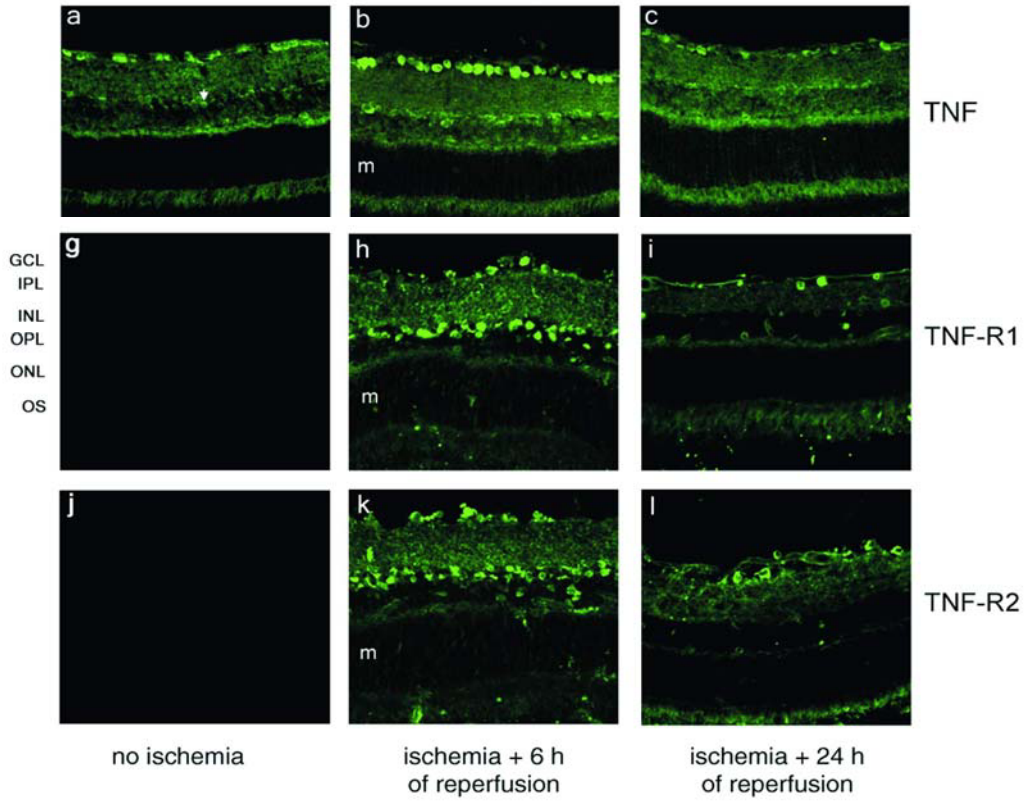

Figure 2. TNF (a-c) and TNFR (g-i) upregulation upon retinal ischemia induction. Note that without ischemia neither TNFR1 nor TNFR2 is detectable (g and j) (modified after Fontaine et al., 2002) (Fontaine, Mohand-Said et al. 2002).

From these results it can be concluded that TNFR1 and TNFR2 have opposite effects and that TNF has to be considered as a reactive cytokine involved in cellular stress responses rather then being part of the pathological process. Interestingly, upon retinal ischemia in the highly protected TNFR1 deficient mice, strong phosphorylation of the protein kinase B (PKB/Akt) was observed, but not in wild type, TNF -, or TNFR2 deficient mice. PKB/Akt is a signaling molecule downstream of the phosphoinositol 3-phosphate dependent kinase (PI3K), which can be pharmacologically blocked by the substances wortmannin and LY249002. Ischemia applied in the presence of LY249002 in TNFR1 deficient mice proved that PKB/Akt phosphorylation is indeed necessary for TNFR2 mediated neuronal protection. The involvement of PKB/Akt phosphorylation in neuroprotection was observed earlier in other neuroprotective signaling pathways (Zheng, Kar et al. 2000; Vincent, Mobley et al. 2004; Wu, Zhu et al. 2004) such as in the IGF and BDNF signaling pathways. However, a TNFR2 dependent PKB/Akt phosphorylation was unknown up to that time point.

From the experiences gathered from TNF transgenic mouse models with more general expression patterns in the brain before and given the fact that TNF expression in human

neurodegenerative diseases is rather restricted to local brain areas, we tried to improve our understanding of TNF function in the brain by using a cell type-restricted neuron-specific promoter to guide TNF expression. A transgenic mouse model (NR2B/TNF) in which TNF is expressed under the control of the promoter of the ionotropic glutamate receptor subunit NMDAR2B led to a deeper insight in TNFR2 mediated neuroprotective signals. Initially, it was observed that TNF expression in the cortex and hippocampus did not lead to severe pathology as observed in other TNF overexpressing mouse models (Campbell, Stalder et al. 1997; Akassoglou, Bauer et al. 1998; Akassoglou, Bauer et al. 1999). However, it proved to be difficult to determine whether the absence of such a forebrain TNF effect should be attributed to a moderate TNF expression in the forebrain or to a regional cell type restricted expression of TNF (or a combination of both). Microglia were activated in the brain areas with TNF expression and the biological activity of transgenic TNF was proven.

According to previous observations TNF and some other cytokines were thought to induce cell death and to be part of the pathological machinery in neurodegenerative diseases. This hypothesis did not hold up when wild type and NR2B/TNF transgenic neuronal cultures were compared for their sensitivity to glutamate. Instead of being prone to cell death, TNF expressing cortical neurons from NR2B/TNF transgenic mice were almost completely resistant to increasing doses of glutamate. In addition, NR2B/TNF neurons exhibit constitutively high levels of PKB/Akt phosphorylation. This neuroprotective effect could be mimicked *in vitro* by pretreatment of wild type cortical neurons with TNF followed by exposure to toxic doses of glutamate. Such a neuroprotective potential triggered by TNF pretreatment was even further enhanced in neurons from TNFR1 knock-out mice, whereas TNFR2 knock-out neurons were more sensitive to TNF-induced apoptosis and could not be protected against glutamate. From these studies we conclude that treatment of neurons with TNF results in resistance to excitotoxic cell death. Apparently this resistance is a result of TNFR2 mediated signaling which can also be demonstrated by specifically triggering TNFR2 in wild type neurons using TNFR specific agonistic antibodies(Figure 3).

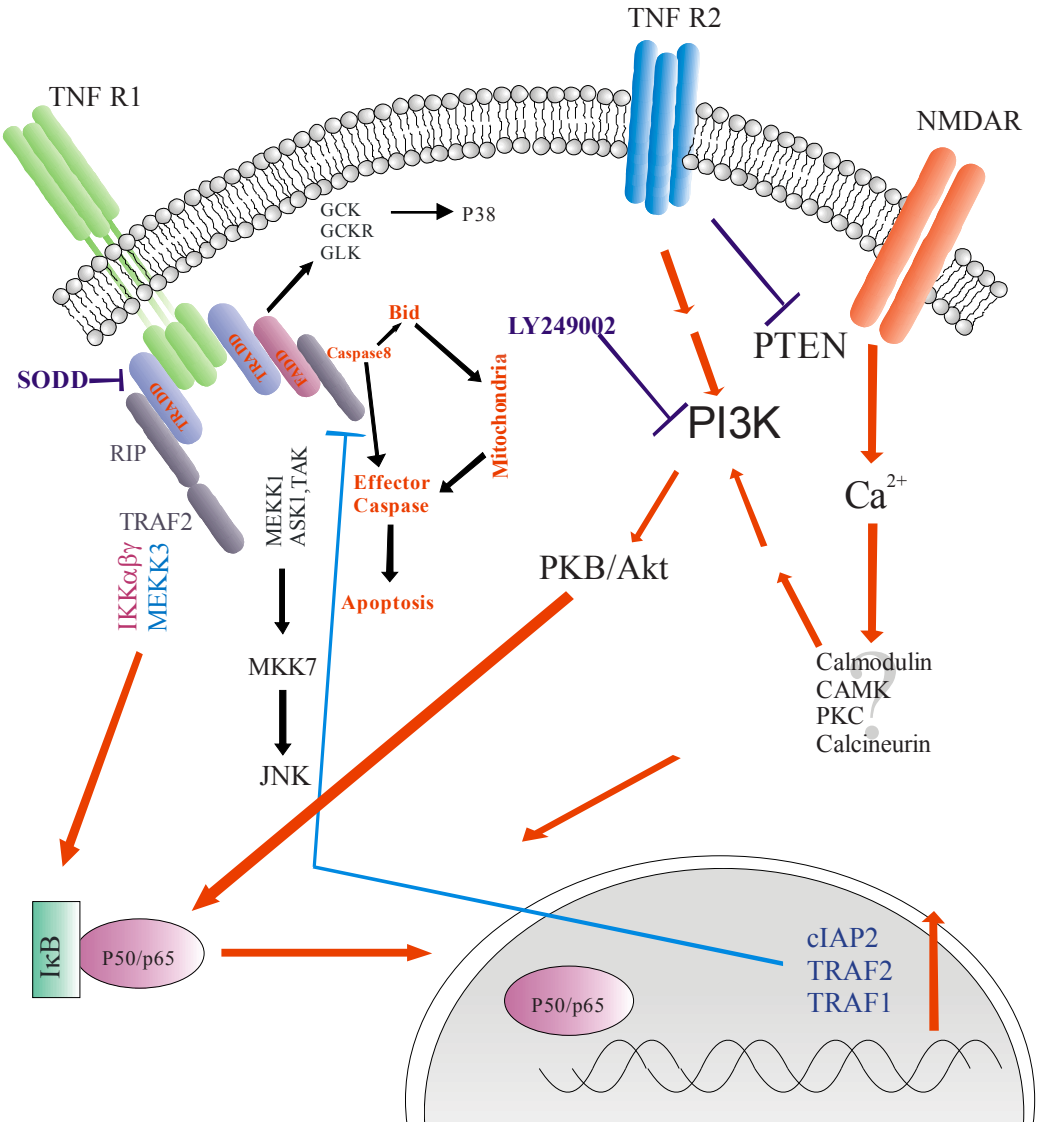

Figure 3. TNFR1 and TNFR2 mediated signaling pathways converge with NMDA receptor signaling on the level of PKB/Akt activation in a model of excitotoxic conditions.

Based on the neuroprotective potential of TNF receptor-mediated signaling we explored the character of the downstream intracellular signaling pathway underlying this protective mechanism. Our studies pointed to the involvement of a PI3K dependent PKB/Akt-mediated NF-κB activation and we were able to demonstrate that NF-κB activation is essential for neuroprotection. We were surprised, however, to find that NF-κB activation by TNFR1 offered no neuroprotection over multiple time points in the experimental setting of primary cortical neurons treated with glutamate plus or minus TNF pretreatment, and, even

more when TNFR1 and TNFR2 signaling p50 and p65 NF-κB molecules were activated. Apparently both TNFR1 and TNFR2 can activate NF-κB but with different cellular effects. This led to the question as to the basis for the difference between TNFR1 and TNFR2-mediated NF-κB activation. It turned out that TNFR1 and TNFR2-mediated NF-κB activations differ strongly in their kinetics. Stimulation of TNFR1 activates NF-κB for roughly 1 to 3 hrs, whereas TNFR2- mediated NF-κB activation under the same conditions lasts for up to 24 hrs. These time differences in NF-κB kinetics could explain why TNFR2, unlike TNFR1, mediates neuroprotection (Marchetti, Klein et al. 2004).

6.2. PKB/Akt and NF-κB: Important Crossroads in Neuroprotection

Several groups have shown previously that PKB/Akt signaling in neurons is neuroprotective. Among the signals that induce PKB/Akt activation are insulin like growth factor (IGF), brain derived neuronal growth factor (BDNF) and TNF (Diem, Meyer et al. 2001; Fontaine, Mohand-Said et al. 2002; Marchetti, Klein et al. 2004). Interestingly, besides neurotrophic factors the ionotropic N-methyl-D-aspartate glutamate receptor (NMDAR), a key player in excitotoxic signaling, also activates PKB/Akt and NF-κB (Lilienbaum and Israel 2003). In TNFR2-mediated neuroprotection experiments it could be shown that TNFR2-mediated phosphorylation of PKB/Akt and NF-κB activation is enhanced by NMDAR signaling (Marchetti, Klein et al. 2004). This is a significant finding in view of the presumed importance of glutamate in many degenerative processes.

The most prevalent form of PKB/Akt in the brain is Akt3 (Yang, Tschopp et al. 2004) and it is therefore likely that Akt3 is a key player in neuroprotection. TNF mediated NF-κB activation via PI3K and PKB/Akt was shown to be dependent on the composition of the inhibitor of κB kinase complex or IKK. Cells with higher levels of the α form of IKK appear to be more responsive to PI3K induced NF-κB activation, whereas IKKα makes TNF-mediated NF-κB activation less responsive to PI3K inhibitors (Gustin, Ozes et al. 2004). In MCF-7 cells, a non-neuronal breast cancer cell line, it was shown that PI3K and PKB/Akt mediated NF-κB activation protects against TNF mediated apoptosis itself (Burow, Weldon et al. 2000). Signaling pathways are obviously highly dependent on the cellular and environmental circumstances. Neurons as non-proliferating cells might be endowed with completely different signaling pathways as compared to proliferating cells (Benn and Woolf 2004). A protective function of TNFR2-mediated signaling, however, might also be present in other tissues as was demonstrated in a mouse model in which overexpression of a non-cleavable murine form of membrane TNF in endothelial cells protected against Con A-mediated liver shock (Willuweit, Sass et al. 2001). The membrane-bound form of TNF is the main activator of TNFR2, whereas the soluble form of TNF binds with much higher affinity to TNFR1 (Grell, Douni et al. 1995) and it is therefore likely that endothelial membrane TNF expression-mediated liver shock protection might be mainly mediated via TNFR2 signaling.
In a very recent report, highly relevant for neuroprotective signaling, the point of transmembrane TNF signaling versus soluble TNF signaling was demonstrated to play a very prominent role. Wang et al. (Wang, Feuerstein et al. 2004) reported that DPH-067517-

mediated inhibition of TACE (the Tumor Necrosis Factor-α converting enzyme) protected rats from focal brain ischemia. TACE is important for cleavage of the membrane-bound form of TNF and its conversion into the soluble form of TNF (therefore TACE inhibits conversion of TNF from a ligand for TNFR2 into the main ligand for TNFR1), Here again TNFR2-mediated neuroprotective signaling mechanisms proved to play a prominent role.

How does TNF mediate PKB/Akt phosphorylation? For the time being there is no direct experimental physical link between TNFR1 or TNFR2 and the PI3K pathway. However, from various tumor cell lines it was reported that the NF-κB-inducing kinase (NIK) and NF-κB activated by TNF lead to downregulation of PTEN (phosphatase and tensin homolog deleted on chromosome ten). PTEN in turn is a strong antagonist of the PI3K/Akt pathway. This may lead to an autoregulatory loop which could result in a sustained increase in PKB/Akt and in turn NF-κB activation (Kim, Domon-Dell et al. 2004). TNFR2-mediated NF-kB and a long lasting upregulation of PKB/Akt, as seen in TNF and glutamate-treated cortical neurons (Marchetti, Klein et al. 2004), could be a result of such an autoregulatory loop. The activation of NF-κB and PKB/Akt by NMDAR was described in an excellent article by Lilienbaum and Israel (Lilienbaum and Israel 2003).

What mechanisms lead to TNF-mediated neuroprotection? Long term TNF treatment of hippocampal neurons was shown to have a modulatory effect on glutamate receptors (NMDA; AMPA, Kainate) (Tancredi, D'Arcangelo et al. 1992; Albensi and Mattson 2000). A great number of protective signaling molecules are known to target both PKB/Akt and NF-κB. The list includes BAD, Bcl-X, the group of inhibitors of apoptosis proteins (IAPs) and other more or less specific molecules - for example, calbindin. Interestingly, Bad phosphorylation, described as an important anti-apoptotic signaling pathway of PKB/Akt, seems not to be affected in primary cortical neurons treated with glutamate and TNF (Marchetti, Klein et al. 2004). It is tempting to speculate that the early response in the form of gene transcription responding to NF-κB as seen with TNFR1-mediated signals may be different from a late or longer lasting response as seen with TNFR2-mediated signals. Does time matter here as well?

7. NEUROINFLAMMATION, MICROGLIAL ACTIVATION AND NEURODEGENERATION: ADDITIONAL PATHWAYS

In dementing disorders with mild cognitive impairment (MCI), as seen in early stage AD, microglial activation with cytokine production are already present (Kropholler, Boellaard et al. 2007; Magaki, Mueller et al. 2007). In the pathogenic pathways initiated by microglial activation, expression of the enzyme indoleamine 2,3-dioxygenase (IDO) may play a pivotal role. IDO catalyzes the first and rate limiting step in the kynurenine pathway, which is the major catabolic pathway for tryptophan, the precursor of the neurotransmitter serotonin (Takikawa 2005). The effects of IDO expression triggered by microglial mobilization are mainly twofold; activation of the kynurenine pathway reduces the biosynthesis of serotonin by decreasing the availability of tryptophan while simultaneously generating neuroactive intermediates, like quinolinic acid (QUIN) and 3-hydroxykynurenine, both implicated in neurotoxicity. Reduced availability of brain serotonin may be associated with behavioral

symptoms like sickness behavior and depression, and may also render neurons more vulnerable to excitotoxic brain damage through increased neuronal excitability (Berends, Luiten et al. 2005).
In general, IDO expression in the brain can be induced by a variety of pro-inflammatory stimuli (O'Connor, Lawson et al. 2009). The main stimulus is the cytokine interferon γ (IFNγ). Human and murine microglia expresses IDO after IFNγ stimulation (Alberati-Giani, Ricciardi-Castagnoli et al. 1996). Neurons and astrocytes can also express IDO after IFNγ stimulation, but levels are much lower than in microglia, and may fail to lead to production of quinolinic acid at a detectable level (Guillemin, Smythe et al. 2005).

Other cytokines can trigger IDO expression. TNF has been shown to induce IDO release synergistically with IFNγ in human macrophages (Currier, Ziegler et al. 2000). IFNβ also activates IDO expression (Guillemin, Kerr et al. 2001). Non-cytokine stimuli such as LPS were demonstrated to induce IDO in monocytes by a mechanism independent of IFNγ (Fujigaki, Saito et al. 2001). Various other pathological stimuli can also induce IDO. E.g. the pathogenic Alzheimer peptide Aβ1-42 can lead to up-regulation of the cellular expression of IDO with, as a consequence, increased quinolinic acid production (Guillemin, Smythe et al. 2003; Guillemin, Brew et al. 2005). Furthermore elevated IDO immunoreactivity was found in human material from AD patients, while quinolinic acid is also implicated in the neuropathology of AD.
The neurotoxic effect of IDO activation is thought to be mediated by the excitotoxicity exerted by the agonist action of quinolinic acid on the NMDA receptor. Indeed, quinolinic acid has long been used as a lesioning agent to reliably ablate specific brain areas (Schwarcz, Whetsell et al. 1983). These neurotoxic effects have been implicated in several neurodegenerative diseases including Huntington's chorea (Estrada Sanchez, Mejia-Toiber et al. 2008), AD (Ting, Brew et al. 2007), stroke (Darlington, Mackay et al. 2007) and amyotrophic lateral sclerosis (Guillemin, Meininger et al. 2005). Quinolinic acid was shown to be able to directly cause apoptosis in astrocytes (Guillemin, Wang et al. 2005) and deregulate glutamate levels by increasing synaptic release and decreasing astrocytic uptake (Tavares, Tasca et al. 2002). Apoptosis is mediated by excitotoxic mechanisms, increased intracellular calcium concentration and deregulated energy balance, followed by oxidative stress and cell death (Foster, Collins et al. 1983). The intermediate metabolite 3-hydroxykynurenine is neurotoxic through oxidative stress (Okuda, Nishiyama et al. 1996), while kynurenic acid, the other major metabolite of the IDO cascade, functions as an NMDA receptor antagonist (Swartz, During et al. 1990) that can counteract quinolinic acid-mediated excitotoxicity and has been described as neuroprotective in ischemia (Gigler, Szenasi et al. 2007).

8. NEUROINFLAMMATION: CAN INFLAMMATION LEAD TO IMPROVEMENTS IN DIAGNOSIS AND TREATMENT OF NEURODEGENERATIVE DISEASES?

Apart from its direct effects on neuronal cell death and neuronal survival, newly disclosed findings also point to unsuspected indirect pathways that link inflammatory mechanisms to neuronal functioning. In this respect we pay specific attention to the interaction between neuroinflammatory mediators and serotonergic neuronal processes and its possible

consequences for the development of neuronal damage in neurodegenerative diseases like cerebral ischemia, PD and AD.

It has been known for more than two decades, that exposure to cytokines, such as IFNγ, leads to strong activation of indoleamine 2,3-dioxygenase (IDO), the rate limiting enzyme in the L-tryptophan-kynurenine pathway (Takikawa, Kuroiwa et al. 1988). High IDO activity converts L-tryptophan to kynurenine and in this way depletes cells in the nervous system of L-tryptophan, the essential precursor for synthesis of the neurotransmitter serotonin or 5-hydroxytryptamine (5-HT). Cerebral ischemia, and probably many other neurodegenerative conditions can lead (Farkas, Donka et al. 2004), via activation of microglia, to IDO expression and in this way to 5-HT depletion. More recent studies have now provided compelling evidence that TNF in particular is a potent activator of IDO (Fujigaki, Saito et al. 2001) and via this pathway may link innate neuroinflammatory mechanisms to loss of 5-HT. With 5-HT depletion the brain loses one of its endogenous neuroprotective transmitter systems as evidenced by our demonstration of the neuroprotective efficacy of 5-HT1A agonists in ischemic stroke experiments (Harkany, Mulder et al. 2001; Oosterink, Harkany et al. 2003). In line with this sequence of events, TNF and its receptor systems can indirectly play a causal role in depletion of 5-HT as an endogenous biological protective mechanism, and may become an unexpected additonal target for neuroprotective therapy. In this respect it was a striking recent finding that we could visualize high levels of IDO immunoreactivity in post-mortem brain samples from AD patients, localized in microglia in and near amyloid plaques in cortex and hippocampus. Ongoing studies on this brain material are now focusing on the interactions between microglial activation, high IDO expression and 5-HT concentrations in this neurodegenerative disease.

The data provided here can give no more than a partial overview of the rich research topic of neuroinflammation in degenerative diseases. The data presented were selected to stress specific points in neuroinflammation:
Firstly, neuroinflammation gives us new and exciting opportunities for development of better diagnostic tools in neuroimaging. This is important in diseases, like AD and other neurodegenerative syndromes that can only be firmly diagnosed at present on post-mortem tissue. Neuroinflammation may also once it gives rise to more precise diagnostic analyses of clinical patients, permit more straightforward therapeutic approaches.
Secondly, there is a question to be resolved. Is the innate immune system part of the problem or part of the solution in the pathogenesis of neurodegenerative diseases, such as stroke, AD or PD. We argue that the protective signaling pathway of TNFR2 opens the road to new concepts in the development of neuroprotective drugs. Agonists triggering TNFR2-mediated responses should be considered as well as antagonists that block TNFR1 function.

CHAPTER 3

Analysis of cognition, motor performance and anxiety in young and aged tumor necrosis factor alpha receptor 1 and 2 deficient mice

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Behavioural Brain Research 2013 Oct.

ABSTRACT

TNFα plays important functional roles in the central nervous system during normal physiological circumstances via intricate signaling mechanisms between its receptors, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). Although the roles of TNFR1 and TNFR2 in the diseased brain have received considerable attention, their functions on behavior and cognition in a non-inflammatory physiological aged environment are still unknown. In the present study we investigated the functional roles of TNFR1 and TNFR2 in learning and memory, motor performance and anxiety-like behavior via several behavioral and cognitive assessments in young and aged mice, deficient of either TNFR1 or TNFR2. Results from this study show that deletion of TNFR2 impairs novel object recognition, spatial memory recognition, contextual fear conditioning, motor performance and can increase anxiety-like behavior in young adult mice. Concerning the functions of TNFR1 and TNFR2 functioning in an aged environment, age caused memory impairment in spatial memory recognition independent of genotype. However, both young and aged mice deficient of TNFR2 performed poorly in the contextual fear conditioning test. These mice displayed decreased anxiety-like behavior, whereas mice deficient of TNFR1 were insusceptible to the effect of aging on anxiety-like behavior. This study provides novel knowledge on TNFR1 and TNFR2 functioning in behavior and cognition in young and aged mice in a non-inflammatory physiological environment.

KEYWORDS: Aging; Cognition; Anxiety; Behavior; Novel object recognition; Spatial recognition memory; Contextual fear conditioning

1. INTRODUCTION

Tumor necrosis factor alpha (TNFα) exerts a broad range of biological functions in the nervous system via its two receptors; tumor necrosis factor α receptor 1 (TNFR1) and tumor necrosis factor α receptor 2 (TNFR2) (Wajant, Pfizenmaier et al. 2003; Naude, den Boer et al. 2011). TNFR1-induced signaling initiates the production of pro-inflammatory factors and can induce a pro-apoptotic environment in neuronal tissue, whereas TNFR2 stimulation promotes neuronal survival (Fontaine, Mohand-Said et al. 2002). TNFα and its receptors have attracted mounting interest regarding their physiological functions in the nervous system on cognition, behavior, neurogenesis of the developing brain and maintenance of neuroplasticity and their roles in the pathology of neurological diseases (Sriram and O'Callaghan 2007; McCoy and Tansey 2008; Clark, Alleva et al. 2010).

Aging is a biological process characterized by time-dependent functional declines, influenced by numerous environmental and physiological factors. A dysregulation of the innate and adaptive immune system is hypothesized as a prominent key player in the aging process which is accompanied by increased inflammatory cytokines, in particular TNFα (Corona, Fenn et al. 2011). TNFα and its receptors have been investigated extensively in aged-associated chronic brain diseases (Mogi, Harada et al. 1994; Paganelli, Di Iorio et al. 2002; Clark, Alleva et al. 2010; Baune B. 2012). However, studies concerning their roles in the healthy aged brain are sparse. It has been shown that ageing can contribute to impaired neuronal TNFα receptor expression *in vitro* (Patel and Brewer 2008), and *in vivo* hippocampal TNFR2 is decreased in aged (22 months) rats (Sama, Mohmmad Abdul et al. 2012). The roles of TNFR1 and TNFR2 on cognition and behavior have been described in young adult TNFR1 or TNFR2 knockout mice concerning their effect on anxiety-like behavior (Simen, Duman et al. 2006; Quintana, Molinero et al. 2007), memory performance (Baune, Wiede et al. 2008) and depressive-like behavior (Simen, Duman et al. 2006). However, the effect of an aged physiological environment on TNFR1 and TNFR2 mediated functioning in behavior and cognition is still unclear. A recent study showed that administering an anti-TNF biologic, that preferentially inhibits TNFR1 signaling, to aged (22 months) rats led to improved memory performance in the Morris Water Maze test (Marques, Mesquita et al. 2012). These studies indicate that age can play an important role on TNFα receptor functioning during non-pathological aging. The aim of this study is to investigate the effect of an aged non-inflammatory physiological environment on TNFR1 and TNFR2 mediated effects on cognitive, behavioral, and motor functioning via various behavioral assessments in young adult and aged WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice.

2. MATERIALS AND METHODS

2.1. Animals

TNFR1$^{-/-}$ (Rothe, Lesslauer et al. 1993) and TNFR2$^{-/-}$ (Lucas, Juillard et al. 1997) mice were initially acquired from Horst Bluethmann (Hoffmann-La Roche, Basel, Switzerland). Genotyping of TNFR1$^{-/-}$ mice was performed using PCR with primers TNF-R1–2883 5'-CTCTCTTGTGATCAGCACTG-3' and Neo-34 5'-CCCGCTTCAGTGACAACGTC-3',

resulting in a 1 kb PCR product for the detection of the TNFR1$^{-/-}$ null allele, and TNFR1–2883 and TNFR1–4938 5'-AGAAATCTCAAGACAATTCTCTGC-3', resulting in a 500 bp fragment for the wild-type allele. Genotyping of the TNFR2$^{-/-}$ mice was performed similarly as for TNFR1$^{-/-}$ mice using TNFR2-A primer 5'-CCTCTCATGCTGTCCCCGGATT-3' and Neo- IL4 5'-GCGCATCGCCTTCTATCGCC-3', resulting in a PCR product of 700 bp for the detection of the TNFR2 null allele, and TNFR2-A and TNFR2-B 5'-AGCTCCAGGCACAAGGGCGGG-3', resulting in a 300 bp fragment for the wild-type allele. All mice were kept in a C57Bl/6 background.

TNFR1 and TNFR2 knockout animals were bred in-house and WT animals were obtained from Harlan laboratories, The Netherlands. All animals were housed in the same room at the animal research facility of the Department of Molecular Neurobiology, at least a month before behavioral experiments were conducted. Animals were individually housed (home cage dimensions, height; 135 mm, width; 150 mm and length; 330 mm) under normal laboratory conditions (air-conditioned, 21±2°C), humidity-controlled room (≈50%), 12/12 h light/dark cycle (light on from 08:00-20:00). Autoclaved wood shavings were used as home cage bedding. Food and water were available *ad libitum*. All animal experiments were approved by the animal ethical committee of the University of Groningen (DEC, application number; 4408C). Animals used for this study were closely monitored for any signs of pathology and inflammatory reactions. Home cage locomotor activity test was performed in 15 months old TNFR1$^{-/-}$ (n=7), TNFR2$^{-/-}$ (n=6) and WT (n=5) control mice. Elevated plus maze, spontaneous alternation test, recognition memory and spatial memory test, balance beam, wire-hanging, contextual fear conditioning test were conducted on 3 months old (indicated as "young" animals in this study) TNFR1$^{-/-}$ (n=10), TNFR2$^{-/-}$ (n=10) and WT (n=10) control mice and concurrently in separate 22 months old (indicated as "aged" animals in this study) TNFR1$^{-/-}$ (n=10), TNFR2$^{-/-}$ (n=10) and WT (n=10) control mice, all of the C57Bl/6 strain.

2.2. Behavioral test procedures

All of the behavioral tests were performed in the same room adjacent to the room where the animals were housed. Experimental results were performed and evaluated by two raters. Since most tests were evaluated visually, all of the animals were provided with a randomized code and one of the two raters was blind to the genotype of the animals in order to minimize biased observation. Mean values of the blinded rater and non-blinded rater were used. After each test mice were given at least 1 week to recover. Tests were performed in the following sequence; elevated plus maze, spontaneous alternation test, recognition memory and spatial memory test, balance beam, wire-hanging, and contextual fear conditioning test. Testing sequence was planned according to the burdensome of an experimental procedure by starting with the least burdensome tests in order to minimize effects of stressful events on subsequent testing procedures (Figure 1). Home cage activity test was performed in a separate group of animals. Home cage bedding was also not replaced at least 2 days prior any of the experimental procedures performed.

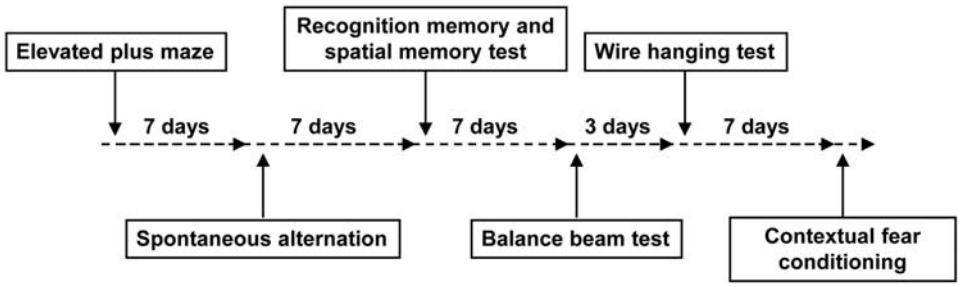

Figure 1. Graphical description of the sequence in which experiments were performed with respective time intervals between the indicated experiments.

2.2.1. Home cage activity test

Mice were tested individually over a 1-week period under non-stressful conditions to assess general locomotor activity. Each home cage was equipped with an overhead infrared sensor, which connected to a computer to register locomotor activity.

2.2.2. Elevated plus maze test

The elevated plus maze test was used to measure anxiety-related exploration. Mice were placed in the center of a plus-shaped maze that was elevated at 60 cm above the ground. Two arms opposite each other were enclosed and the other two served as the open arms without walls. Mice were allowed to explore the maze freely for eight minutes. Transitions were scored as the mice moved from one arm to the other. Arm entry was considered to be complete when the mouse had placed at least three paws in the arm. Furthermore, the total amount of time spent in the center zone, dark arms or open arms were recorded visually (Rodgers, Cao et al. 1997).

2.2.3. Spontaneous alternation test

Short-term spatial memory performance as an expression of working memory was investigated by recording spontaneous (since it is not reinforced) alternation behavior in a Y-maze paradigm. The test was performed in a symmetrical, transparent plastic tubular Y maze. Mice were placed in a Y maze for eight min and the entrances chosen were recorded. Arm entry was considered to be complete when the mouse had placed at least three paws in the arm. The series of arm entries was recorded visually. Percentage alternation is the number of triads containing entries into all three arms divided by the maximum possible alternations (the total number of arms entered minus 2) X 100 (Lalonde 2002).

2.2.4. Recognition memory and spatial memory test

Novel object recognition and spatial recognition tests were carried out to investigate memory performance for subtle visual cues. The test is based on the assumption that rodents explore a novel object more than a familiar one but only if they remember the familiar object. The recognition memory and spatial memory task was performed as described (Williams, Herring et al. 2007) with modifications. The test was conducted in a circular shaped arena (80 cm in diameter and 40 cm in height) on three consecutive days. An

overhead camera was used to record the animal's behavior for subsequent analysis. Prior to the start of testing, animals individually received one habituation session where they were allowed to explore the arena for 10 min (day 1). During the sample phase (day 2), each animal was allowed to explore two identical non-transparent black glass objects with the same shape and size that were placed in a symmetrical position 10 cm away from the barrier. On the following day (day 3) the mouse was returned to the arena that now contained a newly introduced non-transparent black glass object with a different shape and an identical copy of the object previously seen during the sample phase. Again the animal was allowed to explore for 10 min with the same fixed location as day 2. On the last day of the test (day 4) the novel object was moved 90° to another location in the arena that the animal again was allowed to explore for 10 min. Exploration of the objects was scored when the mouse's nose was oriented within one cm toward the object and its vibrissae were moving (Clark, Zola et al. 2000). The exploratory preference was expressed as the ratio of the time spent exploring the novel object over that spent on the two objects. The entire arena and all of the objects were cleaned with 95% ethanol (and completely dried) before another mouse was tested.

2.2.5. Balance beam test

The balance beam test was used to assess motor coordination and balance of mice. Mice were placed 100 cm from a safety platform on an 11 mm thick square wooden beam that was 50 cm above surface level. Each animal was given three trials during a single day of testing. Measurement was taken during the last trial. Latency was measured by the time taken to reach the platform and used as an indicator of the mice's motor functioning.

2.2.6. Wire-hanging test

The wire-hanging test is based on the animal's prehensile reflex and refers to an animal's ability to grasp a horizontal wire with its forepaws and to remain suspended. The test is used to measure the motor function and deficits of rodents. A metal wire 80 cm in length was placed horizontally between two vertical bars at a height of 50 cm. The mice were brought close to the wire by the experimenter until they could grasp it with their front paws; they were then let go. The time they could hang on and their hanging method was recorded and used as a measure for their motor functioning and scoring was performed as described by Hall (Hall 1985). See Table 1 for scoring criteria.

Score	Observation
0	Fell off the wire within the first 30 seconds
1	Held on to the wire for more than 30 seconds
2	Held on to the wire with four paws for at least 5 seconds
3	Held on to the wire with four paws and able to place tail on the wire for at least 30 seconds
4	Held on with four paws, able to get tail on, and traveled along the wire in either direction for at least 5 seconds
5	Traveled to one of the vertical points within the 30-sec test period

Table 1. Scoring criteria for assessment of the wire-hanging test

2.2.7. Contextual fear conditioning

Freezing response upon an unconditioned stimulus, like a mild foot shock, is measured. Mice were individually put into a transparent cage with metallic bars at the bottom, through which mild electric shocks could be applied. Mice were put in the cage and left to explore freely. After 3 minutes an electrical shock of 0.7 mA was administered for two seconds. Mice remained in the cage for an additional 30 seconds after the shock was given. Mice were placed in the same chamber 24 hours later and their freezing behavior was observed for three minutes, in the absence of a shock. To measure their recollection of the shock, freeze response was scored for every 10 seconds. Total amount of freezing is expressed in percentage of time (Chen, Kim et al. 1996).

2.3. Statistical analysis

Differences among multiple means were assessed, as indicated, by one-way or two-way analysis of variance (ANOVA), followed by a Bonferroni test performed with GraphPad Prism version 5.0 for Windows. Differences between two means were assessed by paired Student's t test. Data are presented as a mean value ± the standard error of the mean (S.E.M.).

3. RESULTS

Results are ordered according to their relevant assessments as follows; learning and memory (spontaneous alternation, novel object and spatial memory, and contextual fear conditioning), motor performance (balance beam and wire hanging), and anxiety-like behavior (elevated plus maze).

3.1. Home cage activity test

General home cage locomotor activity was assessed under normal housing conditions as previously described in this manuscript. These measures were performed to control for altered locomotor activity associated with genotype that might affect performance in most of the experimental assessments. No significant differences were found in movement activity (number of running wheel revolutions) in between WT vs. TNFR1$^{-/-}$, WT vs. TNFR2$^{-/-}$ and

TNFR1$^{-/-}$vs. TNFR2$^{-/-}$ mice (Table 2). These results are in accordance with previous studies (Simen, Duman et al. 2006; Baune, Wiede et al. 2008).

Table 2

	Detections	S.E.M.	Group differences	p-values
WT	2756	214	TNFR1$^{-/-}$ vs. wild type	0.24
TNFR1$^{-/-}$	3523	463	TNFR2$^{-/-}$ vs. wild type	0.89
TNFR2$^{-/-}$	2803	230	TNFR1$^{-/-}$ vs. TNFR2$^{-/-}$	0.19

Table 2. Home cage activity test

3.2. Spontaneous alternation

Short-term memory was measured with the Y maze spontaneous alternation test in young and aged WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice. No significant differences between the young WT vs. TNFR1$^{-/-}$, WT vs. TNFR2$^{-/-}$, and, TNFR1$^{-/-}$ vs. TNFR2$^{-/-}$ were observed. No differences were observed between these groups in the aged mice (Table 3). Aging did not have an effect on short term memory performance between any of the experimental groups. Aged WT mice had nevertheless significantly fewer entries into the arms of the Y maze compared to the young WT mice (Table 3).

Table 3

	Young animals			Aged animals		
	Wild type Mean± S.E.M.	TNFR1$^{-/-}$ Mean± S.E.M.	TNFR2$^{-/-}$ Mean± S.E.M.	Wild type Mean± S.E.M.	TNFR1$^{-/-}$ Mean± S.E.M.	TNFR2$^{-/-}$ Mean± S.E.M.
Alternations (%)	60.3±3.0	57.7±3.6	57.9±2.9	61.6±3.4	59.5±2.6	57.3±2.7
Number of entries	30.1±1.8	27.6±1.4	31.2±1.7	22.7±1.9[a]	25.4±2.2	32.6±2.2

[a]$p<0.05$, aged wild type to young wild type.

Table 3. Spontaneous alternation test

3.3. Novel object recognition and spatial memory

Equal amount of interactions (~50%) to both introduced objects were observed in all of experimental groups (Figures 2A, 2B, 2D, and 2E), indicating that the animals did not have a preference to a particular object introduced to the arena. A significant greater interest in the novel introduced object was observed in the young WT and TNFR1$^{-/-}$ mice, whereas no difference was observed in young TNFR2$^{-/-}$ animals. Further analysis revealed a significant difference in novel object recognition ability between WT, TNFR1$^{-/-}$, and TNFR2$^{-/-}$ mice when the interaction of genotype and age was investigated (Figure 2A). In the aged animals, only WT animals showed a significant greater interest to the novel object (Figure 2B). No

effect of aging was found in the novel object recognition test between the young and aged WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice, indicating that TNFR2$^{-/-}$ mice displayed reduced novel object recognition memory compared to WT and TNFR1$^{-/-}$ mice (Figure 2C). However, further analysis for the interaction of age and genotype in the novel object recognition task revealed a significant difference between WT, TNFR1$^{-/-}$, and TNFR2$^{-/-}$ mice (Figure 2C). Young WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice all had significant more interactions with the relocated object (Figure 2D). Analysis for the interaction of age and genotype in the spatial recognition task revealed a significant difference between young WT, TNFR1$^{-/-}$, and TNFR2$^{-/-}$ mice, indicating that young TNFR2$^{-/-}$ mice displayed reduced spatial memory performance compared to WT and TNFR1$^{-/-}$ mice (Figure 2D). Aged WT, TNFR1$^{-/-}$, and TNFR2$^{-/-}$ mice did not display more interest to the spatially relocated object (Figure 2E). Aged WT, TNFR1$^{-/-}$, and TNFR2$^{-/-}$ mice performed significantly worse in the spatial memory test compared to their respective younger counterparts (Figure 2F). The total mobility was determined by the% of time mice were mobile over all three days. No significant differences in the% of mobility between young WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ and young mice vs. their aged genotype was observed (Figure 2G).

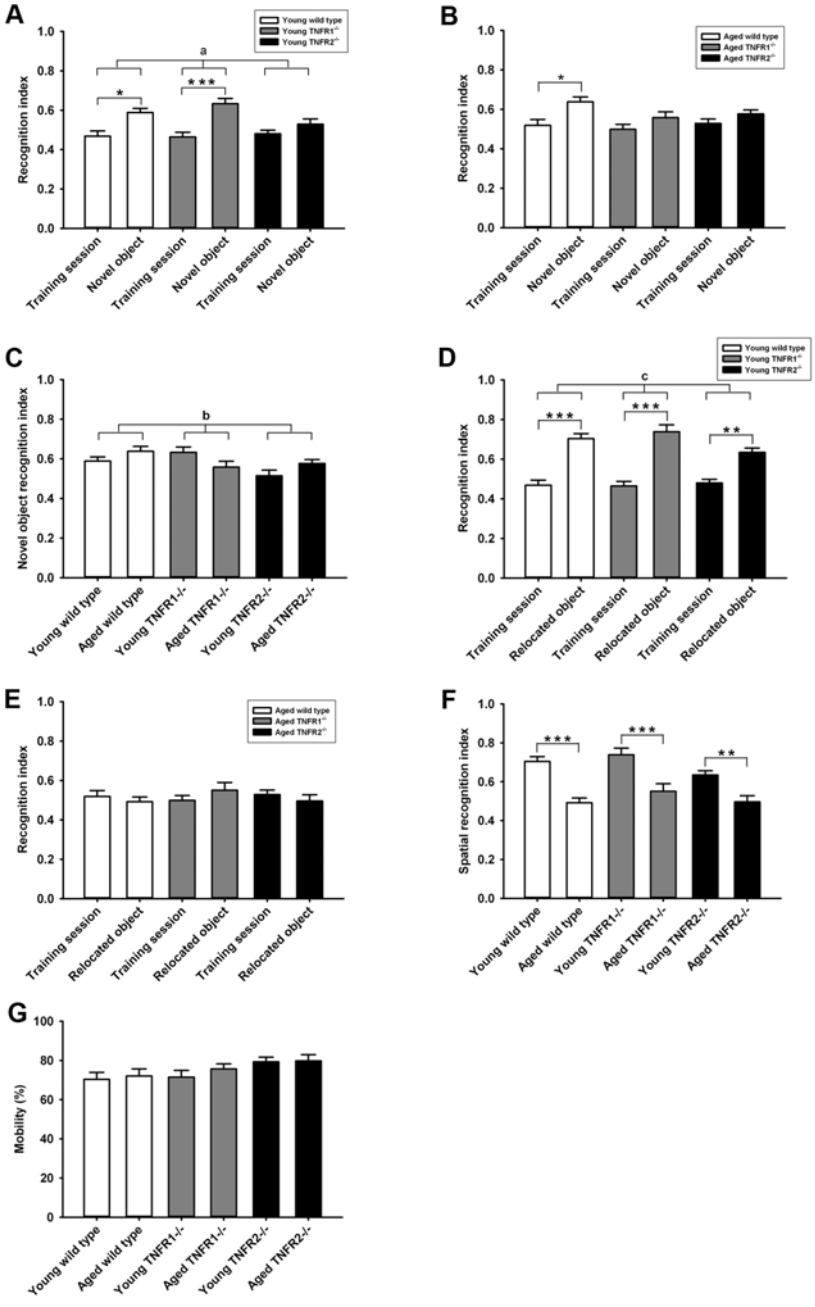
A
Young wild type
Young TNFR1-/-
Young TNFR2-/-
Recognition index
a
*
***
Training session
Novel object
B
Aged wild type
Aged TNFR1-/-
Aged TNFR2-/-
Recognition index
*
C
Novel object recognition index
b
Young wild type
Aged wild type
Young TNFR1-/-
Aged TNFR1-/-
Young TNFR2-/-
Aged TNFR2-/-
D
c
***
**
Relocated object
E
F
Spatial recognition index
G
Mobility (%)

Figure 2. The effect of aging and TNF receptors on novel object recognition and spatial memory. Data are expressed as ratio of interactions with new object compared to object that served as invariable. Ratio of interactions spent with newly introduced object in (A) young adult WT, TNFR1-/- and TNFR2-/ and (B) aged WT, TNFR1-/- and TNFR2-/- mice. (C) Comparison of novel object recognition between young and aged WT, TNFR1-/- and TNFR2-/- mice. Spatial memory recognition performance (D) between young adult WT, TNFR1-/- and TNFR2-/ mice and (E) aged WT, TNFR1-/- and TNFR2-/ mice. (F) Difference between young and aged WT, TNFR1-/- and TNFR2-/- mice in spatial memory recognition performance. (G) Mobility is expressed as% of movement in young and aged WT, TNFR1-/- and TNFR2-/- mice. Error bars indicate the standard S.E.M. (*$p < 0.05$, ** $p < 0.001$, *** $p<0.0001$). Two-way ANOVA revealed a significant interaction between novel object recognition index and genotype in young mice (a, $F(2.53) = 3.72$, $p = 0.03$). Two-way ANOVA also revealed a significant interaction between age and genotype in the novel object recognition test (b, $F(2.54) = 4.46$, $p = 0.016$). Two-way ANOVA revealed a significant interaction between spatial recognition index and genotype in young mice (c, $F(2.54) = 3.42$, $p = 0.04$).

3.4. Contextual fear conditioning

Young TNFR2$^{-/-}$ exhibited a significant reduced freezing response compared to the young WT and TNFR1$^{-/-}$ mice (Table 4). The same effect was also observed in aged mice where aged TNFR2$^{-/-}$ mice displayed a significant impaired freezing response compared to the aged TNFR1$^{-/-}$ and aged WT mice (Table 4).

Table 4

	Young animals			**Aged animals**		
	Wild type Mean± S.E.M	TNFR1$^{-/-}$ Mean± S.E.M	TNFR2$^{-/-}$ Mean± S.E.M	Wild type Mean± S.E.M	TNFR1$^{-/-}$ Mean± S.E.M	TNFR2$^{-/-}$ Mean± S.E.M
Contextual fear conditioning						
Freeze response (%)	50.0±3.4	43.8±5.9	25.9±3.6[a,b]	56.8±5.1	48.8±3.9	22.2±4.7[c,d]
Balance beam						
Crossing time (sec)	11.3±0.8	8.9±0.7	8.4±1.0	29.2±6.2[e]	24.0±4.0[f]	32.9±7.2[g]
Wire-hanging						
Score (out of 5)	4.2±0.4	4.3±0.3	2.2±0.7[h,i]	0.7±0.3	0.2±0.2	0.4±0.3

[a]$p<0.001$, young TNFR2$^{-/-}$ to young wild type;
[b]$p<0.05$, young TNFR2$^{-/-}$ to young TNFR1$^{-/-}$;
[c]$p<0.0001$, aged TNFR2$^{-/-}$ to aged wild type;
[d]$p<0.001$, aged TNFR2$^{-/-}$ to aged TNFR1;
[e]$p<0.05$, aged wild type to young wild type;
[f]$p<0.05$, aged TNFR1$^{-/-}$ to young TNFR1$^{-/-}$;
[g]$p<0.0001$, aged TNFR2$^{-/-}$ to young TNFR2$^{-/-}$;
[h]$p<0.05$, young TNFR2$^{-/-}$ to young wild type;
[i] $p<0.05$, young TNFR2$^{-/-}$ to young TNFR1$^{-/-}$.

Table 4. Balance beam, wire-hanging and contextual fear conditioning test

3.5. Balance beam

Young WT, TNFR1-/- and TNFR2-/- mice passed the balance beam at an equal speed (Table 4). No significant differences between aged WT, TNFR1-/- and TNFR2-/- mice were found for time of crossing. Aging significantly increased the latency of the WT, TNFR1-/- and TNFR2-/-mice to cross the beam when compared to their younger counterparts (Table 4).

3.6. Wire-hanging

Young TNFR2-/- mice performed significantly worse compared to young WT mice and TNFR1-/- mice in the wire-hanging test (Table 4). Because all of the aged mice performed poorly, no significant differences between the aged groups were observed (Table 4). Of note, no significant differences in body weights between groups were observed.

3.7. Elevated plus maze

The elevated plus maze test was performed in order to determine anxiety-like behavior. All mice spent more time in the dark than in the light, or the center of the maze. In the young group, it was observed that young TNFR2-/- mice spent significantly more time in the dark arms and less time in the center, compared to young WT control mice (Figure 3A). Interestingly, aged TNFR1-/- and TNFR2-/- mice on the other hand, spent significantly less time in the dark arms of the maze and more time in the center of the maze compared to aged WT mice (Figure 3B). Aged WT control mice spent significantly less time in the light and center arm and more time in the dark arms compared to the young WT control mice. No affect was observed between young and aged TNFR1-/- mice. Aged TNFR2-/- mice spent significantly less time in the dark arm and more time in the center compared to young TNFR2-/- mice (Figure 3C). Interestingly, a significant difference of time spent in the dark and center was observed between WT, TNFR1-/-, and TNFR2-/- mice when the interaction of genotype and age was investigated (Figure 3C). Aged WT mice exhibited significantly less activity assessed by number of entries into the arms of the maze compared to young WT controls. Young and aged TNFR1-/- mice did not display differences in number of entries. Aged TNFR2-/- mice had significantly more entries than their younger counterparts (Figure 3D). Further analysis indicated a significant interaction of age and genotype on the number of entries between WT, TNFR1-/- and, TNFR2-/- mice (Figure 3D).

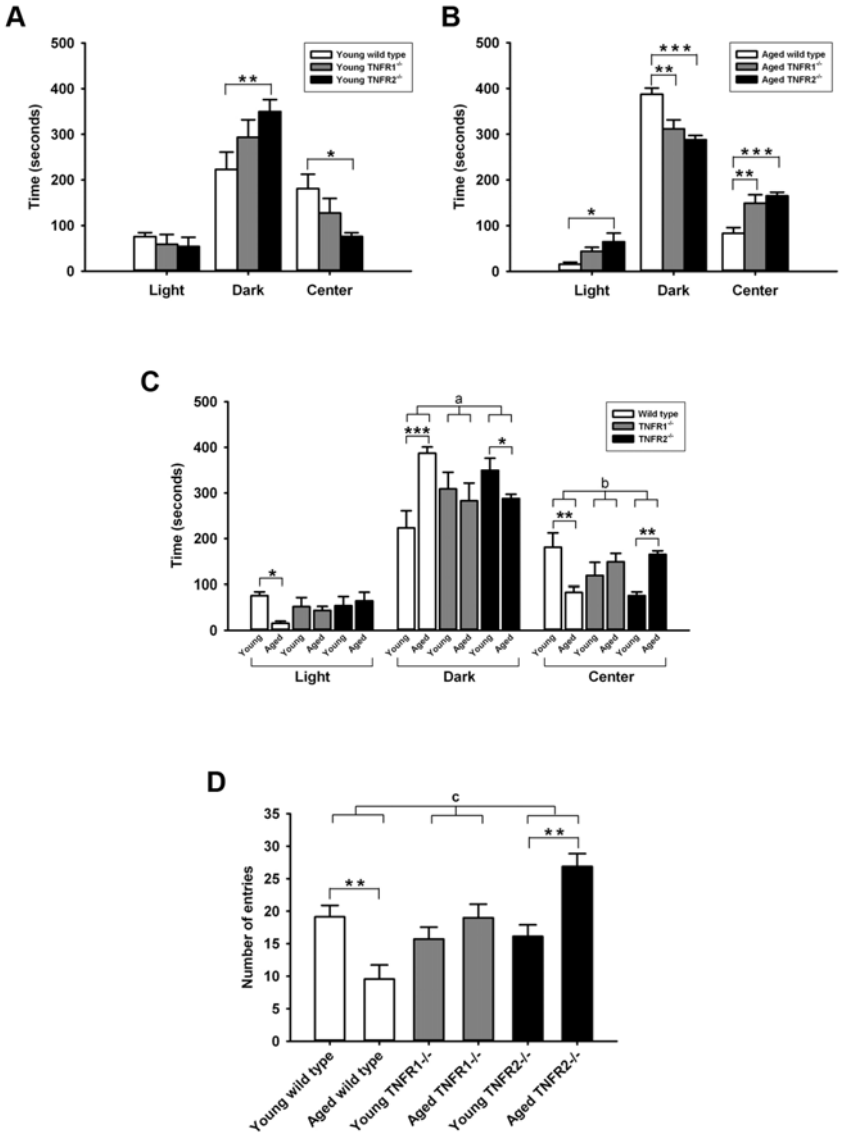

Figure 3. Evaluation of anxiety-like behavior in the elevated plus maze using **(A)** young adult and **(B)** aged WT, TNFR1 $^{-/-}$ and TNFR2$^{-/-}$ mice. **(C)** Comparison between young adult and aged mice in anxiety-like behavior. **(D)** The number of entries into individual arms of the plus maze of all groups tested. Error bars indicate S.E.M. (*$p < 0.05$, ** $p < 0.001$, *** $p<0.0001$). Two-way ANOVA revealed a significant interaction between age and genotype (*a*, $F_{(2.41)} = 10.62$, $p = 0.0002$; *b*, $F_{(2.41)} = 11.95$, $p < 0.0001$; *c*, $F_{(2.41)} = 13.74$, $p = 0.0038$).

Table 5

	Behavioral test	Age group	Wild type	TNFR1$^{-/-}$	TNFR2$^{-/-}$
Learning and memory	Spontaneous alternation (working memory	Young adult	Control group	0	0
		Old	0	0	0
	Novel object recognition	Young adult	Control group	0	↓[a]
		Old	0	0	0
	Spatial recognition	Young adult	Control group	0	↓[b]
		Old	↓↓↓	↓↓↓	↓↓↓
	Contextual fear conditioning	Young adult	Control group	0	↓↓↓
		Old	0	0	0
Motor performance	Balance beam	Young adult	Control group	0	0
		Old	↓	↓↓	↓↓
	Wire hanging	Young adult	Control group	0	↓
		Old	↓↓↓	↓↓↓	↓↓↓
Anxiety-like behavior	Elevated plus maze (anxiety-like behavior)	Young adult	Control group	0	↑↑
		Old	↑↑↑	0	↓

Table. Summary of behavioral phenotypes. Table represents young adult TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice compared with young adult mice and old animals compared with young adults of their respective genotype. **↑,** represent increased behavioral phenotype relative to the behavioral test (↑, $p<0.05$; ↑↑, $p<0.001$, ↑↑↑, $p<0.0001$). ↓ represent decreased behavioral phenotype relative to the behavioral test (↓, $p<0.05$; ↓↓, $p<0.001$, ↓↓↓, $p<0.0001$). *a*, young adult TNFR2$^{-/-}$ mice had impaired novel object recognition compared with young adult wild type and TNFR1$^{-/-}$ mice, determined by two-way ANOVA analyses for interaction between novel object recognition index and genotype ($p=0.03$). *b,* young adult TNFR2$^{-/-}$ mice had impaired spatial recognition compared with young adult wild type and TNFR1$^{-/-}$ mice, determined by two-way ANOVA analyses for interaction between novel object recognition index and genotype ($p=0.016$).

4. DISCUSSION

The goal of this study was to determine if a non-inflammatory, aged physiological environment alters TNFR1 and TNFR2 dependent functioning in behavior and cognition. This was determined via a behavioral approach, assessing several behavioral and cognitive tasks in young adult and aged WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice. No differences in home cage locomotor activity were found between WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice which is in accordance with previous studies (Simen, Duman et al. 2006; Baune, Wiede et al. 2008). Therefore, results from the behavioral tests performed in this study were not affected by alterations in activity due to genotype during testing as opposed to results under home cage

and unchallenged condition. Of note, studies have shown that TNFR1 can have detrimental effects on memory and neuronal survival (Shen and Pervaiz 2006; He, Zhong et al. 2007). The functions induced by TNFα signaling are complex and is highly dependable on the environment in which it occurs. In this manuscript, experiments were performed in TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice that were not subjected to any disease or injury. Some caution should be taken when interpreting our findings, since these animals were born with a permanent lack of either TNFR1 or TNFR2. This might contribute to adaptive processes during aging. Therefore pharmacological approaches to antagonize either TNFR1 or TNFR2 or inducible TNFR1 and TNFR2 animal knockout models will be a sensible approach to further verify our data.
The main findings of this study are summarized as follows (see Table 5): (1) Memory functioning; TNFR2 may play an important function in novel object-, spatial-memory and contextual fear conditioning test in younger animals. Recognition ability in novel object recognition and contextual fear conditioning decreased in aged animals independent of genotype. Thus, age did not have a genotype-specific effect on memory dependent functioning. (2) TNFR2 may play an important role in muscular functioning in young adult mice, observed in the wire hanging task. Furthermore, (3) anxiety-like behavior; young adult TNFR2$^{-/-}$ mice displayed increased anxiety-like behavior compared to young WT and TNFR1$^{-/-}$ mice, whereas old TNFR2$^{-/-}$ mice had decreased anxiety-like behavior compared with old WT and TNFR1$^{-/-}$ mice.

Learning and memory
This study shows that young TNFR2$^{-/-}$ mice display impaired performance compared to the young WT and TNFR1$^{-/-}$ mice in the novel object recognition test. Findings from this study are not in accordance with a previous study by Baune and co-workers, showing that TNFR1$^{-/-}$ and TNFR2$^{-/-}$ (8-12 weeks of age) mice did not exhibit significant learning deficits in the novel object recognition task compared to WT mice (Baune, Wiede et al. 2008). In the aged animals, WT mice spent a significant amount of time with the newly introduced object, whereas the same trend was observed in aged TNFR1$^{-/-}$ and TNFR2$^{-/-}$. Our data indicate that the aged WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$animals did display impaired novel object recognition compared to their respective younger counterparts. With respect to the WT animals, our finding is in accordance with a previous study that illustrated that C57BL/6 mice with similar ages used as the current study did not display age-associated impairment in the novel object recognition test (Vaucher, Reymond et al. 2002).

In the spatial memory assessment (spatial object recognition), young TNFR2$^{-/-}$ mice, similarly to the novel object recognition test, displayed spatial memory impairments compared to young WT and TNFR1$^{-/-}$ mice. A study by Baune reported that TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice of 8-12 weeks of age displayed impaired recognition ability in a spatial recognition compared to WT animals tested with the Barnes Maze [15]. However, the variance outcomes of our results as opposed to that reported by Baune might be due to the different experimental methodologies used. Aged WT, TNFR1$^{-/-}$ and TNFR2$^{-/-}$mice showed impaired spatial memory recognition compared to their younger correspondent genotypes. This may be indicative as a result of aging and not genotype. Our data are in accordance with other studies, in which age related hippocampal changes have been associated with age-

related spatial memory impairment (Begega, Cienfuegos et al. 2001; Havekes, Abel et al. 2011; Klencklen, Despres et al. 2012).
Aged animals did not display deficits in retention of memories for contextual fear conditioning. This finding is in agreement with a previous study that showed that aged C57BL/6 mice did not display impaired memory performance for contextual fear conditioning (Gould and Feiro 2005). Both young and aged TNFR2$^{-/-}$ animals displayed deficits in retention of memories for contextual fear conditioning. Our findings contradict that of a previous study, showing that young adult TNFR1$^{-/-}$ but not TNFR2$^{-/-}$ displayed impaired freezing response in a fear conditioning test [14]. The results showing that both young and aged TNFR2$^{-/-}$ mice displayed deficits in retention of memories for contextual fear conditioning indicate that the absence of TNFR2 and not age causes impaired memory performance in the contextual fear conditioning test. TNFR2 may play a role in amygdala-mediated functioning independent on the effect of aging since memory of the contextual fear conditioning test is dependent hippocampus and amygdala brain regions (Eichenbaum 1996; Raineki, Holman et al. 2010; Orsini, Kim et al. 2011).

Results from our study supports that the absence of TNFR2 or sole presence of TNFR1 can impair learning and memory. TNFα receptors may play important functional roles in neuronal development and maintenance. TNFR2 is important in neurogenesis and cell proliferation, while activation via TNFR1 is the responsible factor of TNFα for negative regulation of neurogenesis in the adult hippocampus (Iosif, Ekdahl et al. 2006). Zhang and colleagues showed in mice that increased TNFα expression in the thalamus is already present during early processes of aging and is mainly produced by microglia (Zhang, Li et al. 2013). Soluble TNFα have been proposed to play a role in microglia-neuron crosstalk that can contribute to neuronal aging (Sama, Mohmmad Abdul et al. 2012; Zhang, Li et al. 2013). The effect of TNFR1 may be increased since increased TNFR1 have been observed in the hippocampus of aged rats (Sama, Mohmmad Abdul et al. 2012). In this regard, a study by Sama and colleagues showed that administration of a soluble dominant negative TNFα, that preferentially inhibits TNFα\TNFR1 signaling, improved learning and memory in aged rats (Sama, Mohmmad Abdul et al. 2012). It would therefore be reasonable to expect that the aged TNFR1$^{-/-}$ mice in this study would have shown improved learning and memory performance. However, aged TNFR1$^{-/-}$ mice exhibited similar cognitive performance than the aged WT mice. This finding might be a result of a mechanistic adaptation during the course of aging due to the absence of TNFR1 since birth of these mice.

Normal and pathological aging is associated with learning and memory deficits, and a decline in working memory has been shown in several animal and human studies (Lamberty and Gower 1992; Mendelsohn and Larrick 2011; Wang, Gamo et al. 2011). We did not observe these age-related changes to working memory between any of our groups, measured with the spontaneous alternation test. Our finding with mice of 22 months of age can be explained by a study by Da Silva, showing that working memory was not affected in aged mice younger than 25 months (Da Silva Costa-Aze, Dauphin et al. 2011).

Motor performance
Aging is not only associated with learning and memory impairment but also with motor dysfunction (Dean, Scozzafava et al. 1981). The motor performance deficit in elderly can be

the result of nervous system or of neuromuscular impairment or both (Seidler, Bernard et al. 2010). The prefrontal cortex, basal ganglia and cerebellum are the main control regions for motor function, and in particular the frontal cortex and basal ganglia are prone to aging effects (Seidler, Bernard et al. 2010). We did not find any significant differences between the young WT and TNFR1$^{-/-}$ or TNFR2$^{-/-}$ animals in the balance beam test. However, aging had a significant effect on coordination skills in all three groups tested.
Muscular strength of the animals was tested in the wire-hanging test. Young TNFR2$^{-/-}$ mice performed significantly worse in this test compared to WT or TNFR1$^{-/-}$ mice. This finding may be explained by the specific functions of TNFα and its receptors in skeletal muscle. Both TNFR1 and TNFR2 are expressed in skeletal muscle tissue (Uysal, Wiesbrock et al. 1997; Zhang, Pilon et al. 2000). The majority of reported data on this topic mainly describes TNFR1 mediated functions in muscle tissue, however the role of TNFR2 is still largely unknown. It has been shown that TNFR1 is responsible for impaired skeletal muscle functioning, especially when TNFα is increased (Hardin, Campbell et al. 2008; Wei, Chen et al. 2008). In this respect, data from our study could lead to the speculation that the sole presence of TNFR1 or the lack of TNFR2 in TNFR2$^{-/-}$ mice may have caused reduced performance in the wire-hanging test or that the presence of TNFR2 may counterbalance the effects of TNFR1 signaling. In accordance with other studies, our findings showed that aging in general has an effect on muscular strength and/or neuromuscular function (Dean, Scozzafava et al. 1981; Joseph, Bartus et al. 1983), possibly also due to increased bodyweight of the aged animals.

Anxiety-like behavior
In this study, young TNFR2$^{-/-}$ mice displayed increased anxiety-like behavior compared to the young WT mice. This finding is also in accordance with a previous study, showing that younger TNFR2$^{-/-}$ mice displayed increased anxiety-like behavior evaluated by the elevated plus maze behavioral test (Gimsa, Kanitz et al. 2012). However, another study by Simen and colleagues showed that there were no significant anxiety-like behavioral differences between WT and TNFR2$^{-/-}$ mice using the elevated plus maze test (Simen, Duman et al. 2006). In the aged mice, WT animals spent significant more time in the dark arms of the elevated plus maze compared to aged TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice. However, aged TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice spent more time in the center of the maze that aged WT mice. This may be an indication that aged TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice displayed reduced anxiety-like behavior or may have been indecisive regarding the preference of arms chosen.
Concerning the effect of age, our results show a clear difference between younger and aged WT animals, namely WT aged mice spent significantly less time in the open arms, while the time spent in the dark arms was dramatically increased. These findings are in accordance with findings as assessed by the elevated plus maze from a previous study done by Fahlstrom and coworkers (Fahlstrom, Zeberg et al. 2011) showing that WT aged mice spent less time in the open arms. Of note, other studies showed that aging did not have an effect on anxiety behavior in rodents based upon observation of defecation and/or urinations as a sign of anxiety (Edstrom and Ulfhake 2005; Altun, Bergman et al. 2007; Fahlstrom, Zeberg et al. 2011). However, the interpretation of anxiety from the different methodologies of testing anxiety and exploratory can be misleading (Fahlstrom, Zeberg et al. 2011).
According to our best knowledge, the effect of aging on TNFα and its receptor functioning in anxiety-like behavior have not been published before. Interestingly, in this setting, young

TNFR1$^{-/-}$ mice did not display significant changes to anxiety-like behavior compared to young WT control, neither did old TNFR1$^{-/-}$ show changes in anxiety-like behavior compared to their young counterparts. This finding indicates that the absence of TNFR1 or sole presence of TNFR2 in TNFR1$^{-/-}$ mice therefore may prevent anxiety-like behavior in an aged environment. Intriguingly, the effect of age had an inverse relationship in TNFR2$^{-/-}$ animals compared to WT mice, in which the effect of age resulted in reduced anxiety-like behavior in TNFR2$^{-/-}$ mice. This same effect was observed regarding the number of entries in the elevated plus maze.

Conclusions

Using a broad spectrum of behavioral tests in this study we were able to reveal that deletion of TNFR2 may impair novel object recognition, spatial memory, contextual fear conditioning, motor functions and can increase anxiety-like behavior in young mice. However, young and aged TNFR2$^{-/-}$ mice performed equally poor in the contextual fear conditioning test. An aged environment caused impaired performance in spatial memory, independent of genotype. Concerning the role of an aged physiological environment on TNFR1 and TNFR2 mediated functioning; aging resulted in decreased anxiety-like behavior in TNFR2$^{-/-}$ mice, whereas TNFR1-/- mice were resistant to changes in anxiety-like behavior mediated by aging. This study indicates that TNFR2 may have important physiological roles in hippocampus-associated memory and muscular functioning during younger age that diminishes with age. Furthermore, TNFR2 may play differential functions in anxiety-like behavior in a young and aged environment. Results from this behavioral study merits further molecular investigations to determine the underlying mechanisms involved in TNFR1 and TNFR2 mediated signaling during normal physiological aging.

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

This study was also supported by the School of Behavioral and Cognitive Neurosciences (BCN) to PJWN, ND and KM.

CHAPTER 4

The role of indoleamine 2,3-dioxygenase in a mouse model of neuroinflammation induced depression

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Journal of Alzheimer's Disease 28 (2011) 1–11

ABSTRACT

Indoleamine 2,3-dioxygenase (IDO), an enzyme which is activated by pro-inflammatory cytokines, has been suggested as a potential link between neuroinflammatory processes in neurodegenerative diseases - like Alzheimer's disease- and depression.
The present study aimed to determine whether neuroinflammation-induced increased IDO levels in the mammalian brain will lead to depressive-like behavior.
Neuroinflammation was initiated in mice by a single intracerebroventricular injection of lipopolysaccharide (LPS). Cerebral inflammation was monitored 1, 2, 3 and 4 days after the injection with small-animal positron emission tomography (PET) using the inflammatory marker [^{11}C]PK11195. In the presence or absence of systemically applied 1-methyl-tryptophan (1-MT), a competitive IDO-inhibitor, we assessed the development of depressive-like behavioral symptoms in parallel with IDO expression and activity.
The PK11195 PET signal reached a highly significant peak 3 days after LPS injection, while these animals displayed a significant increase of depressive-like behavior in the forced swim test (FST) compared to vehicle injected animals. These findings were paralleled by a significant increase of IDO in the brainstem, and an increased kynurenine/tryptophan ratio in the serum. Moreover, we report here for the first time, that inhibition of IDO by 1-MT in centrally induced neuroinflammation under experimental conditions can prevent the development of depressive-like behavior.

KEYWORDS: indoleamine 2,3-dioxygenase (IDO), neuroinflammation, depression, positron emission tomography (PET), lipopolysaccharide (LPS)

1. INTRODUCTION

Neuroinflammation is defined as the activation of an immune response in the central nervous system (CNS) (Streit 2006). During neuroinflammation microglia, the most important resident immune cells, become activated (Dobos, Korf et al. 2010) and as a consequence their morphology starts to change and secretion of pro-inflammatory cytokines, such as interferon γ (IFNγ) (Kawanokuchi, Mizuno et al. 2006), tumor necrosis factor α (TNFα) (Nishioku, Matsumoto et al. 2010) or interleukin 6 (Kakimura, Kitamura et al. 2002), is initiated. An important hallmark of activated microglia is the expression of peripheral benzodiazepine receptors (PBRs) on the outer membrane of the mitochondria, which allows the non-invasive detection their activated state by means of labeled PBR ligands. Ligands such as the isoquinoline PK11195 (1-[2-chlorophenyl]-N-methyl-N-[1-methylpropyl]-3-isoquinoline carboxamide) bind the 18-kDa subunit of the PBRs (Doorduin, de Vries et al. 2008). When labeled with carbon-11, PK11195 can be used as a sensitive marker for positron emission tomography (PET) to visualize and quantify microglial activation *in vivo* associated with various conditions triggered by inflammatory or neurodegenerative processes (Cagnin, Kassiou et al. 2007; Doorduin, de Vries et al. 2008).

Chronic inflammatory changes have been shown to play a key role in the pathogenesis of neurodegenerative diseases, like Alzheimer's disease (AD) (Heneka, O'Banion et al. 2010). The typical hallmarks of AD are extracellular depositions of amyloid β, intracellular aggregates of the protein tau, but besides these features, activation of microglia, elevated cytokine and chemokine levels have been shown as well (Heneka, O'Banion et al. 2010). It has been shown, that AD is often accompanied by symptoms of depression, and patients are under a long-time treatment against depression before clinically diagnosed for AD (Jost and Grossberg 1996). Meta-analysis on retrospective and prospective studies revealed that the history of depression approximately doubles the risk of developing dementia. Several studies support the hypothesis that depression is a likely risk factor for dementia in general and for Alzheimer's disease specifically (Jorm 2000; Jorm 2001).

Inflammation is an important hallmark of sepsis as well. It has been recently shown that severe sepsis can lead to cognitive impairment and functional disability (Iwashyna, Ely et al. 2010).

During peripheral infection, either acute or chronic, the immune system is activated e.g. by macrophage stimulation, and pro-inflammatory cytokines are released, which act also on the brain causing sickness behavior. Sickness behavior shares similar features with major depression. Both are characterized by malaise, weakness, loss of appetite, lethargy, or decreased interest in the surroundings. However, sickness behavior breaks off when the pathogen is eliminated. When the peripheral immune system is continuously activated, like in chronic inflammatory diseases, such as rheumatoid arthritis, osteoarthritis or inflammatory lung disease, the resulting chronic activation of the immune system in the brain can lead to the development of depression. Depression may thus represent a maladaptive version of cytokine-induced sickness behavior (Dantzer, O'Connor et al. 2008). In 1991, R.S. Smith proposed the macrophage theory of depression (Smith 1991) which triggered a growing body of evidence supporting the notion that inflammation can increase the risk of developing major depression (Miller, Maletic et al. 2009). The overall idea being that the overexpression of pro-inflammatory agents is associated with increased activity of the

ubiquitous intracellular enzyme indoleamine 2,3-dioxygenase (IDO), which catalyzes tryptophan catabolism through the kynurenine pathway (Leonard 2007; Dantzer, O'Connor et al. 2008). The depletion of tryptophan in brain cells reduces the production of brain serotonin (5-HT) (Leonard 2007; Dantzer, O'Connor et al. 2008). The degradation of tryptophan along the kynurenine pathway also generates neurotoxins, like quinolinic acid (QUIN) an NMDA receptor agonists, or 3-hydroxykynurenine (3-HK), which leads to apoptosis in neurons, which can add to local excitotoxic neuronal overstimulation next to modulating serotonergic neurotransmission (Muller and Schwarz 2007). Taken together, cytokine-induced IDO-mediated tryptophan depletion (Russo, Kema et al. 2009) and QUIN-mediated neurotoxicity (Maes, Mihaylova et al. 2007; Muller and Schwarz 2007) are hypothesized to be involved in the pathophysiology of mood disorders, like major depression. Nevertheless, it has been also shown that the metabolism of tryptophan by IDO through the kynurenine pathway is increased in AD (Guillemin, Brew et al. 2005). Lipopolysaccharide (LPS) injection in rodents is often used as an experimental model for systemic immune challenges. LPS-injected mice display immediate sickness behavior after peripheral injection of LPS whereas depressive-like behavior seems to emerge in a shifted temporal frame, when sickness is already abated (Dantzer, O'Connor et al. 2008; Moreau, Andre et al. 2008). Accordingly, IDO activation and concomitant depressive-like behavior has been shown to be causally interrelated in this mouse model (O'Connor, Lawson et al. 2009).

Previous studies demonstrated that systemic exposure to LPS induces chronic central neuroinflammation (Qin, Wu et al. 2007) as well as cerebral IDO activation (Lestage, Verrier et al. 2002; Andre, O'Connor et al. 2008). In our study, we measured IDO activity and expression after intracerebroventricular LPS injection and assessed the development of depressive-like symptoms in the presence or absence of 1-methyl tryptophan (1-MT), a competitive IDO-inhibitor. Neuroinflammation was monitored over time by small animal positron emission tomography (PET).

We report here for the first time that the inhibition of IDO in centrally induced neuroinflammation is sufficient to prevent the development of depressive-like behavior in mice.

2. MATERIALS AND METHODS

Ethics statement

All animal care and treatments were reviewed and approved by the Ethical Committee for the use of experimental animals of the University of Groningen under licence number DEC5461.

2.1. Animals

Three months old male C57Bl/6J mice (n=8-10/group, groups: Placebo/PBS, 1-MT/PBS, Placebo/LPS, 1-MT/LPS) were obtained from Harlan (Horst, The Netherlands). Animals were individually housed under normal laboratory conditions (air-conditioned (21±2°C), humidity-controlled room, 12/12 h light/dark cycle (light on from 08:00-20:00). Food and water were available *ad libitum*. In all cases the weight of the animals was monitored on a daily basis.

2.2. Intracerebroventricular LPS injection

Before fixation in a stereotactic apparatus mice were anaesthetized with avertin (250 mg/kg by intraperitoneal injection). Periprocedural analgesia was provided with finadyne (2.5 mg/kg, subcutaneously). The coordinates for the bilateral intracerebroventricular (ICV) injections were -2.5 mm dorsal/ventral, -1.0 mm lateral and -0.5 mm anterior/posterior from bregma (Keith B. J. Franklin 1997). The holes were drilled perpendicularly to the previously exposed skull. 1 µl endotoxin-free phosphate buffered saline (PBS) or 5 µg lipopolysaccharide (LPS, L-6529, serotype 055:B5, Sigma-Aldrich) dissolved in 1 µl PBS was injected into the lateral ventricles using a 1 µl Hamilton needle (cat. nr.170431, Omnilabo) fitted to a 25 µl Hamilton syringe. This dose of LPS was previously shown to induce significant neuroinflammation (Milatovic, Zaja-Milatovic et al. 2004; Aid, Langenbach et al. 2008; Choi, Langenbach et al. 2008; Aid, Parikh et al. 2010). A syringe pump (TSE system, Bad Homburg, Germany) was used for injection at a constant rate of 0.3 µl/min. After injection the needle was kept in the ventricles for an additional 5 minutes and subsequently slowly removed from the brain. The incision was sealed with dental cement.

2.3. Slow-release pellet implantation

Four days prior to ICV injection, mice were initially anaesthetized by 5% isoflurane in an induction chamber. After anaesthetic induction, the animals were placed in an open circuit system and 2% isoflurane was administered via a nose-cone. The competitive IDO inhibitor 1-methyl-DL-tryptophan (1-MT) was applied via a slow release pellet implanted subcutaneously beneath the dorsal skin surface. Pellets were designed to continuously release 5 mg/day of drug for 21 days (Innovative Research of America, Sarasota, FL, USA) (O'Connor, Lawson et al. 2009). Similar drug free pellets served as placebo.

2.4. Synthesis of [^{11}C]-PK11195

The PET tracer [^{11}C]-PK11195 was produced at the University Medical Center Groningen according to the procedure described by Cremer et al. (Cremer, Hume et al. 1992). The product was obtained in 32±10% radiochemical yield as a sterile solution in 10% ethanol in saline. The radiochemical purity was always >95% and the specific activity was 110±69 GBq/µmol.

2.5. Micro-PET imaging

Mice were anaesthetized by 5% isoflurane (Pharmachemie, The Netherlands) mixed with medical air in an induction chamber. Anesthesia was maintained with 2% isoflurane in medical air at a flow of 2 ml/min via a nose-cone. Mice were injected intravenously with 7.5±2.5 MBq [^{11}C]-PK11195 via the penile vein and positioned in a small animal PET camera (Focus 220, Siemens Medical Solutions USA, Inc.) in a transaxial position with their heads in the field of view. A transmission scan with a ^{57}Co point source was acquired for the correction of attenuation by tissue. Ten minutes after tracer injection, a 30-minute static emission scan was started. After completion of the emission scan, the animals were allowed to recover. Emission sinograms were iteratively reconstructed (OSEM2d, 4 iterations) after

being normalized, corrected for attenuation and decay of radioactivity. Regions-of-interest were manually drawn over the whole mouse brain in the reconstructed images using the microPET® ASIPro™ VM6.6.2.0 software. Tracer uptake in regions-of-interest (ROI) was obtained in Bq/cm^3 and converted into the standardized uptake value (SUV), defined as: [tissue activity concentration (MBq/cm^3)]/[(injected dose (MBq)/body weight (g)].

2.6. Elevated plus maze

The elevated plus maze test was used to measure anxiety-related exploration (Ostroveanu, van der Zee et al. 2009). The test consisted of a plus-shaped maze, 50 cm above the floor, with two opposite arms closed by high side walls and two opposite open arms without walls (50 cm long, 5 cm wide). The mouse was placed in the center of the maze and was allowed to explore the maze freely for 8 min. Exploratory activity, time spent in the dark arms, open arms and center zone were recorded visually by two raters (one of them was blind to the experiment). The ratio of time spent in the open (light) and closed (dark) arms and in the center to total time spent in the maze was calculated for each group. A lower ratio of time spent in the open arms and total time is indicative for higher anxiety levels.

2.7. Spontaneous alternation

Short-term spatial memory performance (working memory) was investigated by recording spontaneous (since it is not reinforced) alternation behavior in a Y-maze paradigm (Dolga, Granic et al. 2009). The maze consisted of three tubular and transparent plexiglas arms forming the Y. All three arms were 5 cm in diameter, 27.5 cm long, and at a 120° angle from each other. The experimental room contained visual cues for spatial orientation. The mouse (naive to the maze) was placed into the centre of the Y maze and allowed to explore the maze freely during an 8-min session. The series of arm entries (considered to be completed when all four paws of the animal entered the arm) was recorded visually by two raters (one of them was blind to the experiment). Alternation was defined as successive entries into the three arms on overlapping triplet sets. Working memory was assessed by the alternation percentage, which was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two). Exploratory activity was assessed by counting the total number of arm entries.

2.8. Forced swim test

The forced swim test is a widely used paradigm to measure antidepressant activity and depressive-like behavior in animal models (Cryan and Mombereau 2004). A cylinder (22 cm height × 11.5 cm diameter) was filled with water (23-25 °C) up to a height of 17 cm. The animal was placed inside the cylinder and immobility time was recorded over a 7 min test period. Afterwards the mouse was dried and returned to its cage. Between testing sessions the water was refreshed.

2.9. Protein analysis

Immediately after the PET scans and behavioral experiments, mice were sacrificed by CO_2 inhalation. Brains were quickly dissected, snap-frozen in liquid nitrogen and stored at -80 °C until homogenization and Western blotting (Ostroveanu, Van der Zee et al. 2007). Antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used for IDO (rat monoclonal antibody), and HPRT (hypoxanthine-guanine phosphoribosyltransferase) as an internal standard to correct for variations in protein content (rabbit polyclonal antibody), and stained with horseradish peroxidase-conjugated secondary antibodies (goat-anti-mouse, donkey-anti-rabbit, respectively) The proteins were detected using Enhanced Chemiluminescence (Pierce ® ECL Western Blotting Substrate, Rockford, IL, USA) and the Molecular Image Chemidoc XRS+ System (Biorad). The results were analyzed using Image Lab software (BioRad).

2.10. Serum tryptophan and kynurenine quantification

We used as an *in vivo* index of IDO activity the ratio of the blood levels of kynurenine/tryptophan. Nevertheless, it is known, that tryptophan 2,3-dioxygenase (TDO) also catabolyzes tryptophan into kynurenine, but since 1-MT specifically inhibits IDO, but not TDO, we could use the results of the ratio of kynurenine/tryptophan as a reflection of IDO activity. (Mellor and Munn 2004) Tryptophan and kynurenine concentrations in serum were determined by an automated on-line solid-phase extraction-liquid chromatographic-tandem mass spectrometric (XLC-MS/MS) method with deuterated internal standards as described previously (de Jong, Smit et al. 2009).

2.11. Statistical analysis

The data in Fig. 1F were analyzed by 2-way analysis of variance (ANOVA), and the data in Fig. 4. were analyzed by 3-way ANOVA with repeated measures. All other data were analyzed by one-way ANOVA, followed by the LSD post-hoc test to determine the differences between the selected groups using the program SPSS 16.0 for Windows. A p-value *<0.05 was considered to be statistically significant (p-value **<0.005: highly significant, a p-value ***<0.0005: extremely significant). Data are presented as a mean value ± the standard error of the mean (SEM).

3. RESULTS

3.1. Intracerebroventricular LPS injection-induced neuroinflammation culminates 3 days after injection

We evaluated the temporal course of *in vivo* cerebral inflammation in the mouse brain using micro-PET. (Fig.1D). PK11195 binding potential peaked 3 days after the ICV injection of either LPS ($F(7,57)=5.099$, $p<0.0005$) or, interestingly, also after injection of PBS ($p\leq0.05$) with similar kinetics. Glial activation was significantly higher at day 3 and 4 in LPS injected mice when compared to PBS injected mice. Already on the fourth post-operative day the inflammatory response started to decline (Fig. 1D).

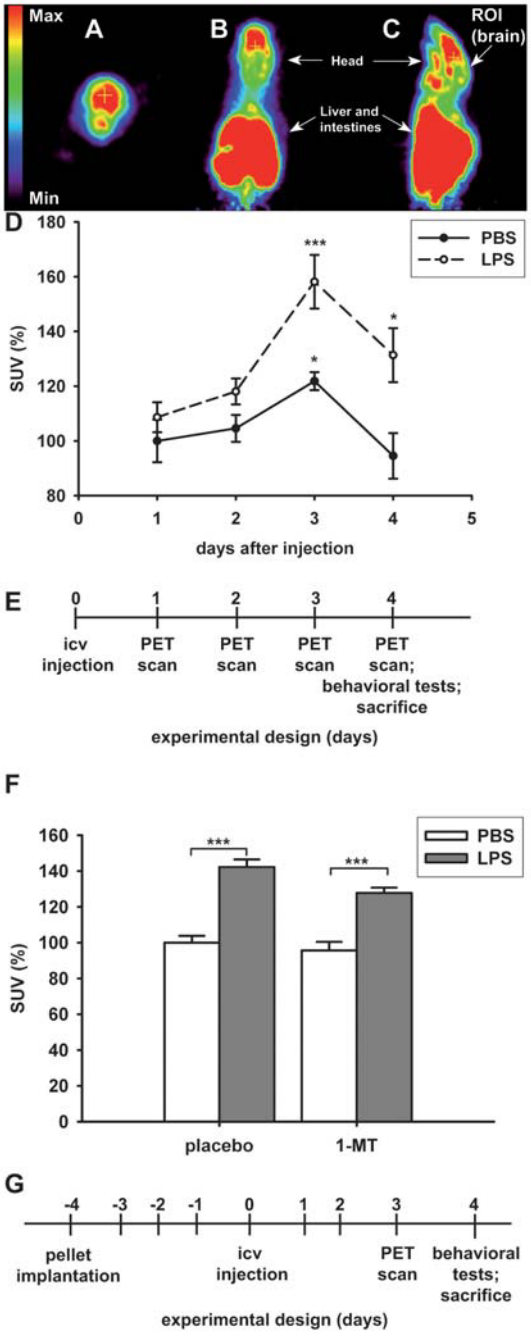

Figure 1. Microglia activation in the brain visualized by small animal PET. Representative pictures of a mouse PET scan (A-C). Microglia activation is depicted using colours for high (red) or low (purple) [^{11}C]-PK11195 uptake. **A)** transversal section, **B)** coronal section, **C)** sagittal section. Brain (ROI) is indicated by the arrow. **D)** Standardized uptake value (SUV) versus days after ICV injection. 1 µl endotoxin-free phosphate buffered saline (PBS) or 5 µg lipopolysaccharide (LPS) dissolved in 1 µl PBS was injected into the lateral ventricles. Data represent mean SUVs ± SEM as a percentage of control, PBS injected animals on day 1. Number of scanned animals: 1. day PBS=13, LPS=14, 2. day: PBS=6, LPS=7, 3. day: PBS=8, LPS=7, 4. day: PBS=5, LPS=5, *p<0.05, **p<0.005, ***p<0.0005. **E)** Diagram of the time course of the PET scanning design. **F)** SUVs 3 days after injection in placebo or 1-MT pretreated, PBS or LPS injected mice. Four days prior to ICV injection, the IDO inhibitor 1-methyl-tryptophan (1-MT) was applied via a slow release pellet (continuously release 5 mg/day of drug/placebo) implanted subcutaneously beneath the dorsal skin surface. Data represent mean SUVs ± SEM as a percentage of the Placebo/PBS group. Number of scanned animals: Placebo/PBS=6, 1-MT/PBS=6, Placebo/LPS=7, 1-MT/LPS=6 *p<0.05, **p<0.005, ***p<0.0005. **G)** Schematic representation of the time course of the experiment.

3.2. Pretreatment with 1-MT does not have a significant influence on LPS-induced neuroinflammation

Microglia activation was measured by PET analysis on the 3rd post-operative day. The results analyzed by 2-way ANOVA, showed a significant effect of pretreatment (placebo or 1-MT) ($F(4.827)$, $p<0.05$) and treatment (PBS or LPS) ($F(74.309)$, $p<0.0005$). These results corroborate previous findings that ICV injection of LPS indeed induces central neuroinflammation. Most interestingly, there was no significant interaction between pretreatment and treatment ($F(1.393)$, $p>0.05$), which means 1-MT does not have a significant influence on LPS-induced neuroinflammation. (Fig.1F).

3.3. LPS induces depressive-like behavior 3 days after ICV injection

A group of animals was tested 3 days after ICV PBS or ICV LPS injection, when neuroinflammation peaked. Depressive-like behavior was significantly increased after ICV LPS injection as tested in the forced swim test ($F(1,11)=5,323$, $p<0.05$, Fig.2G).

3.4. Inhibition of IDO abrogates the development of LPS-induced depressive-like behavior

On day 4 after ICV injection, LPS increased the immobility time in the forced swim test ($F(3,26)=3.36$, $p<0.05$) in placebo/LPS mice, compared to either placebo/PBS ($p<0.05$) or 1-MT/PBS ($p<0.05$) mice. This effect of LPS was completely inhibited by 1-MT pretreatment ($p<0.05$) (Fig.2A). These results demonstrated that IDO inhibition prior to ICV LPS injection was sufficient to prevent the development of LPS-induced depression-like behavior.

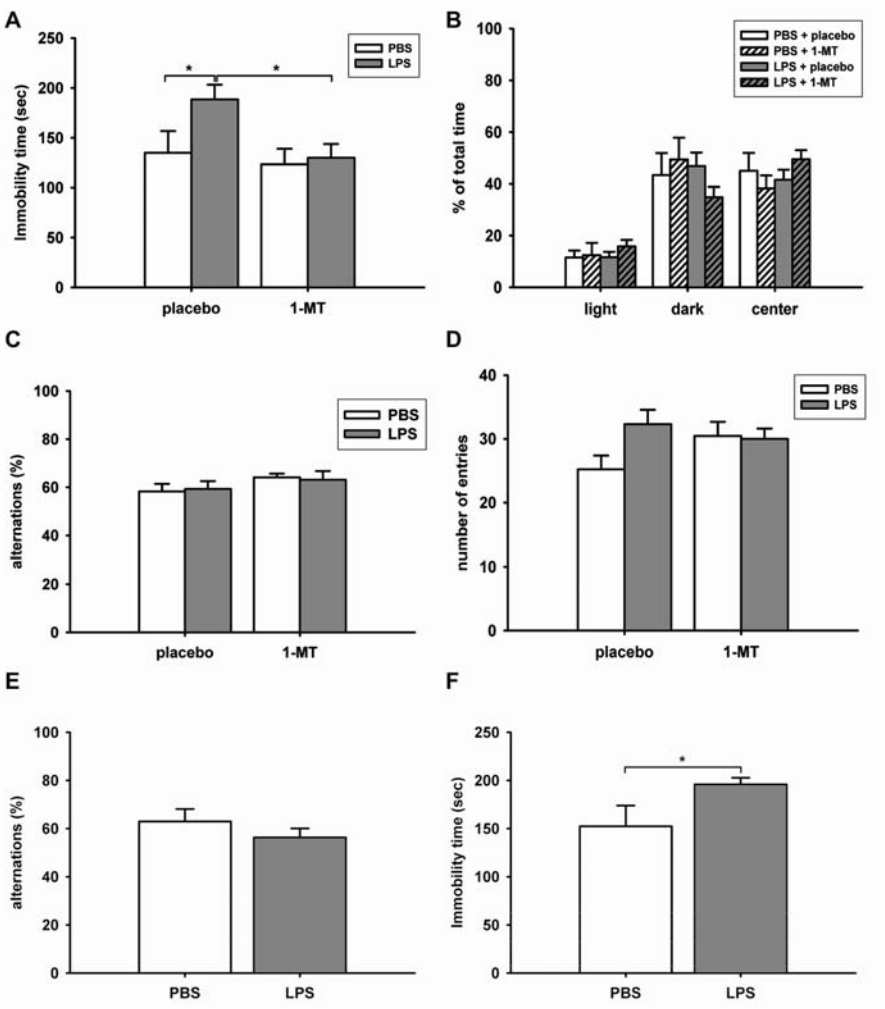

Figure 2. The effect of neuroinflammation on behavior and the consequences of IDO inhibition. A) Forced swim test, a widely used paradigm to measure antidepressant activity and depressive-like behavior in animal models. The animal was placed inside the cylinder and immobility time was recorded over a 7 min test period. Data represent mean values ± SEM, *p<0.05 **B) Elevated plus maze test** was used to measure anxiety-related exploration. The mouse was placed in the center of the maze and was allowed to explore the maze freely for 8 min. Exploratory activity, time spent in the dark arms, open arms and center zone were recorded visually. The ratio of time spent in the open and closed arms and in the center was calculated for each group. A lower ratio of time spent in the open arms and total time is indicative for higher anxiety levels. Data represent mean values ± SEM in percentage.
C,D) Spontaneous alternation test. Short-term spatial memory performance (working memory) was investigated by recording spontaneous alternation behavior in a Y-maze paradigm. The mouse was placed into the centre of the Y maze and allowed to explore the

maze freely during an 8-min session. The series of arm entries was recorded visually. Alternation was defined as successive entries into the three arms on overlapping triplet sets. Working memory was assessed by the alternation percentage, which was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two). Exploratory activity was assessed by counting the total number of arm entries. Data represent mean values ± SEM, *p<0.05. E) Spontaneous alternation test 3 days after ICV PBS or ICV LPS injection. Data represent mean values ± SEM in percentage. F) Forced swim test 3 days after ICV PBS or ICV LPS injection. Data represent mean values ± SEM, *p<0.05.

3.5. ICV administered LPS did not affect anxiety-related behavior or working memory

ICV administered LPS did not affect anxiety behavior in the elevated plus maze on day 4 (time spent in the open arms ($F(3,31)=0.41$, $p>0.05$), in the center ($F(3,31)=0.84$, $p>0.05$), in the dark ($F(3,31)=0.82$, $p>0.05$) when compared to placebo/PBS injected mice (Fig.2B). 1-MT also did not affect anxiety behavior (time spent in the open arms ($F(3,31)=0.41$, $p>0.05$), in the center ($F(3,31)=0.84$, $p>0.05$), in the dark ($F(3,31)=0.82$, $p>0.05$) when compared to placebo/PBS or placebo/LPS injected animals. Working memory, measured in the spontaneous alternation task (number of alternations ($F(3,33)=0.89$, $p>0.05$, Fig.2C)) and exploratory behavior (number of entries ($F(3,33)=2.08$, $p>0.05$, Fig.2D) were not affected by LPS treatment nor by application of 1-MT. Overall, these results showed that neither ICV injected LPS nor concurrent IDO inhibition could affect anxiety-like behavior and working memory performances.
Also, a separate group of animals were tested 3 days after ICV PBS or ICV LPS injection. No abnormalities were found in the working memory (number of alternations: $F(1,13)=1.117$, $p>0.05$, Fig.2E) and exploratory behavior (number of entries: $F(1,13)=1.859$, $p>0.05$, data not shown) as tested in spontaneous alternation test.

3.6. LPS treatment increases IDO levels in the brainstem 4 days after injection

A significant increase of IDO ($F(3,131)=6.388$, $p<0.05$) was detected in the brainstem of placebo/LPS ($p<0.05$) and 1-MT/LPS ($p<0.005$), compared to both PBS groups (Fig.3A). In the other brain regions, no significant differences among the four groups was observed (cerebellum ($F(3,49)=0.37$, $p>0.05$), cortex ($F(3,52)=0.24$, $p>0.05$), hippocampus ($F(3,93)=0.36$, $p>0.05$), hypothalamus ($F(3,51)=0.70$, $p>0.05$), olfactory bulb ($F(3,109)=0.69$, $p>0.05$), and striatum ($F(3,56)=0.815$, $p>0.05$).

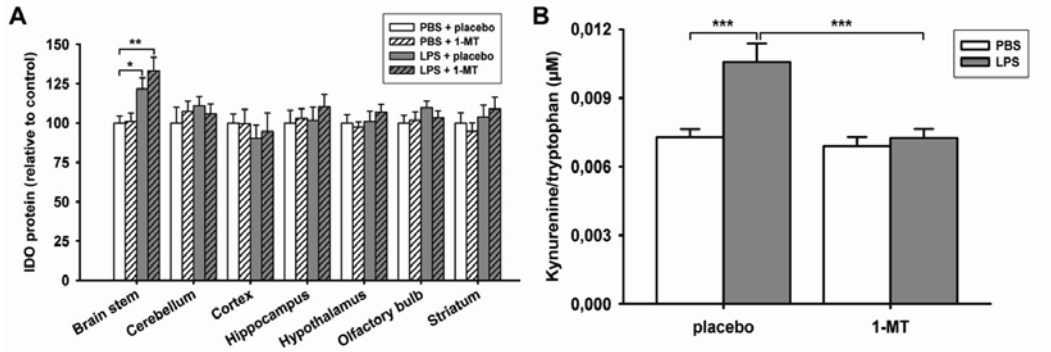

Figure 3. A) IDO protein expression in different regions of the brain. Mice were sacrificed, brains were quickly dissected, snap-frozen and Western blotting was done. Data represent mean values ± SEM as a percentage of the Placebo/PBS group. *p<0.05, **p<0.005. **B) IDO activity in serum**. Tryptophan and kynurenine concentrations in serum were determined by an automated on-line solid-phase extraction-liquid chromatographic-tandem mass spectrometric (XLC-MS/MS) method. IDO activity is defined as the ratio of the concentration of kynurenine and tryptophan. Data represent mean values ± SEM as a percentage of the Placebo/PBS group. ***p<0.0005.

3.7. 1-MT inhibits IDO activity in the serum after ICV LPS injection

ICV LPS injection led to increased IDO activity, defined as a ratio of kynurenine and tryptophan, measured in the serum, which could be completely inhibited by 1-MT ($F(3,26)=10.611$, $p<0.0005$). (Fig.3B).

3.8. ICV injection of LPS results in a robust reduction in body weight

Cerebral inflammation was paralleled by considerable weight reductions in both LPS groups after injection, but was normalized by the time of behavioral assessment (Fig.4). The data were analyzed by 3-way ANOVA with repeated measures, showing a significant effect of time (days) ($F(36.101)$, $p<0.0005$) and also, there was a significant interaction between time and treatment (PBS or LPS) ($F(22.041)$, $p<0.0005$). There was no interaction effect between pretreatment (placebo or 1-MT) and treatment (PBS or LPS) ($F(6.000)$, $p>0.05$). Source of significance was analyzed by univariate measures. No significant difference was observed on day -4 ($F(0.454)$, $p>0.05$), day -3 ($F(0.453)$, $p>0.05$), day 0 ($F(0.777)$, $p>0.05$) and day 4 ($F(4.022)$, $p>0.05$). There was a significant difference on day 1 ($F(8.661)$, $p<0.05$), day 2 ($F(10.662)$, $p<0.05$) and day 3 ($F(7.162)$, $p<0.05$).

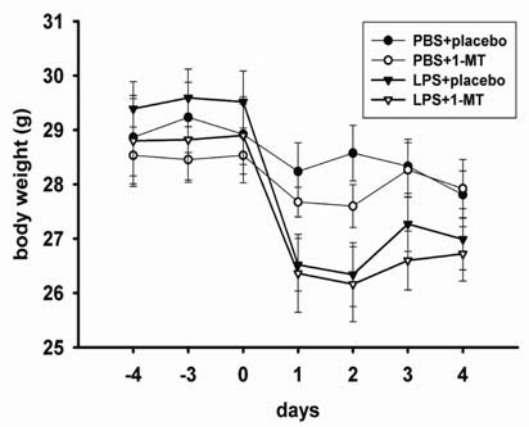

Figure 4. Changes of the body weight of the animals during the experiment. ICV LPS injection took place at day 0. Data represent mean values ± SEM.

4. DISCUSSION

In the study presented here we used an animal model for depression in neuroinflammatory conditions, such as Alzheimer's disease. Our results provide some evidence that LPS-induced neuroinflammation leads to increased IDO protein levels in the brainstem, which is accompanied by the onset of depressive-like behavior. This behavioral phenomenon can be completely blocked by IDO inhibition suggesting a causal relation between the behavioral expression and IDO activation.

Several studies have shown that peripheral inflammation can cause central neuroinflammatory responses which might be causal for depressive-like behavior. Although the question still remains, whether central inflammation alone is sufficient to lead to depressive-like behavior.

O'Connor et al. showed that the inhibition of IDO by 1-MT in the context of systemic LPS administration can normalize plasma and brain kynurenine/tryptophan ratios, while only kynurenine levels were altered (O'Connor, Lawson et al. 2009), which is consistent with the data presented here. We also found a significant increase of the kynurenine/tryptophan ratio in the serum of ICV LPS-injected animals, whereas tryptophan levels remained unchanged.

We used microPET to monitor the ongoing cerebral innate immune responses and dissociate cytokine-induced sickness from depressive-like behavior. Our findings clearly showed that cerebral inflammation was considerably higher, as expected, in ICV LPS injected mice and reached its peak 3 days after the ICV injection. The 4th post-injection day - when sickness-behavior is imperceptible - was chosen to perform behavioral studies to measure depressive-like behavior. On the 4th post-injection day - when the placebo/LPS treated animals exhibited increased depressive-like behavior in the forced swim test – cerebral inflammation was almost gone. This was confirmed by reduced body weight in both LPS groups during the first two post-injection days and subsequent restoration by the time of behavioral assessment (Fig.4.). The reduced food intake might affect the tryptophan levels in the blood, but that can not be the reason of the higher kynurenine/tryptophan ratio, since the 1-MT/LPS group showed a dramatic decrease in the body weight as well, but did not show increased kynurenine/tryptophan ratio. No differences were observed in the general

activity of the mice when the behavioral tests were done, as seen also in the total arm entries in the elevated plus maze test (data not shown) or in the spontaneous alternation test (Fig.2.). We also did not observe any differences in working memory, exploratory behavior and anxiety-like behavior between PBS and LPS-treated groups (Fig.2.), indicating that locomotor activity was normal by the time mice were tested in the forced swim test.
Behavioral tests were also performed on day 3 (Fig.2), showing the same results, namely: working memory and exploratory behavior were not affected, as tested in the spontaneous alternation test, but significantly increased immobility was observed in the forced swim test in the LPS-treated group. Statistical analysis showed that depressive-like behavior after ICV LPS injection was more robust on the 4th post-operative day than on the 3rd (p values are: 0.02, and 0.04, respectively), making the hypothesis stronger, that depressive-like behavior lasts longer after a neuroinflammatory response.
It is important to mention that a limitation of the present study, especially in relation to neurodegenerative diseases, e.g. AD, is that no long-term consequences of ICV LPS-injection and/or IDO inhibition were assessed. Indeed, it has been previously shown that chronic LPS infusion directly in the 4th ventricle in rats can result in impaired cognitive performance (Hauss-Wegrzyniak, Dobrzanski et al. 1998). We therefore hypothesize that an acute and transient LPS challenge might not be sufficient to unequivocally confound the elaborate and pleiotropic physiology of brain cytokines normally involved in cognitive processes (McAfoose and Baune 2009).
Taken together, our findings further support the hypothesis that depression-like behavior remains even when temporally induced sickness behavior has waned. The temporal profile showed a different pattern from that described in previous studies (Frenois, Moreau et al. 2007; Fu, Zunich et al. 2010), but it should be noted that we used larger doses of LPS to induce the immunological challenge in our study. More importantly, we mainly measured reactivity of resident brain cells in response to ICV LPS injection, which might be delayed when compared to the immediate sickness-inducing vagal afference and monocytic infiltration to the CNS following systemic LPS exposure.
We further demonstrate for the first time that the inhibition of IDO by 1-MT pretreatment in the context of centrally LPS-induced neuroinflammation is sufficient to prevent the development of depressive-like behavior. Since IDO in our study was inhibited systemically (O'Connor, Lawson et al. 2009), we cannot ascertain whether this finding is associated with direct central IDO inhibition or reduction of CNS influx of peripheral kynurenine, which has been previously shown to induce depressive-like behavior (O'Connor, Lawson et al. 2009). It may very well be the result of a combined effect of peripheral and central IDO inhibition. It has been shown that even much lower doses of LPS can cross the brain to blood direction (Banks and Robinson 2010), leading to a subsequent peripheral inflammation, as it has been already also shown that lower dose of ICV injected LPS leads to increased production of central and peripheral TNFα (Faggioni, Fantuzzi et al. 1995). We also measured TNFα expression in the liver, and we found an increase of TNFα in both LPS treated groups (Results in %: Placebo/PBS: 100±12, 1-MT/PBS: 99±12, Placebo/LPS: 131±15, 1-MT/LPS: 154±15) indicating peripheral inflammation in our paradigm, although this effect is not because of the dose of LPS we used in this study, but because the immune privilege of the brain is not absolute and there is a constitutive communication of the brain and the periphery (Galea, Bechmann et al. 2007).

It was shown that IDO mRNA expression was reduced in whole brain homogenates of 1-MT/LPS treated mice (O'Connor, Lawson et al. 2009), which was however not observed in the context of *Bacille Calmette-Guérin*-induced infection (O'Connor, Lawson et al. 2009). In any case, those measurements were conducted in whole brain homogenates, so we cannot exclude the possibility that brain IDO expression or cytokine signaling is regionally altered. Indeed, our data show that IDO protein expression is increased in the brainstem of LPS injected mice, independently of pretreatment with placebo or 1-MT. Although we did not measure cytokine expression levels in the brain, our results clearly argue in favor of a post-translational inhibition of IDO by 1-MT. Down-regulation of IDO expression in other brain regions was not found, but even if it was, this does not necessarily mean it would be a direct effect of 1-MT.

Our PET findings are consistent with a moderate, but not significant anti-inflammatory effect of 1-MT, as shown by the reduction of peripheral benzodiazepine receptors expressed in the brains of 1-MT/LPS relative to placebo/LPS treated mice. O'Connor et al. (O'Connor, Lawson et al. 2009) have shown that the presence of 1-MT did not modify the expression of pro-inflammatory cytokines in mice upon peripheral injection of LPS. However, direct as well as accumulative immune effects of the various neuroactive tryptophan catabolites have to be taken into account. Indeed, Maes et al. (Maes, Mihaylova et al. 2007) have shown *in vitro* that kynurenine and kynurenic acid (KYNA) display anti-inflammatory properties via reduction of stimulated IFNγ and TNFα production. In addition, Doorduin et al. (Doorduin, de Vries et al. 2008) comment that PBRs are also expressed in activated astrocytes, which preferentially produce KYNA (Guillemin, Smith et al. 2000; Muller and Schwarz 2007). We therefore hypothesize that IDO inhibition in microglia mitigates the production of pro-inflammatory QUIN, which in combination with a shortage of anti-inflammatory astrocyte-derived KYNA upon LPS challenge, might leave the brain of 1-MT/LPS mice in a neuroinflammatory state lower than placebo/LPS mice, but greater than the placebo/PBS animals.

We have found significantly higher levels of IDO in the brainstem of LPS injected mice. It has been shown that physical stressors – such as trauma or infection – rapidly affect the brainstem, where coordinated processing of autonomic, immune and neuroendocrine information takes place (Ulrich-Lai and Herman 2009). Interestingly, injection of mice with LPS selectively increases immediate-early gene expression within serotonergic neurons of the interfascicular part of the dorsal raphe nucleus (Hollis, Evans et al. 2006). These neurons comprise a unique, anatomically and functionally distinct immune-responsive subpopulation within the brainstem Raphe complex that essentially differs from the neuronal population responding to anxiety- and stress-related stimuli (Lowry, Hollis et al. 2007). This may explain the "paradox" of decreased behavioral activity but increased (Linthorst, Flachskamm et al. 1995) or unchanged (O'Connor, Lawson et al. 2009) serotonergic neurotransmission following acute immune stimulation.

In conclusion, our findings indicate that neuroinflammation induces depressive-like symptoms in an animal model, which can be abolished by inhibiting the effects of IDO. Moreover, this study does not only provide evidence of a pathogenic role of cerebral inflammation in the precipitation of depression, but suggests also that symptoms often concurring with depression, such as anxiety, feelings of misery and impaired working memory are the consequences of some other processes and apparently dissociated from brainstem serotonin neurotransmission.

ACKNOWLEDGEMENTS

This work was supported by grants from the International Foundation for Alzheimer Research (ISAO), grant no.06511, the Gratama Foundation and the EU-grant FP6 NeuroproMiSe LSHM-CT-2005-018637. The skillful technical assistance of Dr. J.Doorduin, W.Douwenga, J.N.Keijser, J.Krijnen, Dr. M.F.Masman and J.W.A.Sijbesma is greatly appreciated.

CHAPTER 5

Interferon β induces the upregulation of indoleamine 2,3-dioxygenase and leads to depressive-like behavior and memory impairment in mice via the interferon α/β receptor

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Manuscript in preparation

ABSTRACT

Interferon beta (IFNβ) has been successfully used for a long time in the treatment of Multiple Sclerosis (MS). Nevertheless, a direct connection between IFNβ-therapy and neuropsychiatric complications, e.g. agitation, depression, anxiety and memory loss comes from recent clinical observations. 30-40% of MS patients treated with IFNβ develop major depression, however after the withdrawal of the treatment, a complete remission of the symptoms was observed.

IFNβ is a potent inducer of indoleamine 2,3-dioxygenase (IDO), a tryptophan catabolising enzyme, which has been shown to play an important role in depression.

The aim of the present study was to determine whether IFNβ-therapy is responsible for neuropsychiatric problems the interferon α/β receptors. To investigate the effect of IFNβ, wildtype and interferon α/β receptor knock out (IFNRKO) mice were injected with IFNβ for seven consecutive days. PBS injected wildtype and IFNRKO animals were used as controls.

We report here for the first time, that IFNβ induces learning and memory problems and depressive-like behavior via the IFNα/β receptors as observed in wildtype IFNβ-injected animals. Furthermore, IDO expression in the brain stem and cortex and IDO activity in the blood of the wildtype, IFNβ injected mice were found to be increased. From these results, we hypothesize, that IFNβ induced IDO expression and higher activity might be responsible for the depressive-like behavior after IFNβ treatment.

KEYWORDS: Interferon beta (IFNβ), indoleamine 2,3-dioxygenase (IDO), depressive-like behavior, Multiple Sclerosis (MS), neuroinflammation

1. INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory disorder of the central nervous system (CNS). The disease is the most common cause of neurological disability in young and middle aged adults (Feinstein 2000). The most important feature of the disease is the damaged myelin sheaths. Demyelination leads to physical and cognitive disability. Hitherto, there is no cure for the disease, only therapies that can treat and guard the relapse of MS. As a first-line therapy in MS, interferon beta (IFNβ) is used successfully. Interferon beta is a member of the interferon type I family. These cytokines have the ability to modulate immune responses (Sternberg, Chadha et al. 2008). IFNβ is able to slow the progression of brain atrophy in MS and reduces the relapse rate by approximately 30% (Farrell and Giovannoni 2010; Rudick and Goelz 2011). However, a growing body of evidence shows that interferon-therapy, including IFNβ can have serious side effects. Especially interferon alpha (IFNα)-induced depression and cognitive deficits have been widely investigated (Merimsky and Chaitchik 1992; Valentine, Meyers et al. 1998). Although not much is known about the psycho-neurological effects of IFNβ clinical observations suggest a direct connection between IFNβ-therapy and neuropsychiatric complications such as agitation, depression, anxiety and memory loss (Moriuchi, Shimizu et al. 1996; Feinstein 2006; Paul, Ricour et al. 2007; Fragoso, Frota et al. 2010). It has been previously shown *in vitro* and *in vivo* that all cell types of the central nervous system can respond to type I interferons (Njenga, Pease et al. 1997; Paul, Ricour et al. 2007). Recently, IFNβ induced apoptosis has been also shown *in vitro* (Yen and Ganea 2009; Dedoni, Olianas et al. 2010).

The transcription of interferons is regulated by interferon regulatory factors (IRFs). IRFs have a key role in antiviral defense, immune response and apoptosis. IRF3 and IRF7 are critical for the transcriptional regulation of IFNβ (Paun and Pitha 2007). Under physiological conditions, IRF3 and IRF7 are expressed constitutively at low levels in the cytosol. Upon a viral infection, in the early phase, IRF3 and IRF7 monomers get phosphorylated and form homo- and heterodimers, which translocate to the nucleus. In the nucleus, IRF dimers interact with the co-activator cyclic-AMP-responsive-element-binding protein (CBP) or p300. This holocomplex binds to the target DNA sequence (e.g. type I IFN genes), resulting in the initiation of the transcription of type I IFN genes (Honda and Taniguchi 2006). In the later phase of the viral infection, type I IFNs bind to the type I IFN receptor (also called interferon α/β receptor) which activates the IFN-stimulated gene factor 3 (ISGF3) which is responsible for the stimulation of the IFN-stimulated response element (ISRE) of a gene promoter, e.g. the IRF7 gene. Similarly to the early phase, the newly synthetized IRF7 forms homo- or heterodimers with IRF3, get phosphorylated, translocate to the nucleus which results in even more expression of type I IFNs. IRF3 only initiates the expression of IFNβ, whereas IRF7 activates IFNα and IFNβ genes (Honda and Taniguchi 2006; Paun and Pitha 2007).

In chronic inflammatory disorders, like MS, the abnormal peripheral autoimmune processes generate autoimmune inflammatory cascades in the brain and this chronic inflammation and activation of the immune system can lead to the development of major depression (Dobos, de Vries et al. 2011). The lifetime prevalence of major depression in people living with MS is approximately 50% (Feinstein 2006). In 1987, Minden et al. showed in their study, that 54%

of MS patients reported at least one major depressive episode after the onset of their illness (Minden, Orav et al. 1987).
It is known, that MS patients show an increased pro-inflammatory cytokine profile (Link 1998; Trenova, Manova et al. 2011). These pro-inflammatory cytokines, e.g. TNF alpha (TNFα), interferon gamma (IFNγ), or interleukin 6 (IL-6) are known to be an effective stimulator of an ubiquitous intracellular enzyme, indoleamine 2,3-dioxygenase (IDO), which catalyzes tryptophan catabolism through the kynurenine pathway (Leonard 2007; Dantzer, O'Connor et al. 2008). IFNβ has been described as a potential stimulator of IDO (Carlin, Borden et al. 1987; Carlin, Borden et al. 1989). Neurotoxins, which are responsible for neuronal death, like quinolinic acid (QUIN), an NMDA receptor agonists, or 3-hydroxykynurenine (3-HK) are generated in the kynurenine-pathway, whereas the decreased tryptophan level in brain cells reduces the production of brain serotonin (5-HT) possibly leading to altered serotonergic neurotransmission (Muller and Schwarz 2007; Dobos, de Vries et al. 2011). Taken together, neuroinflammation-induced increased IDO expression and activity is accompanied by the onset of depressive-like behavior (Dobos, de Vries et al. 2011).
Moreover, it has been shown that stimulation of ISRE can lead to IDO expression as well (Suh, Zhao et al. 2007).
Also, cognitive impairment has been associated with MS. Rao et. al showed (Rao, Leo et al. 1991), that approximately 40% of MS patients suffer from cognitive decline (Rao, Leo et al. 1991; Rao 1995; Feinstein 2006).
Although there is clear evidence that IFNβ-treatment in patients increases the risk of the development of major depression and cognitive impairment, the site of action of systemic IFNβ remains undefined with regard to its central nervous system consequences. To investigate whether IFNβ-treatment can lead to depressive-like behavior and cognitve impairment and whether this effect is via the IFNα/β receptor we used wildtype and mutant mice with a deletion of IFNα/β receptor (IFNRKO). We tested the behavioral changes of wildtype and IFNRKO animals after 1 week of intraperitoneal (IP) injection of IFNβ. Afterwards, IDO expression and activity was measured. We report here for the first time, that 7 consecutive days of IP injected IFNβ is able to increase significantly the activity and expression of IDO and leads to the development of depressive-like behavior and deficits in learning and memory.

2. MATERIALS AND METHODS

2.1. Animals

Approximately 3 months old male C57Bl/6J (wildtype, wt) mice (n=10/group) were obtained from Harlan (Horst, The Netherlands). 24 weeks old male conditional mutant mice with a deletion of interferon α/β receptor (IFNRKO) (n=10/group) were kindly provided by Dr. T. Blank and Prof. Dr. M. Prinz (Freiburg, Germany). The following groups were used: wildtype mice treated with PBS (wt/PBS), wildtype mice treated with IFNβ (wt/IFNβ), IFNRKO mice treated with PBS (IFNRKO/PBS) and IFNRKO mice treated with IFNβ (IFNRKO/IFNβ). Upon arrival the animals were individually housed under normal laboratory conditions (air-conditioned (21±2°C), humidity-controlled room, 12/12 h light/dark cycle (light on from 08:00-20:00)). Food and water were available *ad libitum*. The

treatments were started after 2 weeks of acclimatization. All animal care and treatments were carried out in accordance with the regulations of the Ethical Committee for the use of experimental animals of the University of Groningen (licence number DEC4408B).

2.2. Generation of interferon α/β receptor knock out mice

Inactivation of the type I IFN receptor gene in embryonic stem (ES) cells was achieved by homologous recombination (Muller, Steinhoff et al. 1994). IFNRKO mice were obtained with a mendelian frequency, proved fertile, and had no apparent phenotypic anomalies by 6 months of age. They were backcrossed 20 times to C57Bl/6 and provided by the Prinz lab, Freiburg.

2.3. Timeline of the experiment

Upon arrival, the animals were housed individually. After 2 weeks acclimatization, animals were injected for 7 consecutive days with IFNβ. Injection was performed every day ~9 a.m. On the 6th day of injection, the training session of the fear conditioning paradigm was performed. On the 7th day, retention of the fear conditioning test and the forced swim test was done. After completion of the behavior tests, animals were sacrificed and brain and blood samples were collected for further analysis (Fig.1).

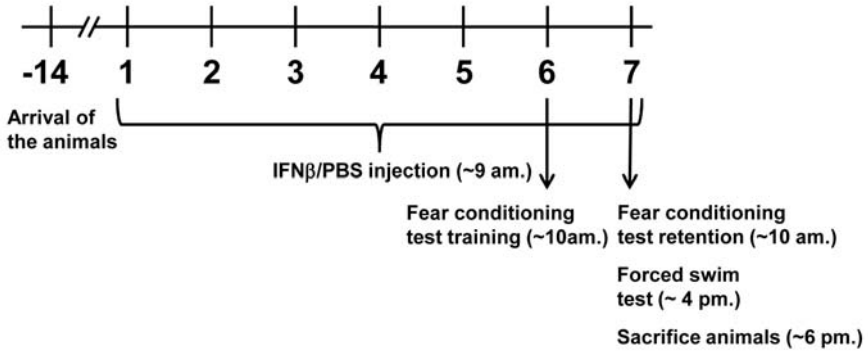

Figure 1. Diagram of the time course of the experiment.

2.4. Intraperitoneal injection

Animals were injected intraperitoneally either with 0.2 ml IFNβ solution (100.000 IU/ml, Hycult Biotechnology) or endotoxin-free phosphate buffered saline (PBS) for 7 consecutive days.

2.5. Fear conditioning

The fear conditioning test is an associative learning test in which mice learn to fear a neutral environment (testing box) because the environment was associated with a mild electrical foot shock during a training session. The contextual fear conditioning experiments were performed as described previously (Ostroveanu, van der Zee et al. 2010) using a computer-

controlled fear conditioning system (Ethovision, Noldus, Wageningen, The Netherlands). The test was done in a Plexiglas cage (44 × 24 × 44 cm) with a constantly illuminated fear conditioning box (12 V, 10 W halogen lamp, 100–500 lux). The training (conditioning) consisted of a single trial. The mouse was exposed to the conditioning context (180 sec), followed by a foot shock (0.7 mA, 2 sec, constant current) delivered through a stainless steel grid floor. The mouse was removed from the fear conditioning box 30 sec after shock termination to avoid an aversive association with the handling procedure. Between testing sessions, the box was cleaned with 70% ethanol. Memory tests were performed 24 h after fear conditioning. Contextual memory was tested in the fear conditioning box for 180 sec without footshock presentation. Freezing, defined as the lack of movement except for respiration and heart beat, was assessed as the behavioral parameter of the defensive reaction of mice by a time-sampling procedure every 10 sec throughout memory tests.

2.6. Forced swim test

The forced swim test is a widely used paradigm to measure antidepressant activity and depressive-like behavior in animal models (Cryan and Mombereau 2004). A cylinder (22 cm height × 11.5 cm diameter) was filled with water (23-25 °C) up to a height of 17 cm. The animal was placed inside the cylinder and immobility time was recorded over a 7 min test period. Afterwards the mouse was dried and returned to its cage.

2.7. Protein analysis

Immediately after the last behavioral experiment, mice were sacrificed by CO_2 inhalation. Brains were quickly dissected, snap-frozen in liquid nitrogen and stored at -80 °C until homogenization and Western blotting (Ostroveanu, Van der Zee et al. 2007; Dobos, de Vries et al. 2011), Antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used for IDO (rat monoclonal antibody), and HPRT (hypoxanthine-guanine phosphoribosyltransferase) as an internal standard to correct for variations in protein content (rabbit polyclonal antibody), and stained with horseradish peroxidase-conjugated secondary antibodies (goat-anti-mouse, donkey-anti-rabbit, respectively) The proteins were detected using Enhanced Chemiluminescence (Pierce ® ECL Western Blotting Substrate, Rockford, IL, USA) and the Molecular Image Chemidoc XRS+ System (Biorad). The results were analyzed using Image Lab software (BioRad).

2.8. Serum tryptophan and kynurenine quantification

We used as an *in vivo* index of IDO activity the ratio of the blood levels of kynurenine/tryptophan. Tryptophan and kynurenine concentrations in serum were determined by an automated on-line solid-phase extraction-liquid chromatographic-tandem mass spectrometric (XLC-MS/MS) method with deuterated internal standards as described previously (de Jong, Smit et al. 2009).

2.9. Statistical analysis

All data were analyzed by one-way analysis of variance (one-way ANOVA), followed by the LSD post-hoc test to determine the differences between the selected groups using the program SPSS 16.0 for Windows. A p-value *<0.05 was considered to be statistically significant (p-value **<0.005: highly significant, a p-value ***<0.0005: extremely significant). Data are presented as a mean value ± the standard error of the mean (SEM).

3. RESULTS

3. 1. IP injection of IFNβ impairs memory but does not influence the activity of mice during training

The contextual fear conditioning test was used to investigate the effect of IP IFNβ injections on hippocampus-dependent learning and memory. 1 week of IP injected IFNβ led to a significant increase in freezing behavior in the wt/IFNβ group when compared either to the wt/PBS or both IFNRKO groups ($F(3,30)=10.867$, $p<0.05$, Fig.2A). IFNβ injections did not influence the basal activity of mice during the training session ($F(3.30)=0.794$, $p>0.05$, Fig.2B).

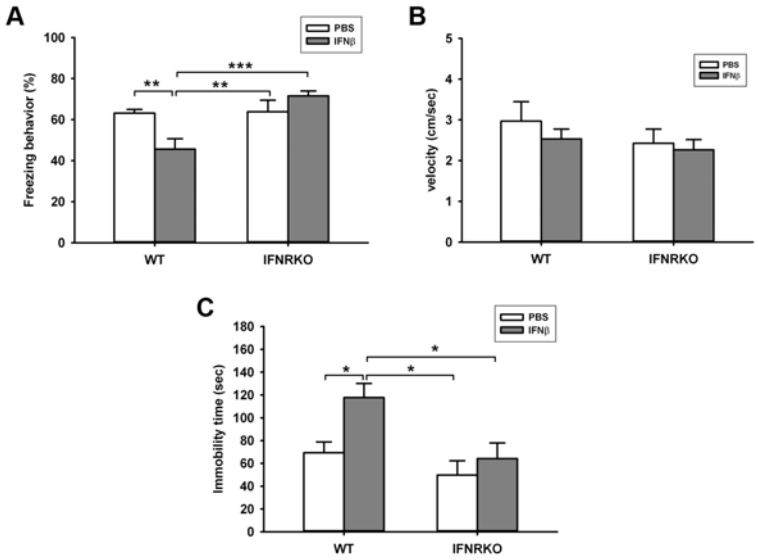

Figure 2. Interferon β induced behavioral changes. A) Fear conditioning paradigm, a widely used test for measuring learning and memory impairment in animal models. Data represent mean values±SEM, *p < 0.05. **B) Basal activity** of the mice during the training session of the fear conditioning test. **C) Forced swim test**, a test for measuring antidepressant activity and depressive-like behavior in rodents. Data represent mean values±SEM, *p < 0.05.

3.2. Intraperitoneal injection of IFNβ induces depressive-like behavior in mice

After one week IP injection of either PBS or IFNβ, depressive-like behavior of the mice was tested in the forced swim test. Significantly increased immobility was observed in the wt/IFNβ group when compared either to the wt/PBS or both IFNRKO groups. ($F(3,31)=6.085$, $p<0.05$, Fig.2C).

3.3. Intraperitoneal IFNβ treatment increases IDO levels in the brainstem and in the cortex

A significant increase of IDO was detected in the brainstem of the wt/IFNβ group when compared either to the wt/PBS and both IFNRKO groups ($F(3,30)=6.335$, $p<0.05$) (Fig.3A). Also, IDO was significantly increased in the cortex of wt/IFNβ group when compared either to the wt/PBS or the IFNRKO/IFNβ groups ($F(3,32)=2.364$, $p<0.05$). Surprisingly, a significant decrease was found in the hypothalamus of both IFNβ treated groups when compared to the wt/PBS group ($F(3,38)=1.934$, $p<0.05$). In the other brain regions, no significant differences among the four groups were observed (cerebellum ($F(3,30)=0.222$, $p>0.05$), hippocampus ($F(3,40)=0.543$, $p>0.05$), olfactory bulb ($F(3,37)=0.747$, $p>0.05$), and striatum ($F(3,39)=1.044$, $p>0.05$).

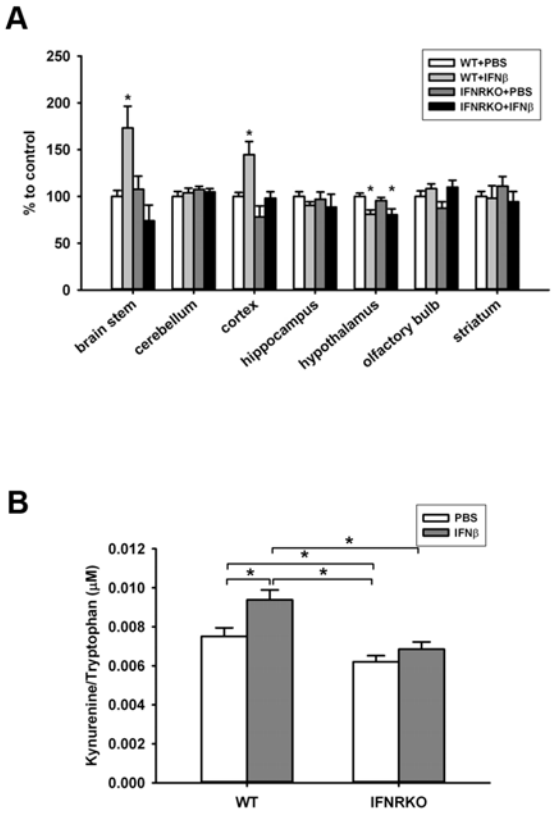

Figure 3. A) IDO protein expression in different brain regions, measured by western blotting. Data represent mean values±SEM as a percentage of the wt/PBS group. *p < 0.05. **B) IDO activity**, defined as the ratio of the concentration of kynurenine and tryptophan measured in the serum. Data represent mean values±SEM, *p < 0.05

3.4. Intraperitoneal IFNβ treatment results in increased IDO activity in the serum

IP IFNβ injection in the wt/IFNβ animals led to increased IDO activity compared to the wt/PBS group and both IFNRKO groups, defined as a ratio of kynurenine and tryptophan measured in the serum ($F(3,31)=10.247$, $p<0.005$) (Fig.3B).

4. DISCUSSION

In the present study, we used wildtype and interferon α/β receptor KO (IFNRKO) mice to investigate whether IFNβ is responsible for the development of depressive-like behavior possibly inducing indoleamine 2,3-dioxygenase (IDO) and cognitive impairment through the interferon α/β receptor.

Interferons are key components of the innate immune system with antiviral, antitumoral and immunomodulatory function. The most pausible therapeutic effect of IFNβ is based on its immunomodulatory property. IFNβ is able to reduce antigen presentation, inhibit T-cell proliferation and modulate cytokine production (Markowitz 2007; Rudick and Goelz 2011). MS is an autoimmune inflammatory disorder. Altered peripherial immune function, damaged blood brain barrier, infiltration of autoreactive T cells into the CNS, activated microglia and astrocytes, release of inflammatory mediators and toxic molecules are main features in MS. Oligodendrocytes and neurons are seriously affected by inflammatory damage, leading to demyelination and axon loss (Hemmer, Nessler et al. 2006; Markowitz 2007). IFNβ has been shown to reduce T-cell activation by downregulation of major histocompatibility complex (MHC) class II expression on antigen-presenting cells. IFNβ reduces TH1 pro-inflammatory cytokines leading to a shift in the immune response towards the TH2 profile, releasing cytokines, such as interleukin 4 and 6 (IL-4 and IL-6), which inhibit the expression of TH1 cytokines, altering the inflammatory response. Furthermore, T cells express VLA-4 molecules on the cell surface, which can bind to the VCAM-1 molecules on the surface of brain vascular endothelium. This interaction ensures, that activated T cells can cross the blood brain barrier. IFNβ prevents T cells getting into the CNS by downregulation of VLA-4 expression, and shedding soluble VCAM-1 molecules on the surface of brain vascular endothelium cells, therefore the binding of VLA-4 to VCAM-1 is arrested (Markowitz 2007). The mechanism of action of interferon β has been reported previously: it binds to the extracellular part of the interferon α/β receptor, which consists of a signaling chain (IFNAR1) and a binding chain (IFNAR2), the newly formed complex allows the intracellular part of the receptor to interact with Janus kinase 1 (JAK1) and Tyrosine kinase 2 (Tyk2). This interaction leads to a phosphorylation cascade and finally to the activation of STATs (Signal Transducer and Activator of Transcription), which translocates into the nucleus to bind ISRE elements and induce gene transcriptions (Rudick and Goelz 2011). The therapeutic effect of IFNβ can be direct, if IFNβ binds to its receptor and induces gene transcriptions, or indirect when gene transcription alters other cells involved in pathogenic mechanisms in MS (Rudick and Goelz 2011).

IFNβ is a safe and effectively used drug as a treatment for multiple sclerosis. However, the neuropsychiatric side effects of IFNβ-therapy must be considered. For instance, Fragoso et. al. (Fragoso, Frota et al. 2010) showed that patients undergoing IFNβ-treatment, without previously reported psychiatric diseases, developed severe depression in a relatively short period. IFNβ withdrawal resulted in the complete reduction of the symptoms.
Cognitive impairment has also been associated with MS. Rao et al. showed (Rao, Leo et al. 1991), that approximately 40% of MS patients suffer from cognitive decline (Rao, Leo et al. 1991; Rao 1995; Feinstein 2006). Results are contradictory in concern of the effect of IFNβ-treatment on cognition. IFNβ-therapy has been shown to have a positive effect on cognitive decline in a dose-dependent manner (Bastianello, Giugni et al. 2011), however in an other study, there was no correlation between IFNβ-treatment and cognitive improvement (Carmona, Masuet et al. 2011). In a cell culture study, Yen and Ganea showed, that IFNβ induces apoptosis in mature dendritic cells via activation of caspase-11/caspase-3, which requires activation of STAT1 (Yen and Ganea 2009). Interestingly, the PI3K/Akt pathway has been reported to be involved in the Tumor Necrosis Factor Receptor 2 (TNFR2)-mediated neuroprotection as well (Marchetti, Klein et al. 2004) while the JAK/STAT signaling pathway is activated by Necrosis Factor Receptor 1 (TNFR1), which is mainly responsible for cell death (Guo, Dunbar et al. 1998). In our recent study, we found an IFNβ-induced learning and memory impairment in the wt/IFNβ group in the fear conditioning test, while we did not see any effect of IFNβ in the IFNRKO group. Taken together, these results indicate that IFNβ might be neurotoxic and responsible for memory impairment via IFNα/β receptors.
IFNβ is known to be an inducer of the tryptophan degrading enzyme, indoleamine 2,3-dioxygenase (IDO) (Carlin, Borden et al. 1987; Carlin, Borden et al. 1989; Suh, Zhao et al. 2007). IDO-mediated tryptophan depletion in brain cells results in reduced serotonin levels, therefore IDO has been proposed in several studies to play a crucial role in depression (Leonard 2007; Muller and Schwarz 2007; Dantzer, O'Connor et al. 2008; Dobos, de Vries et al. 2011). Furthermore, in a previous paper we showed that the inhibition of IDO by 1-methyl-tryptophan (1-MT) can prevent the development of depressive-like behavior (Dobos, de Vries et al. 2011). We tested here, whether IFNβ is able to induce depression via the IFNα/β receptors. Our forced swim test results clearly show that the wt/IFNβ group, but not the IFNRKO/IFNβ group developed depressive-like behavior. We also analysed brain and blood of the animals, and we found an increase of IDO expression in the brain stem and in the cortex and also increased IDO activity in the serum of the wt/IFNβ group.
To our best knowledge, we show here the first time that IFNβ induces learning and memory problems and depressive-like behavior via the IFNα/β receptors. Although we did not use IDO inhibitor, previous and recent results suggest, that IFNβ-induced IDO expression and higher activity might be responsible for the depressive-like behavior after IFNβ-treatment.

ACKNOWLEDGEMENTS

The authors are grateful for Dr. M.F. Masman, K. van der Bij, E. Postma and P. Slagter, for the skillful practical assistence.

CHAPTER 6

General discussion

ABSTRACT

Major depression (MD) is the second largest cause of disability in the developed world (Belmaker and Agam 2008). Apart from the individual suffering, the lost productivity of MD patients and the rising healthcare costs create enormous financial consequences. Also, MD, especially its chronic recurrent form, is associated with a severe risk suicide (Belmaker and Agam 2008). Furthermore, MD is also a major risk factor and/or source of comorbidity with several other disorders, such as cardiovascular diseases (Carney, Freedland et al. 2002), neurological disorders, e.g. Alzheimer's disease (AD), Parkinson's disease (PD) or multiple sclerosis (MS) (Minden, Orav et al. 1987; Jorm 2001; Kanner 2005; Feinstein 2006). Therefore, the importance of research on the neurobiology of MD and the mechanisms of antidepressant therapy cannot be overstated.

In light of the fact that neuroinflammation is a quite common finding in various brain disorders, particularly neurodegenerative diseases, it might not be surprising that neuroinflammation is also implicated in MD. Therefore, the objective of the present thesis was to gain more insight in the potential role of neuroinflammation in major depression with special emphasis on the activation of the kynurenine pathway.

NEUROINFLAMMATION IN NEURODEGENERATION AND MD

Chapters 1 and 2 provide extensive information on neuroinflammation, its neurodegenerative and behavioral consequences, and some of the underlying molecular mechanisms, with particular focus on the tumor necrosis factor α (TNFα) pathway.
What makes neuroinflammation a potential pathogenic factor in MD, however, is a series of findings indicating that anti-inflammatory treatment may have antidepressant action (Wager-Smith and Markou 2011). It has also been shown that continuous activation of the immune system in inflammatory diseases can lead to the development of major depression (Dantzer, O'Connor et al. 2008). Other studies have revealed a positive correlation between depressive symptoms and increased levels of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and TNFα in the serum and brain of depressed patients (Maes, Bosmans et al. 1991; Mikova, Yakimova et al. 2001; Dean, Tawadros et al. 2010; Raison, Dantzer et al. 2010). It is also well-known, that effective antidepressant therapy is characterized by a strong immunomodulatory effect, leading to decreased levels of circulating pro-inflammatory cytokines (Szelenyi and Selmeczy 2002).
Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, is a robust inflammatory agent and frequently applied in experimental inflammatory models. It has been revealed that both peripheral and central administration of LPS induces chronic neuroinflammation and generates depressive behavior in rodents (Lestage, Verrier et al. 2002; Qin, Wu et al. 2007; Andre, O'Connor et al. 2008; Dobos, de Vries et al. 2011).

Chapter 1 generally focuses on the activation of the kynurenine pathway, the neurodegenerative and behavioral consequences of neuroinflammatory conditions, which has been experimentally approached in Chapters 4 and 5.
Also, in Chapter 1, the particular functions of TNFα, having both neuroprotective as well as pro-inflammatory and pro-apoptotic features and being a potent activator of the kynurenine pathway has been described. The complex role of TNFα and its two receptors, tumor necrosis factor 1 (TNFR1) and tumor necrosis factor 2 (TNFR2), together with the signaling pathways in neuroprotection and neurodegeneration has been further dealth with in Chapter 2. This chapter also emphasizes the importance of *in vivo* structural and functional neuroimaging techniques. Visualizing neuroinflammation is of interest for diagnosing pathological changes in the brain. Neuroimaging techniques are not only reliable tools for monitoring disease processes but also offer the option to selectively target certain molecules, brain areas or brain disorders. In that respect, the use of radiopharmaceuticals by positron emission tomography (PET) and single photon emission computed tomography (SPECT) has been discussed. Microglia, the resident immune cells of the brain are the specific target of neuroinflammatory imaging (Banati, Goerres et al. 1999). The radiolabeled PK11195 (1-[2-chlorophenyl]- N -[1-methyl-propyl]-3-isoquinoline carboxamide), a specific and selective high-affinity ligand of the peripheral benzodiazepine receptors (PBRs), which are expressed on glial cells, is a good marker for neuroinflammation as also detailed in chapter 1, 2 and 4. The PK11195 compound has been successfully used in various neuroinflammatory diseases, for example to detect active lesions in Multiple Sclerosis patients (Vowinckel, Reutens et al. 1997), to distinguish Alzheimer's disease patients from control subjects (Kuhlmann and Guilarte 2000), or to prove the involvement of the inflammatory process in the pathologic

state of migraine (Cui, Takashima et al. 2009). However, we can argue with good reasons, that the detection and analysis of neuroinflammation in an early state of brain disorders would serve novel and more specifically targeted therapeutical approaches.
The obvious role of TNFα in neuroinflammation has been extensively characterized in these chapters, therefore approaching the TNF receptors and modulating the TNFα signaling pathway by pharmaceutical interventions opened new avenues in the development of novel drugs for treatment of certain neurodegenerative and chronic inflammatory diseases as well as major depression (Russo and Polosa 2005; Smolen, Aletaha et al. 2007; Raison, Rutherford et al. 2013).
It should be noted, that aging itself has neurodegenerative and neuroinflammatory features without any remarkable appearance, and affects the signaling pathways, including the TNFα pathway. Therefore it is important to investigate the non-pathological physiological effects of TNFα and its main two receptors - TNFR1 and TNFR2 - during aging. In chapter 3 we applied a broad range of behavioral tests to assess behavioral, cognitive and physical changes induced by aging on TNFR1 and TNFR2 functioning in the central nervous system. We also found that TNFR2 plays an essential role in normal physiological functioning and hippocampus-dependent learning and memory, which was found not to be affected by aging.
Therapeutical approaches of the TNFα pathway carry many pros and cons. Neuroprotective targeting TNFα itself, or selectively TNFR1 by antagonists or TNFR2 by agonists seems to be reasonable, however, the complexity of the signaling pathway present several difficulties. For instance, detrimental neurological side-effects, like demyelination have been related to anti-TNFα therapy (Andreadou, Kemanetzoglou et al. 2013; Kaltsonoudis, Voulgari et al. 2013). Moreover, the lack of activated TNFR1 eventually results in decreased DNA synthesis, delayed restoration of liver mass, and increased mortality (Yamada, Kirillova et al. 1997; Bradham, Plumpe et al. 1998). Furthermore, overexpression or long-term activation of TNFR2 has been associated with cellular apoptosis and autoimmune diseases (Bigda, Beletsky et al. 1994; Medvedev, Sundan et al. 1994; Depuydt, van Loo et al. 2005).
In conclusion, the biological environment is largely influencing the TNFα signaling. Due to this fact, our study should be extended to investigate the role of TNFα, TNFR1 and TNFR2 in an artificial neuroinflammatory condition. For instance, it would be of interest to use mouse models of neurodegeneration or neuroinflammation.

THE MONOAMINE HYPOTHESIS OF MD

For decades, neurotransmitter dysregulation has been thought to be the major component of neuropsychiatric disorders like depression. According to the well-known monoamine hypothesis of depression, MD is a result of the underactivity of monoamines, especially serotonin, in the brain. Alteration in serotonergic neurotransmission, decrease in serotonin levels or availability of serotonin and elevated serotonin transport have been linked to depression (Risch and Nemeroff 1992). Moreover, the first line therapies in depression, like the monoamine oxidase inhibitors or the selective serotonin reuptake inhibitors are also focusing on the augmentation of neurotransmission or the availability of serotonin (DeVane 2000). Although the monoamine hypothesis has been the major focus of depression and antidepressant research, mounting evidence suggest that this hypothesis is insufficient to provide an absolute explanation for the neurobiology of major depression.

THE KYNURENINE PATHWAY AND THE NEUROINFLAMMATORY HYPOTHESIS OF MD

In 1992, Smith reported the macrophage theory of depression which is based on the findings that inflammation is a major component in the development of depression (Smith 1991; Dantzer, O'Connor et al. 2008).
Indoleamine 2,3-dioxygenase (IDO) has been suggested as a potential link between neuroinflammation and major depression. IDO catalyzes the first and rate limiting step in the degradation of the essential amino acid, tryptophan, through the kynurenine pathway (Leonard 2007; Dantzer, O'Connor et al. 2008). Upregulation of IDO by pro-inflammatory mediators causes tryptophan depletion in brain cells, resulting in reduced production of serotonin, which may lead to MD (Leonard 2007; Dantzer, O'Connor et al. 2008).

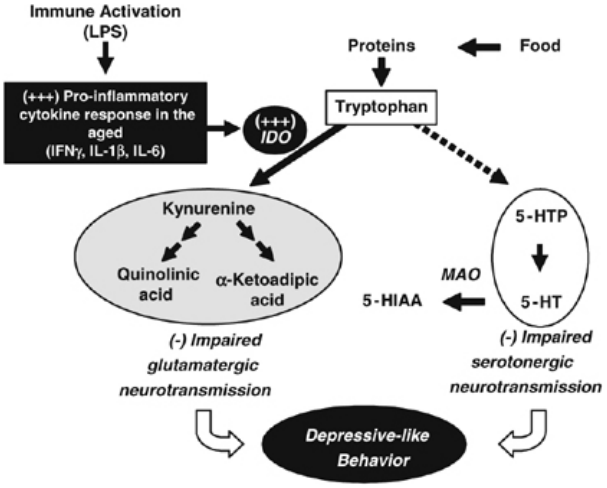

Figure 1. Aging exacerbates depressive-like behavior in mice in response to activation of the peripheral innate immune system. (Godbout, Moreau et al. 2008)

However, studies on the above mentioned hypothesis of inflammation-induced IDO-mediated tryptophan depletion resulted in contradictory findings.
LPS has been reported to generate cytokine release and play a role in the development of depression via activating the kynurenine pathway and upregulating IDO (O'Connor, Andre et al. 2009). For instance, it has been shown that LPS-induced inflammation leads to IDO activation and depressive-like behavior but inconsistently results in elevated tryptophan and serotonin levels (Godbout, Moreau et al. 2008; Walker, Budac et al. 2013). This phenomena was interpreted as a stress response and is not specific to the LPS-induced depressive-like behavior (Dunn and Welch 1991; Dunn, Wang et al. 1999; Godbout, Moreau et al. 2008; Walker, Budac et al. 2013).
Thus, the inconclusive monoamine hypothesis of depression should be carefully re-thought and rather focus on the overproduction and neurotoxicity of tryptophan catabolites (TRYCATs) in the development of depression.

It has been shown that the TRYCATs can initiate behavioral effects on their own, independently from the serotonin levels. For instance, kynurenine administration was found to induce depressive-like behavior in mice (O'Connor, Lawson et al. 2009) and anxiety (Salazar, Gonzalez-Rivera et al. 2012). In addition, kynurenine is converted into quinolinic acid, a potent N-methyl-D-aspartate (NMDA) agonist and stimulator of glutamate release, that exerts its neurotoxicity by inducing excitotoxicity, calcium overload and associated oxidative stress (Rios and Santamaria 1991; Schwarcz and Pellicciari 2002; Muller and Schwarz 2007; McNally, Bhagwagar et al. 2008; Catena-Dell'Osso, Bellantuono et al. 2011). In a vicious circle, glutamate receptor activity can further participate in the development of an inflammatory environment within the brain and enhance the cytokine production.
In Chapter 4 we injected LPS intracerebroventricularly and induced neuroinflammation in C57Bl/6 mice. We showed in this experimental model, that LPS-induced neuroinflammation peaks 3 days after administration, leads to IDO activation and manifests in depressive-like behavior. We could also demonstrate that the depressive-like behavior is dependent on the upregulation of the kynurenine pathway, since inhibition of IDO by 1-methyl-tryptophan (1-MT) could prevent the depressive phenomena. Even though our IDO inhibitory experiments in this chapter clearly prove a certain relationship between neuroinflammation, IDO activation and depressive-like behavior, the final conclusion should be drawn with caution, because the mechanism, how the kynurenine pathway is involved in the development of depressive-like behavior, needs further investigations. Therefore, it is important to be aware of the limitations of our study.

To date, the consequences of a long-term neuroinflammation in line with chronic IDO inhibition is not known to the best of our knowledge. Since IDO plays also a major role in general immune processes, the development of brain-specific IDO inhibitors would be an option to treat neuropsychiatric disorders. However, before focusing on the problems of administration, dosage and side effects of IDO inhibitors in antidepressant therapies, it is essential to know the distribution and regulation of IDO in the brain (Leonard 2010).
In our study we blocked IDO by 1-methyl-tryptophan, however, targeting other enzymes of the kynurenine pathway - like the mostly microglia dependent kynurenine converting kynurenine-3-hydroxylase (KMO), or others, like kynureninase or 3-hydroxyanthraniate-3,4-dioxygenase - and applying specific inhibitors would serve also an interesting therapeutical approach. Indeed, it has been reported that the inhibition of KMO results in amelioration of several brain disorders (Cozzi, Carpenedo et al. 1999; Moroni, Carpenedo et al. 2003; Richter and Hamann 2003; Samadi, Gregoire et al. 2005).
For instance, it has been shown in experimental autoimmune encephalomyelitis (EAE), which is an experimental model of MS, that the expression and activity of KMO are significantly increased in the spinal cord of rats resulting in neurotoxic levels of 3-hydroxykynurenine and quinolinic acid (Chiarugi, Cozzi et al. 2001). Selective inhibition of KMO leads to the reduction of 3-hydroxykynurenine and quinolinic acid as well as in the accumulation of the neuroprotective kynurenic acid.

It is known, that MS patients show elevated pro-inflammatory cytokine levels and often suffer from cognitive decline and depressive symptoms (Link 1998; Trenova, Manova et al. 2011). Besides the increased pro-inflammatory cytokines, e.g. TNFα, IFNγ and IL-6, the well established IFNβ-therapy may also contribute to the development of depression by

activating the kynurenine pathway (Carlin, Borden et al. 1987; Carlin, Borden et al. 1989; Leonard 2007; Dantzer, O'Connor et al. 2008). Therefore, in Chapter 5 we have investigated the involvement of the kynurenine pathway in depressive-like behavior following IFNβ administration. Wildtype and mutant mice with a deletion of IFNα/β receptor (IFNRKO) were injected with IFNβ for 7 consecutive days to see whether IFNβ is accountable for the depressive symptoms and cognitive impairment and whether its effect is via the IFNα/β receptors. Our results clearly indicate that IFNβ-treatment leads to the activation of the kynurenine pathway, significantly increase the activity and expression of IDO which might be responsible for the development of depressive-like behavior and deficits in learning and memory through the IFNα/β receptors. The main limitation of our study is, that we did not use IDO inhibitors for unequivocal evidence of the role of IDO underlying the neuropsychiatric impairments. Therefore extension of the study using IDO inhibitors would be fundamental. Furthermore, proceeding with the previously presented concept of the association between KMO, neurodegeneration and neuroinflammation, the inhibition of other enzymes of the kynurenine pathway, particularly KMO, would be of interest.

IMPLICATIONS FOR THE INVOLVEMENT OF GLUTAMATERGIC NEUROTRANSMISSION AND THE KYNURENINE PATHWAY IN NEUROPSYCHIATRIC DISORDERS

Glutamatergic release results in the activation of glutamate receptors at the post-synaptic neurons. There are two types of glutamatergic receptors, the ionotropic glutamate receptors, (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA; *N*-methyl-D-aspartate, NMDA; and kainic acid, KA receptors) are opening cation ion channels into the neurons leading to depolarisation/excitation while the other type of glutamatergic receptors are metabotropic receptors, which are bound to guanosine-5'-triphosphate (GTP) binding proteins (Lees 2000).

It has been shown, that AMPA receptors potentiate synaptic degeneration and dendritic spine loss (Centonze, Muzio et al. 2009; Rossi, Lo Giudice et al. 2012). In MS, AMPA receptor mediated cell death of oligodendrocytes leads to demyelination, which is the main feature of the disease (Ruiz, Matute et al. 2010). The NMDA receptor has been also implicated in synaptic impairment and in the progression of EAE (Grasselli, Rossi et al. 2013). Grasselli et al. showed that the NMDA receptor hyperactivity results in enhanced glutamate release in the synaptic cleft and contributes to mitochondrial dysfunction and severe disease course (Grasselli, Rossi et al. 2013).

It should be noted, that the neurotoxic metabolites produced along the kynurenine pathway, such as quinolinic acid or 3-hydroxykynurenine, a pro-apoptotic agent, can robustly contribute to the development of MD. Recently, is has been shown in mice that LPS-induced inflammation which activates the kynurenine pathway leading to depressive-like behavior, is mediated by NMDA receptor activation, which can be blocked by administration of the NMDA receptor antagonist ketamine in mice (Walker, Budac et al. 2013).

THE EFFECT OF NEUROTOXICITY ON SYNAPTIC PLASTICITY: THE SYNAPTOGENIC HYPOTHESIS OF MD

Cognitive deficits in neurodegenerative diseases and major depression have been associated with neuroinflammation (Leonard 2007). Learning and memory problems are early manifestations of neurodegeneration in many diseases, such as Alzheimer's disease, Parkinson's disease or Multiple sclerosis and are linked to hippocampal dysfunction due to synaptic loss (Schindowski, Bretteville et al. 2006; Scheff, Price et al. 2007; Sicotte, Kern et al. 2008; Nistico, Pignatelli et al. 2012; Calabresi, Castrioto et al. 2013; Marxreiter, Ettle et al. 2013; Nistico, Mango et al. 2013).

Neuroinflammation-induced microglia activation and the subsequently released cytokines, e.g. TNFα or IL-1β have been shown to alter synaptic transmission (Mandolesi, Grasselli et al. 2010; Mandolesi, Grasselli et al. 2012). Furthermore, it has been revealed that inflammation enhances the glutamate neurotransmission in EAE (Mandolesi, Grasselli et al. 2010).

Besides the fact that depressed patients have decreased volumes of cortical and limbic brain areas, suggesting neuronal degeneration (Duman and Aghajanian 2012), a decrease in the number of synapses have been determined by electron microscopy (Kang, Voleti et al. 2012). Interestingly, NMDA neurotoxicity [31-33] and pro-apoptotic mechanisms [34] are both prime causes of synaptic loss.

The synaptogenic hypothesis, a novel theory of MD, is based on the findings that the severity of depressive symptoms is negatively correlated with the number of hippocampal and prefrontal cortical spine synapses (Hajszan, Szigeti-Buck et al. 2010). However, the mechanisms underlying the loss of synapses in MD are much less understood, necessitating further investigation of this issue.

Previously, it has been demonstrated that chronic antidepressant treatment slowly enhances synaptic plasticity by increasing the expression of neurotrophic factors, like brain derived neurotrophic factor (BDNF) (Bath, Jing et al. 2012). Furthermore, the rapid antidepressant action of ketamine, an NMDA receptor antagonist has been linked to synaptogenesis (Li, Lee et al. 2010). This study confirmed that ketamine rapidly activated the mammalian target of rapamycin pathway (mTOR), resulting in increased synaptic signaling proteins and increased number and function of new spine synapses (Li, Lee et al. 2010).

Additionally, suggesting the crucial role of the kynurenine pathway in synaptic plasticity, Forrest et al. reported recently, that inhibition of the kynurenine pathway *in utero* results in synaptic changes in the offsprings via altering the expression of the NMDA receptors (Forrest, Khalil et al. 2013).

These data further lend support to the hypothesis that the kynurenine pathway has a significant role in the development and the homeostasis of the central nervous system.

OVERALL CONCLUSION

In summary, current state of knowledge in the field of depression research suggests that inflammation contributes to the development of MD by interfering with synaptogenesis in different brain areas. It appears that IDO is a critical component of this process by converting inflammatory signals into production of synaptotoxic metabolites.
Advanced future investigations aimed at unraveling the underlying molecular mechanisms will be required for a better and solid understanding the complex kynurenine pathway as a target for novel antidepressant therapies.

REFERENCES

(1996). "Assessment of brain SPECT. Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology." Neurology 46(1): 278-285.

(1999). "TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group." Neurology 53(3): 457-465.

Abraham, I., T. Harkany, et al. (2000). "Chronic corticosterone administration dose-dependently modulates Abeta(1-42)- and NMDA-induced neurodegeneration in rat magnocellular nucleus basalis." J Neuroendocrinol 12(6): 486-494.

Aid, S., R. Langenbach, et al. (2008). "Neuroinflammatory response to lipopolysaccharide is exacerbated in mice genetically deficient in cyclooxygenase-2." J Neuroinflammation 5: 17.

Aid, S., N. Parikh, et al. (2010). "Neuronal overexpression of cyclooxygenase-2 does not alter the neuroinflammatory response during brain innate immune activation." Neuroscience Letters 478(3): 113-118.

Akassoglou, K., J. Bauer, et al. (1999). "Transgenic models of TNF induced demyelination." Adv Exp Med Biol 468: 245-259.

Akassoglou, K., J. Bauer, et al. (1998). "Oligodendrocyte apoptosis and primary demyelination induced by local TNF/p55TNF receptor signaling in the central nervous system of transgenic mice: models for multiple sclerosis with primary oligodendrogliopathy." Am J Pathol 153(3): 801-813.

Albensi, B. C. and M. P. Mattson (2000). "Evidence for the involvement of TNF and NF-kappaB in hippocampal synaptic plasticity." Synapse 35(2): 151-159.

Alberati-Giani, D., P. Ricciardi-Castagnoli, et al. (1996). "Regulation of the kynurenine metabolic pathway by interferon-gamma in murine cloned macrophages and microglial cells." J Neurochem 66(3): 996-1004.

Altun, M., E. Bergman, et al. (2007). "Behavioral impairments of the aging rat." Physiol Behav 92(5): 911-923.

Andre, C., J. C. O'Connor, et al. (2008). "Spatio-temporal differences in the profile of murine brain expression of proinflammatory cytokines and indoleamine 2,3-dioxygenase in response to peripheral lipopolysaccharide administration." J Neuroimmunol 200(1-2): 90-99.

Andreadou, E., E. Kemanetzoglou, et al. (2013). "Demyelinating Disease following Anti-TNFa Treatment: A Causal or Coincidental Association? Report of Four Cases and Review of the Literature." Case Rep Neurol Med 2013: 671935.

Arnett, H. A., J. Mason, et al. (2001). "TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination." Nat Neurosci 4(11): 1116-1122.

Association, A. P. (2000). Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition - Text Revision (DSMIV-TR) Washington DC., American Psychiatric Association.

Athens, J. W., A. M. Mauer, et al. (1959). "Leukokinetic studies. I. A method for labeling leukocytes with diisopropyl-fluorophosphate (DFP32)." Blood 14(4): 303-333.

Banati, R. B., G. W. Goerres, et al. (1999). "[11C](R)-PK11195 positron emission tomography imaging of activated microglia in vivo in Rasmussen's encephalitis." Neurology 53(9): 2199-2203.

Banati, R. B., R. Myers, et al. (1997). "PK ('peripheral benzodiazepine')--binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [3H]PK11195 binding to activated microglia." J Neurocytol 26(2): 77-82.

Banati, R. B., J. Newcombe, et al. (2000). "The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative in vivo imaging of microglia as a measure of disease activity." Brain 123 (Pt 11): 2321-2337.

Banks, W. A. (2005). "Blood-brain barrier transport of cytokines: a mechanism for neuropathology." Curr Pharm Des 11(8): 973-984.

Banks, W. A. and S. M. Robinson (2010). "Minimal penetration of lipopolysaccharide across the murine blood-brain barrier." Brain Behav Immun 24(1): 102-109.

Barger, S. W., D. Horster, et al. (1995). "Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca2+ accumulation." Proc Natl Acad Sci U S A 92(20): 9328-9332.

Barker, C. F. and R. E. Billingham (1977). "Immunologically privileged sites." Adv Immunol 25: 1-54.

Bastianello, S., E. Giugni, et al. (2011). "Changes in magnetic resonance imaging disease measures over 3 years in mildly disabled patients with relapsing-remitting multiple sclerosis receiving interferon beta-1a in the COGnitive Impairment in MUltiple Sclerosis (COGIMUS) study." BMC Neurol 11: 125.

Bath, K. G., D. Q. Jing, et al. (2012). "BDNF Val66Met impairs fluoxetine-induced enhancement of adult hippocampus plasticity." Neuropsychopharmacology 37(5): 1297-1304.
Baune B., C. M.-L., Eyre H., Jawahar C., Anscomb H., Körner H. (2012). "Tumour necrosis factor-alpha mediated mechanisms of cognitive dysfunction." Translational Neuroscience 3: 263-277.
Baune, B. T., F. Wiede, et al. (2008). "Cognitive dysfunction in mice deficient for TNF- and its receptors." Am J Med Genet B Neuropsychiatr Genet 147B(7): 1056-1064.
Beattie, E. C., D. Stellwagen, et al. (2002). "Control of synaptic strength by glial TNFalpha." Science 295(5563): 2282-2285.
Begega, A., S. Cienfuegos, et al. (2001). "Effects of ageing on allocentric and egocentric spatial strategies in the Wistar rat." Behav Processes 53(1-2): 75-85.
Belmaker, R. H. and G. Agam (2008). "Major depressive disorder." N. Engl. J. Med. 358(1): 55-68.
Benn, S. C. and C. J. Woolf (2004). "Adult neuron survival strategies--slamming on the brakes." Nat Rev Neurosci 5(9): 686-700.
Berends, A. C., P. G. Luiten, et al. (2005). "A review of the neuroprotective properties of the 5-HT1A receptor agonist repinotan HCl (BAYx3702) in ischemic stroke." CNS Drug Rev 11(4): 379-402.
Bigda, J., I. Beletsky, et al. (1994). "Dual role of the p75 tumor necrosis factor (TNF) receptor in TNF cytotoxicity." J Exp Med 180(2): 445-460.
Bitsch, A., H. Bruhn, et al. (1999). "Inflammatory CNS demyelination: histopathologic correlation with in vivo quantitative proton MR spectroscopy." AJNR Am J Neuroradiol 20(9): 1619-1627.
Black, R. A., C. T. Rauch, et al. (1997). "A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells." Nature 385(6618): 729-733.
Bluthe, R. M., S. Laye, et al. (2000). "Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice." Eur J Neurosci 12(12): 4447-4456.
Bluthe, R. M., B. Michaud, et al. (2000). "Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice." Physiol Behav 70(3-4): 367-373.
Bradham, C. A., J. Plumpe, et al. (1998). "Mechanisms of hepatic toxicity. I. TNF-induced liver injury." Am J Physiol 275(3 Pt 1): G387-392.
Bribes, E., D. Carriere, et al. (2004). "Immunohistochemical assessment of the peripheral benzodiazepine receptor in human tissues." J Histochem Cytochem 52(1): 19-28.
Brown, R. R. (2003). Closing remarks. New York, Kluwer Academic/Plenum Publishers.
Brown, R. R., Y. Ozaki, et al. (1991). "Implications of interferon-induced tryptophan catabolism in cancer, auto-immune diseases and AIDS." Adv Exp Med Biol 294: 425-435.
Bruce, A. J., W. Boling, et al. (1996). "Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors." Nat Med 2(7): 788-794.
Burow, M. E., C. B. Weldon, et al. (2000). "PI3-K/AKT regulation of NF-kappaB signaling events in suppression of TNF-induced apoptosis." Biochem Biophys Res Commun 271(2): 342-345.
Byrne, G. I., L. K. Lehmann, et al. (1986). "Induction of tryptophan catabolism is the mechanism for gamma-interferon-mediated inhibition of intracellular Chlamydia psittaci replication in T24 cells." Infect Immun 53(2): 347-351.
Cacquevel, M., N. Lebeurrier, et al. (2004). "Cytokines in neuroinflammation and Alzheimer's disease." Curr Drug Targets 5(6): 529-534.
Cagnin, A., A. Gerhard, et al. (2002). "In vivo imaging of neuroinflammation." Eur Neuropsychopharmacol 12(6): 581-586.
Cagnin, A., M. Kassiou, et al. (2007). "Positron emission tomography imaging of neuroinflammation." Neurotherapeutics 4(3): 443-452.
Calabresi, P., A. Castrioto, et al. (2013). "New experimental and clinical links between the hippocampus and the dopaminergic system in Parkinson's disease." Lancet Neurol 12(8): 811-821.
Campbell, I. L., A. K. Stalder, et al. (1997). "Transgenic models to assess the pathogenic actions of cytokines in the central nervous system." Mol Psychiatry 2(2): 125-129.
Capuron, L., A. Ravaud, et al. (2001). "Association between immune activation and early depressive symptoms in cancer patients treated with interleukin-2-based therapy." Psychoneuroendocrinology 26(8): 797-808.
Carlin, J. M., E. C. Borden, et al. (1987). "Biologic-response-modifier-induced indoleamine 2,3-dioxygenase activity in human peripheral blood mononuclear cell cultures." J Immunol 139(7): 2414-2418.
Carlin, J. M., E. C. Borden, et al. (1989). "Interferon-induced indoleamine 2,3-dioxygenase activity in human mononuclear phagocytes." J Leukoc Biol 45(1): 29-34.

Carlson, N. G., A. Bacchi, et al. (1998). "Nicotine blocks TNF-alpha-mediated neuroprotection to NMDA by an alpha-bungarotoxin-sensitive pathway." J Neurobiol 35(1): 29-36.
Carmona, O., C. Masuet, et al. (2011). "Multiple sclerosis and cognitive decline: is ApoE-4 a surrogate marker?" Acta Neurol Scand 124(4): 258-263.
Carney, R. M., K. E. Freedland, et al. (2002). "Depression as a risk factor for cardiac mortality and morbidity: a review of potential mechanisms." J. Psychosom. Res. 53(4): 897-902.
Casserly, I. and E. Topol (2004). "Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins." Lancet 363(9415): 1139-1146.
Catena-Dell'Osso, M., C. Bellantuono, et al. (2011). "Inflammatory and neurodegenerative pathways in depression: a new avenue for antidepressant development?" Curr Med Chem 18(2): 245-255.
Cecil, K. M. and R. E. Lenkinski (1998). "Proton MR spectroscopy in inflammatory and infectious brain disorders." Neuroimaging Clin N Am 8(4): 863-880.
Centonze, D., L. Muzio, et al. (2009). "Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis." J Neurosci 29(11): 3442-3452.
Chavant, F., J. Deguil, et al. (2010). "Imipramine, in part through tumor necrosis factor alpha inhibition, prevents cognitive decline and beta-amyloid accumulation in a mouse model of Alzheimer's disease." J Pharmacol Exp Ther 332(2): 505-514.
Chen, C., J. J. Kim, et al. (1996). "Hippocampal lesions impair contextual fear conditioning in two strains of mice." Behav Neurosci 110(5): 1177-1180.
Chen, M. K., K. Baidoo, et al. (2004). "Peripheral benzodiazepine receptor imaging in CNS demyelination: functional implications of anatomical and cellular localization." Brain 127(Pt 6): 1379-1392.
Chen, N. J., Chio, II, et al. (2008). "Beyond tumor necrosis factor receptor: TRADD signaling in toll-like receptors." Proc Natl Acad Sci U S A 105(34): 12429-12434.
Cheng, B., S. Christakos, et al. (1994). "Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis." Neuron 12(1): 139-153.
Chertoff, M., N. Di Paolo, et al. (2011). "Neuroprotective and neurodegenerative effects of the chronic expression of tumor necrosis factor alpha in the nigrostriatal dopaminergic circuit of adult mice." Exp Neurol 227(2): 237-251.
Chiarugi, A. (2002). "Characterization of the molecular events following impairment of NF-kappaB-driven transcription in neurons." Brain Res Mol Brain Res 109(1-2): 179-188.
Chiarugi, A., A. Cozzi, et al. (2001). "Kynurenine 3-mono-oxygenase activity and neurotoxic kynurenine metabolites increase in the spinal cord of rats with experimental allergic encephalomyelitis." Neuroscience 102(3): 687-695.
Choi, S. H., R. Langenbach, et al. (2008). "Genetic deletion or pharmacological inhibition of cyclooxygenase-1 attenuate lipopolysaccharide-induced inflammatory response and brain injury." FASEB Journal 22(5): 1491-1501.
Choi, S. J., K. H. Lee, et al. (2005). "Differential expression, shedding, cytokine regulation and function of TNFR1 and TNFR2 in human fetal astrocytes." Yonsei Med J 46(6): 818-826.
Chong, Y. H., S. A. Shin, et al. (2002). "Molecular mechanisms underlying cyclic AMP inhibition of macrophage dependent TNF-alpha production and neurotoxicity in response to amyloidogenic C-terminal fragment of Alzheimer's amyloid precursor protein." J Neuroimmunol 133(1-2): 160-174.
Clark, I. A., L. M. Alleva, et al. (2010). "The roles of TNF in brain dysfunction and disease." Pharmacol Ther 128(3): 519-548.
Clark, R. E., S. M. Zola, et al. (2000). "Impaired recognition memory in rats after damage to the hippocampus." J Neurosci 20(23): 8853-8860.
Clarkson, A. N., R. Rahman, et al. (2004). "Inflammation and autoimmunity as a central theme in neurodegenerative disorders: fact or fiction?" Curr Opin Investig Drugs 5(7): 706-713.
Clementi, E., G. Martino, et al. (1994). "Intracellular Ca2+ stores of T lymphocytes: changes induced by in vitro and in vivo activation." Eur J Immunol 24(6): 1365-1371.
Cooper, N. R., R. N. Kalaria, et al. (2000). "Key issues in Alzheimer's disease inflammation." Neurobiol Aging 21(3): 451-453.
Corona, A. W., A. M. Fenn, et al. (2011). "Cognitive and Behavioral Consequences of Impaired Immunoregulation in Aging." J Neuroimmune Pharmacol.
Corstens, F. H. and J. W. van der Meer (1999). "Nuclear medicine's role in infection and inflammation." Lancet 354(9180): 765-770.

Cozzi, A., R. Carpenedo, et al. (1999). "Kynurenine hydroxylase inhibitors reduce ischemic brain damage: studies with (m-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[-N-4-(nitrophenyl)thiazol-2yl]-benzenesulfonamide (Ro 61-8048) in models of focal or global brain ischemia." J Cereb Blood Flow Metab 19(7): 771-777.
Cremer, J. E., S. P. Hume, et al. (1992). "The distribution of radioactivity in brains of rats given [N-methyl-11C]PK 11195 in vivo after induction of a cortical ischaemic lesion." Int J Rad Appl Instrum B 19(2): 159-166.
Cryan, J. F. and C. Mombereau (2004). "In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice." Mol Psychiatry 9(4): 326-357.
Cui, Y., T. Takashima, et al. (2009). "11C-PK11195 PET for the in vivo evaluation of neuroinflammation in the rat brain after cortical spreading depression." J Nucl Med 50(11): 1904-1911.
Cummings, J. L. (2000). "Cognitive and behavioral heterogeneity in Alzheimer's disease: seeking the neurobiological basis." Neurobiol Aging 21(6): 845-861.
Currier, A. R., M. H. Ziegler, et al. (2000). "Tumor necrosis factor-alpha and lipopolysaccharide enhance interferon-induced antichlamydial indoleamine dioxygenase activity independently." J Interferon Cytokine Res 20(4): 369-376.
Cserr, H. F. and P. M. Knopf (1992). "Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view." Immunol Today 13(12): 507-512.
Da Silva Costa-Aze, V., F. Dauphin, et al. (2011). "Serotonin 5-HT6 receptor blockade reverses the age-related deficits of recognition memory and working memory in mice." Behav Brain Res 222(1): 134-140.
Dantzer, R., J. C. O'Connor, et al. (2008). "From inflammation to sickness and depression: when the immune system subjugates the brain." Nat Rev Neurosci 9(1): 46-56.
Darlington, L. G., G. M. Mackay, et al. (2007). "Altered kynurenine metabolism correlates with infarct volume in stroke." Eur J Neurosci 26(8): 2211-2221.
Davis, S. and S. Laroche (2003). "What can rodent models tell us about cognitive decline in Alzheimer's disease?" Mol Neurobiol 27(3): 249-276.
Davis, S. M. and G. A. Donnan (2004). "Steroids for stroke: another potential therapy discarded prematurely?" Stroke 35(1): 230-231.
de Jong, W. H., R. Smit, et al. (2009). "Plasma tryptophan, kynurenine and 3-hydroxykynurenine measurement using automated on-line solid-phase extraction HPLC-tandem mass spectrometry." J Chromatogr B Analyt Technol Biomed Life Sci 877(7): 603-609.
de la Torre, J. C. (2004). "Alzheimer's disease is a vasocognopathy: a new term to describe its nature." Neurol Res 26(5): 517-524.
De Reuck, J., H. Stevens, et al. (1999). "Cobalt-55 positron emission tomography of ipsilateral thalamic and crossed cerebellar hypometabolism after supratentorial ischaemic stroke." Cerebrovasc Dis 9(1): 40-44.
Dean, B., N. Tawadros, et al. (2010). "Regionally-specific changes in levels of tumour necrosis factor in the dorsolateral prefrontal cortex obtained postmortem from subjects with major depressive disorder." J Affect Disord 120(1-3): 245-248.
Dean, R. L., 3rd, J. Scozzafava, et al. (1981). "Age-related differences in behavior across the life span of the C57BL/6J mouse." Exp Aging Res 7(4): 427-451.
Dedoni, S., M. C. Olianas, et al. (2010). "Interferon-beta induces apoptosis in human SH-SY5Y neuroblastoma cells through activation of JAK-STAT signaling and down-regulation of PI3K/Akt pathway." J Neurochem 115(6): 1421-1433.
Depino, A. M., M. Alonso, et al. (2004). "Learning modulation by endogenous hippocampal IL-1: blockade of endogenous IL-1 facilitates memory formation." Hippocampus 14(4): 526-535.
Depuydt, B., G. van Loo, et al. (2005). "Induction of apoptosis by TNF receptor 2 in a T-cell hybridoma is FADD dependent and blocked by caspase-8 inhibitors." J Cell Sci 118(Pt 3): 497-504.
DeVane, C. L. (2000). "Pharmacologic characteristics of ideal antidepressants in the 21st century." J Clin Psychiatry 61 Suppl 11: 4-8.
Di Patre, P. L., S. L. Read, et al. (1999). "Progression of clinical deterioration and pathological changes in patients with Alzheimer disease evaluated at biopsy and autopsy." Arch Neurol 56(10): 1254-1261.
Diem, R., R. Meyer, et al. (2001). "Reduction of potassium currents and phosphatidylinositol 3-kinase-dependent AKT phosphorylation by tumor necrosis factor-(alpha) rescues axotomized retinal ganglion cells from retrograde cell death in vivo." J Neurosci 21(6): 2058-2066.
Diorio, D., S. A. Welner, et al. (1991). "Peripheral benzodiazepine binding sites in Alzheimer's disease frontal and temporal cortex." Neurobiol Aging 12(3): 255-258.
Dirnagl, U. (2004). "Inflammation in stroke: the good, the bad, and the unknown." Ernst Schering Res Found Workshop(47): 87-99.

Dobos, N., E. F. de Vries, et al. (2011). "The Role of Indoleamine 2,3-Dioxygenase in a Mouse Model of Neuroinflammation-Induced Depression." J Alzheimers Dis.
Dobos, N., J. Korf, et al. (2010). "Neuroinflammation in Alzheimer's disease and major depression." Biol Psychiatry 67(6): 503-504.
Dolga, A. M., I. Granic, et al. (2009). "Pretreatment with lovastatin prevents N-methyl-D-aspartate-induced neurodegeneration in the magnocellular nucleus basalis and behavioral dysfunction." J Alzheimers Dis 17(2): 327-336.
Doorduin, J., E. F. de Vries, et al. (2008). "PET imaging of the peripheral benzodiazepine receptor: monitoring disease progression and therapy response in neurodegenerative disorders." Curr Pharm Des 14(31): 3297-3315.
Dowlati, Y., N. Herrmann, et al. (2010). "A meta-analysis of cytokines in major depression." Biol Psychiatry 67(5): 446-457.
Dubinsky, J. M. (1993). "Examination of the role of calcium in neuronal death." Ann N Y Acad Sci 679: 34-42.
Duman, R. S. and G. K. Aghajanian (2012). "Synaptic dysfunction in depression: potential therapeutic targets." Science 338(6103): 68-72.
Dunn, A. J., J. Wang, et al. (1999). "Effects of cytokines on cerebral neurotransmission. Comparison with the effects of stress." Adv Exp Med Biol 461: 117-127.
Dunn, A. J. and J. Welch (1991). "Stress- and endotoxin-induced increases in brain tryptophan and serotonin metabolism depend on sympathetic nervous system activity." J Neurochem 57(5): 1615-1622.
Eagger, S., G. M. Syed, et al. (1992). "Morphologic (CT) and functional (rCBF-SPECT) correlates in Alzheimer's disease." Nucl Med Commun 13(9): 644-647.
Edstrom, E. and B. Ulfhake (2005). "Sarcopenia is not due to lack of regenerative drive in senescent skeletal muscle." Aging Cell 4(2): 65-77.
Eichenbaum, H. (1996). "Is the rodent hippocampus just for 'place'?" Curr Opin Neurobiol 6(2): 187-195.
Eikelenboom, P. and F. C. Stam (1982). "Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study." Acta Neuropathol 57(2-3): 239-242.
Eisel, U. L. (2002). "Cytokines in degenerative brain diseases: lessons from transgenic animals." Curr Top Microbiol Immunol 265: 49-62.
Eisel, U. L. M., Biber, K., Luiten, P.G.M. (2006). "Life and Death of Nerve Cells: Therapeutic Cytokine Signaling Pathways." Current Signal Transduction Therapy Volume 1: 133-146.
Estrada Sanchez, A. M., J. Mejia-Toiber, et al. (2008). "Excitotoxic neuronal death and the pathogenesis of Huntington's disease." Arch Med Res 39(3): 265-276.
Faggioni, R., G. Fantuzzi, et al. (1995). "Independent down-regulation of central and peripheral tumor necrosis factor production as a result of lipopolysaccharide tolerance in mice." Infection and Immunity 63(4): 1473-1477.
Fahlstrom, A., H. Zeberg, et al. (2011). "Changes in behaviors of male C57BL/6J mice across adult life span and effects of dietary restriction." Age (Dordr).
Farkas, E., G. Donka, et al. (2004). "Experimental cerebral hypoperfusion induces white matter injury and microglial activation in the rat brain." Acta Neuropathol 108(1): 57-64.
Farkas, E. and P. G. Luiten (2001). "Cerebral microvascular pathology in aging and Alzheimer's disease." Prog Neurobiol 64(6): 575-611.
Farrell, R. A. and G. Giovannoni (2010). "Current and future role of interferon beta in the therapy of multiple sclerosis." J Interferon Cytokine Res 30(10): 715-726.
Faustman, D. and M. Davis (2010). "TNF receptor 2 pathway: drug target for autoimmune diseases." Nat Rev Drug Discov 9(6): 482-493.
Feinstein, A. (2000). "Multiple sclerosis, disease modifying treatments and depression: a critical methodological review." Mult Scler 6(5): 343-348.
Feinstein, A. (2006). "Mood disorders in multiple sclerosis and the effects on cognition." J Neurol Sci 245(1-2): 63-66.
Ferger, B., A. Leng, et al. (2004). "Genetic ablation of tumor necrosis factor-alpha (TNF-alpha) and pharmacological inhibition of TNF-synthesis attenuates MPTP toxicity in mouse striatum." J Neurochem 89(4): 822-833.
Feuerstein, G. Z., X. Wang, et al. (1997). "Inflammatory gene expression in cerebral ischemia and trauma. Potential new therapeutic targets." Ann N Y Acad Sci 825: 179-193.
Fillit, H., W. H. Ding, et al. (1991). "Elevated circulating tumor necrosis factor levels in Alzheimer's disease." Neurosci Lett 129(2): 318-320.

Fontaine, V., S. Mohand-Said, et al. (2002). "Neurodegenerative and neuroprotective effects of tumor Necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2." J Neurosci 22(7): RC216.
Forrest, C. M., O. S. Khalil, et al. (2013). "Prenatal inhibition of the tryptophan-kynurenine pathway alters synaptic plasticity and protein expression in the rat hippocampus." Brain Res 1504: 1-15.
Foster, A. C., J. F. Collins, et al. (1983). "On the excitotoxic properties of quinolinic acid, 2,3-piperidine dicarboxylic acids and structurally related compounds." Neuropharmacology 22(12A): 1331-1342.
Fragoso, Y. D., E. R. Frota, et al. (2010). "Severe depression, suicide attempts, and ideation during the use of interferon beta by patients with multiple sclerosis." Clin Neuropharmacol 33(6): 312-316.
Frankola, K. A., N. H. Greig, et al. (2011). "Targeting TNF-alpha to elucidate and ameliorate neuroinflammation in neurodegenerative diseases." CNS Neurol Disord Drug Targets 10(3): 391-403.
Frenois, F., M. Moreau, et al. (2007). "Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior." Psychoneuroendocrinology 32(5): 516-531.
Fu, X., S. M. Zunich, et al. (2010). "Central Administration of Lipopolysaccharide Induces Depressive-like Behavior in Vivo and Activates Brain Indoleamine 2,3 Dioxygenase In Murine Organotypic Hippocampal Slice Cultures." J Neuroinflammation 7(1): 43.
Fujigaki, S., K. Saito, et al. (2001). "Lipopolysaccharide induction of indoleamine 2,3-dioxygenase is mediated dominantly by an IFN-gamma-independent mechanism." Eur J Immunol 31(8): 2313-2318.
Furukawa, K. and M. P. Mattson (1998). "The transcription factor NF-kappaB mediates increases in calcium currents and decreases in NMDA- and AMPA/kainate-induced currents induced by tumor necrosis factor-alpha in hippocampal neurons." J Neurochem 70(5): 1876-1886.
Galea, I., I. Bechmann, et al. (2007). "What is immune privilege (not)?" Trends Immunol 28(1): 12-18.
Gibbons, S. J., J. R. Brorson, et al. (1993). "Calcium influx and neurodegeneration." Ann N Y Acad Sci 679: 22-33.
Gigler, G., G. Szenasi, et al. (2007). "Neuroprotective effect of L-kynurenine sulfate administered before focal cerebral ischemia in mice and global cerebral ischemia in gerbils." Eur J Pharmacol 564(1-3): 116-122.
Gimsa, U., E. Kanitz, et al. (2012). "Tumour necrosis factor receptor deficiency alters anxiety-like behavioural and neuroendocrine stress responses of mice." Cytokine 59(1): 72-78.
Glazner, G. W. and M. P. Mattson (2000). "Differential effects of BDNF, ADNF9, and TNFalpha on levels of NMDA receptor subunits, calcium homeostasis, and neuronal vulnerability to excitotoxicity." Exp Neurol 161(2): 442-452.
Godbout, J. P., M. Moreau, et al. (2008). "Aging exacerbates depressive-like behavior in mice in response to activation of the peripheral innate immune system." Neuropsychopharmacology 33(10): 2341-2351.
Godin-Ethier, J., L. A. Hanafi, et al. (2011). "Indoleamine 2,3-dioxygenase expression in human cancers: clinical and immunologic perspectives." Clin Cancer Res 17(22): 6985-6991.
Goeb, J. L., C. Even, et al. (2006). "Psychiatric side effects of interferon-beta in multiple sclerosis." Eur Psychiatry 21(3): 186-193.
Goodman, J. C., C. S. Robertson, et al. (1990). "Elevation of tumor necrosis factor in head injury." J Neuroimmunol 30(2-3): 213-217.
Gould, T. J. and O. R. Feiro (2005). "Age-related deficits in the retention of memories for cued fear conditioning are reversed by galantamine treatment." Behav Brain Res 165(2): 160-171.
Gramsbergen, J. B., L. Veenma-van der Duin, et al. (1988). "Imaging of the degeneration of neurons and their processes in rat or cat brain by 45CaCl2 autoradiography or 55CoCl2 positron emission tomography." J Neurochem 50(6): 1798-1807.
Grasselli, G., S. Rossi, et al. (2013). "Abnormal NMDA receptor function exacerbates experimental autoimmune encephalomyelitis." Br J Pharmacol 168(2): 502-517.
Grell, M., E. Douni, et al. (1995). "The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor." Cell 83(5): 793-802.
Griffin, W. S. (2008). "Perispinal etanercept: potential as an Alzheimer therapeutic." J Neuroinflammation 5: 3.
Groom, G. N., L. Junck, et al. (1995). "PET of peripheral benzodiazepine binding sites in the microgliosis of Alzheimer's disease." J Nucl Med 36(12): 2207-2210.
Guilarte, T. R., A. C. Kuhlmann, et al. (1995). "Enhanced expression of peripheral benzodiazepine receptors in trimethyltin-exposed rat brain: a biomarker of neurotoxicity." Neurotoxicology 16(3): 441-450.
Guillemin, G. J., B. J. Brew, et al. (2005). "Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus." Neuropathol Appl Neurobiol 31(4): 395-404.

Guillemin, G. J., S. J. Kerr, et al. (2001). "IFN-beta1b induces kynurenine pathway metabolism in human macrophages: potential implications for multiple sclerosis treatment." J Interferon Cytokine Res 21(12): 1097-1101.
Guillemin, G. J., V. Meininger, et al. (2005). "Implications for the kynurenine pathway and quinolinic acid in amyotrophic lateral sclerosis." Neurodegener Dis 2(3-4): 166-176.
Guillemin, G. J., D. G. Smith, et al. (2000). "Characterisation of kynurenine pathway metabolism in human astrocytes and implications in neuropathogenesis." Redox Report 5(2-3): 108-111.
Guillemin, G. J., G. Smythe, et al. (2005). "Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons." Glia 49(1): 15-23.
Guillemin, G. J., G. A. Smythe, et al. (2003). "A beta 1-42 induces production of quinolinic acid by human macrophages and microglia." Neuroreport 14(18): 2311-2315.
Guillemin, G. J., L. Wang, et al. (2005). "Quinolinic acid selectively induces apoptosis of human astrocytes: potential role in AIDS dementia complex." J Neuroinflammation 2: 16.
Guo, D., J. D. Dunbar, et al. (1998). "Induction of Jak/STAT signaling by activation of the type 1 TNF receptor." J Immunol 160(6): 2742-2750.
Gustin, J. A., O. N. Ozes, et al. (2004). "Cell type-specific expression of the IkappaB kinases determines the significance of phosphatidylinositol 3-kinase/Akt signaling to NF-kappa B activation." J Biol Chem 279(3): 1615-1620.
Hajszan, T., K. Szigeti-Buck, et al. (2010). "Effects of estradiol on learned helplessness and associated remodeling of hippocampal spine synapses in female rats." Biol. Psychiatry 67(2): 168-174.
Hall, E. D. (1985). "High-dose glucocorticoid treatment improves neurological recovery in head-injured mice." J Neurosurg 62(6): 882-887.
Hammoud, D. A., C. J. Endres, et al. (2005). "Imaging glial cell activation with [11C]-R-PK11195 in patients with AIDS." J Neurovirol 11(4): 346-355.
Hansson, O., H. Zetterberg, et al. (2006). "Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study." Lancet Neurol 5(3): 228-234.
Hardin, B. J., K. S. Campbell, et al. (2008). "TNF-alpha acts via TNFR1 and muscle-derived oxidants to depress myofibrillar force in murine skeletal muscle." J Appl Physiol (1985) 104(3): 694-699.
Harkany, T., I. Abraham, et al. (2000). "beta-amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis." Eur J Neurosci 12(8): 2735-2745.
Harkany, T., J. Mulder, et al. (2001). "Oral post-lesion administration of 5-HT(1A) receptor agonist repinotan hydrochloride (BAY x 3702) attenuates NMDA-induced delayed neuronal death in rat magnocellular nucleus basalis." Neuroscience 108(4): 629-642.
Hart, M. N. and Z. Fabry (1995). "CNS antigen presentation." Trends Neurosci 18(11): 475-481.
Hartley, D. M., M. C. Kurth, et al. (1993). "Glutamate receptor-induced 45Ca2+ accumulation in cortical cell culture correlates with subsequent neuronal degeneration." J Neurosci 13(5): 1993-2000.
Hauss-Wegrzyniak, B., P. Dobrzanski, et al. (1998). "Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease." Brain Res 780(2): 294-303.
Havekes, R., T. Abel, et al. (2011). "The cholinergic system and neostriatal memory functions." Behav Brain Res 221(2): 412-423.
Haverstick, D. M. and L. S. Gray (1993). "Increased intracellular Ca2+ induces Ca2+ influx in human T lymphocytes." Mol Biol Cell 4(2): 173-184.
He, P., Z. Zhong, et al. (2007). "Deletion of tumor necrosis factor death receptor inhibits amyloid beta generation and prevents learning and memory deficits in Alzheimer's mice." J Cell Biol 178(5): 829-841.
Head, J. R. and W. S. Griffin (1985). "Functional capacity of solid tissue transplants in the brain: evidence for immunological privilege." Proc R Soc Lond B Biol Sci 224(1236): 375-387.
Hemmer, B., S. Nessler, et al. (2006). "Immunopathogenesis and immunotherapy of multiple sclerosis." Nat Clin Pract Neurol 2(4): 201-211.
Heneka, M. T., M. K. O'Banion, et al. (2010). "Neuroinflammatory processes in Alzheimer's disease." J Neural Transm 117(8): 919-947.
Hickey, W. F., B. L. Hsu, et al. (1991). "T-lymphocyte entry into the central nervous system." J Neurosci Res 28(2): 254-260.
Higuchi, K. and O. Hayaishi (1967). "Enzymic formation of D-kynurenine from D-tryptophan." Arch Biochem Biophys 120(2): 397-403.
Hofman, F. M., D. R. Hinton, et al. (1989). "Tumor necrosis factor identified in multiple sclerosis brain." J Exp Med 170(2): 607-612.

Hollis, J. H., A. K. Evans, et al. (2006). "Lipopolysaccharide has indomethacin-sensitive actions on Fos expression in topographically organized subpopulations of serotonergic neurons." Brain Behav Immun 20(6): 569-577.
Honda, K. and T. Taniguchi (2006). "IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors." Nat Rev Immunol 6(9): 644-658.
Howren, M. B., D. M. Lamkin, et al. (2009). "Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis." Psychosom Med 71(2): 171-186.
Intiso, D., M. M. Zarrelli, et al. (2004). "Tumor necrosis factor alpha serum levels and inflammatory response in acute ischemic stroke patients." Neurol Sci 24(6): 390-396.
Iosif, R. E., C. T. Ekdahl, et al. (2006). "Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis." J Neurosci 26(38): 9703-9712.
Iwashyna, T. J., E. W. Ely, et al. (2010). "Long-term cognitive impairment and functional disability among survivors of severe sepsis." JAMA 304(16): 1787-1794.
Jansen, H. M., R. A. Dierckx, et al. (1997). "Positron emission tomography in primary brain tumours using cobalt-55." Nucl Med Commun 18(8): 734-740.
Jansen, H. M., S. Knollema, et al. (1996). "Pharmacokinetics and dosimetry of cobalt-55 and cobalt-57." J Nucl Med 37(12): 2082-2086.
Jansen, H. M., J. van der Naalt, et al. (1996). "Cobalt-55 positron emission tomography in traumatic brain injury: a pilot study." J Neurol Neurosurg Psychiatry 60(2): 221-224.
Jansen, H. M., A. T. Willemsen, et al. (1995). "Cobalt-55 positron emission tomography in relapsing-progressive multiple sclerosis." J Neurol Sci 132(2): 139-145.
Jiang, Y., J. D. Woronicz, et al. (1999). "Prevention of constitutive TNF receptor 1 signaling by silencer of death domains." Science 283(5401): 543-546.
Joachim, C. L., J. H. Morris, et al. (1989). "Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease." Am J Pathol 135(2): 309-319.
Jorm, A. F. (2000). "Is depression a risk factor for dementia or cognitive decline? A review." Gerontology 46(4): 219-227.
Jorm, A. F. (2001). "History of depression as a risk factor for dementia: an updated review." Aust. N. Z. J. Psychiatry 35(6): 776-781.
Jorm, A. F. (2001). "History of depression as a risk factor for dementia: an updated review." Aust N Z J Psychiatry 35(6): 776-781.
Joseph, J. A., R. T. Bartus, et al. (1983). "Psychomotor performance in the senescent rodent: reduction of deficits via striatal dopamine receptor up-regulation." Neurobiol Aging 4(4): 313-319.
Jost, B. C. and G. T. Grossberg (1996). "The evolution of psychiatric symptoms in Alzheimer's disease: a natural history study." J Am Geriatr Soc 44(9): 1078-1081.
Kakimura, J. I., Y. Kitamura, et al. (2002). "Microglial activation and amyloid-Î² clearance induced by exogenous heat-shock proteins." FASEB Journal 16(6): 601-603.
Kalaria, R. N. (2003). "Comparison between Alzheimer's disease and vascular dementia: implications for treatment." Neurol Res 25(6): 661-664.
Kaltsonoudis, E., P. V. Voulgari, et al. (2013). "Demyelination and other neurological adverse events after anti-TNF therapy." Autoimmun Rev.
Kang, H. J., B. Voleti, et al. (2012). "Decreased expression of synapse-related genes and loss of synapses in major depressive disorder." Nat. Med. 18: 1413-1417.
Kannan, S., B. Balakrishnan, et al. (2009). "Positron emission tomography imaging of neuroinflammation." J Child Neurol 24(9): 1190-1199.
Kanner, A. M. (2005). "Depression and the risk of neurological disorders." Lancet 366(9492): 1147-1148.
Kassiotis, G. and G. Kollias (2001). "Uncoupling the proinflammatory from the immunosuppressive properties of tumor necrosis factor (TNF) at the p55 TNF receptor level: implications for pathogenesis and therapy of autoimmune demyelination." J Exp Med 193(4): 427-434.
Kawanokuchi, J., T. Mizuno, et al. (2006). "Production of interferon-γ by microglia." Multiple Sclerosis 12(5): 558-564.
Keith B. J. Franklin, G. P. (1997). The mouse brain in stereotaxic coordinates, Academic Press.
Ketonen, L. and M. J. Tuite (1992). "Brain imaging in human immunodeficiency virus infection." Semin Neurol 12(1): 57-69.
Kiger, N., A. Khalil, et al. (1980). "Tumor-necrotizing serum production by administration of BCG + Pseudomonas: its application in treatment of fibrosarcoma in mice." Recent Results Cancer Res 75: 220-225.

Kim, D. M., R. Tien, et al. (1996). "Imaging in acquired immune deficiency syndrome dementia complex (AIDS dementia complex): a review." Prog Neuropsychopharmacol Biol Psychiatry 20(3): 349-370.
Kim, G. M., J. Xu, et al. (2001). "Tumor necrosis factor receptor deletion reduces nuclear factor-kappaB activation, cellular inhibitor of apoptosis protein 2 expression, and functional recovery after traumatic spinal cord injury." J Neurosci 21(17): 6617-6625.
Kim, S., C. Domon-Dell, et al. (2004). "Down-regulation of the tumor suppressor PTEN by the tumor necrosis factor-alpha/nuclear factor-kappaB (NF-kappaB)-inducing kinase/NF-kappaB pathway is linked to a default IkappaB-alpha autoregulatory loop." J Biol Chem 279(6): 4285-4291.
Klencklen, G., O. Despres, et al. (2012). "What do we know about aging and spatial cognition? Reviews and perspectives." Ageing Res Rev 11(1): 123-135.
Klohs, J. and M. Rudin (2011). "Unveiling molecular events in the brain by noninvasive imaging." Neuroscientist 17(5): 539-559.
Kropholler, M. A., R. Boellaard, et al. (2007). "Evaluation of reference regions for (R)-[(11)C]PK11195 studies in Alzheimer's disease and mild cognitive impairment." J Cereb Blood Flow Metab 27(12): 1965-1974.
Kuhlmann, A. C. and T. R. Guilarte (2000). "Cellular and subcellular localization of peripheral benzodiazepine receptors after trimethyltin neurotoxicity." J Neurochem 74(4): 1694-1704.
Laabich, A., G. Li, et al. (2002). "Enhanced expression of TNF-R1 protein in NMDA-mediated cell death in the retina." Brain Res Mol Brain Res 109(1-2): 239-246.
Lalonde, R. (2002). "The neurobiological basis of spontaneous alternation." Neurosci Biobehav Rev 26(1): 91-104.
Lamberty, Y. and A. J. Gower (1992). "Age-related changes in spontaneous behavior and learning in NMRI mice from middle to old age." Physiol Behav 51(1): 81-88.
Langston, J. W., L. S. Forno, et al. (1999). "Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure." Ann Neurol 46(4): 598-605.
Larson, M. E. and S. E. Lesne (2012). "Soluble Abeta oligomer production and toxicity." J Neurochem 120 Suppl 1: 125-139.
Lassmann, H., K. Rossler, et al. (1991). "Expression of adhesion molecules and histocompatibility antigens at the blood-brain barrier." Brain Pathol 1(2): 115-123.
Lee, T. H., Q. Huang, et al. (2003). "The death domain kinase RIP1 is essential for tumor necrosis factor alpha signaling to p38 mitogen-activated protein kinase." Mol Cell Biol 23(22): 8377-8385.
Lees, G. J. (2000). "Pharmacology of AMPA/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders." Drugs 59(1): 33-78.
Leonard, B. E. (2007). "Inflammation, depression and dementia: are they connected?" Neurochem Res 32(10): 1749-1756.
Leonard, B. E. (2010). Modern trends in pharmacopsychiatry (formerly published as Modern problems in pharmacopsychiatry). Depression: From Psychopathology to Paharmacotherapy. B. E. L. J.F. Cryan, Karger. 27: 53-71.
Lestage, J., D. Verrier, et al. (2002). "The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen." Brain Behav Immun 16(5): 596-601.
Li, N., B. Lee, et al. (2010). "mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists." Science 329(5994): 959-964.
Li, R., L. Yang, et al. (2004). "Tumor necrosis factor death receptor signaling cascade is required for amyloid-beta protein-induced neuron death." J Neurosci 24(7): 1760-1771.
Liebetrau, M., B. Steen, et al. (2004). "Silent and symptomatic infarcts on cranial computerized tomography in relation to dementia and mortality: a population-based study in 85-year-old subjects." Stroke 35(8): 1816-1820.
Lilienbaum, A. and A. Israel (2003). "From calcium to NF-kappa B signaling pathways in neurons." Mol Cell Biol 23(8): 2680-2698.
Linde, R., H. Laursen, et al. (1996). "Is calcium accumulation post-injury an indicator of cell damage?" Acta Neurochir Suppl 66: 15-20.
Link, H. (1998). "The cytokine storm in multiple sclerosis." Mult Scler 4(1): 12-15.
Linthorst, A. C., C. Flachskamm, et al. (1995). "Effect of bacterial endotoxin and interleukin-1 beta on hippocampal serotonergic neurotransmission, behavioral activity, and free corticosterone levels: an in vivo microdialysis study." J Neurosci 15(4): 2920-2934.
Liu, T., R. K. Clark, et al. (1994). "Tumor necrosis factor-alpha expression in ischemic neurons." Stroke 25(7): 1481-1488.

Lob, S., A. Konigsrainer, et al. (2009). "Inhibitors of indoleamine-2,3-dioxygenase for cancer therapy: can we see the wood for the trees?" Nat Rev Cancer 9(6): 445-452.
Loetscher, H., Y. C. Pan, et al. (1990). "Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor." Cell 61(2): 351-359.
Lowry, C. A., J. H. Hollis, et al. (2007). "Identification of an immune-responsive mesolimbocortical serotonergic system: potential role in regulation of emotional behavior." Neuroscience 146(2): 756-772.
Lucas, R., P. Juillard, et al. (1997). "Crucial role of tumor necrosis factor (TNF) receptor 2 and membrane-bound TNF in experimental cerebral malaria." Eur J Immunol 27(7): 1719-1725.
Maes, M., E. Bosmans, et al. (1991). "Depression-related disturbances in mitogen-induced lymphocyte responses and interleukin-1 beta and soluble interleukin-2 receptor production." Acta Psychiatr Scand 84(4): 379-386.
Maes, M., I. Mihaylova, et al. (2007). "The immune effects of TRYCATs (tryptophan catabolites along the IDO pathway): relevance for depression - and other conditions characterized by tryptophan depletion induced by inflammation." Neuro Endocrinol Lett 28(6): 826-831.
Magaki, S., C. Mueller, et al. (2007). "Increased production of inflammatory cytokines in mild cognitive impairment." Exp Gerontol 42(3): 233-240.
Mandolesi, G., G. Grasselli, et al. (2012). "GABAergic signaling and connectivity on Purkinje cells are impaired in experimental autoimmune encephalomyelitis." Neurobiol Dis 46(2): 414-424.
Mandolesi, G., G. Grasselli, et al. (2010). "Cognitive deficits in experimental autoimmune encephalomyelitis: neuroinflammation and synaptic degeneration." Neurol Sci 31(Suppl 2): S255-259.
Mann, U. M., E. Mohr, et al. (1992). "Heterogeneity in Alzheimer's disease: progression rate segregated by distinct neuropsychological and cerebral metabolic profiles." J Neurol Neurosurg Psychiatry 55(10): 956-959.
Marchetti, L., M. Klein, et al. (2004). "Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway." J Biol Chem 279(31): 32869-32881.
Markowitz, C. E. (2007). "Interferon-beta: mechanism of action and dosing issues." Neurology 68(24 Suppl 4): S8-11.
Marques, F., S. D. Mesquita, et al. (2012). "Lipocalin 2 is present in the EAE brain and is modulated by natalizumab." Front Cell Neurosci 6: 33.
Marx, F., I. Blasko, et al. (1998). "The possible role of the immune system in Alzheimer's disease." Exp Gerontol 33(7-8): 871-881.
Marxreiter, F., B. Ettle, et al. (2013). "Glial A30P alpha-synuclein pathology segregates neurogenesis from anxiety-related behavior in conditional transgenic mice." Neurobiol Dis 59: 38-51.
Mattson, M. P. (2004). "Pathways towards and away from Alzheimer's disease." Nature 430(7000): 631-639.
Mattson, M. P. and S. Camandola (2001). "NF-kappaB in neuronal plasticity and neurodegenerative disorders." J Clin Invest 107(3): 247-254.
Mattson, M. P. and S. L. Chan (2003). "Neuronal and glial calcium signaling in Alzheimer's disease." Cell Calcium 34(4-5): 385-397.
Mattson, M. P., C. Culmsee, et al. (2000). "Roles of nuclear factor kappaB in neuronal survival and plasticity." J Neurochem 74(2): 443-456.
Mattson, M. P., F. M. LaFerla, et al. (2000). "Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders." Trends Neurosci 23(5): 222-229.
McAfoose, J. and B. T. Baune (2009). "Evidence for a cytokine model of cognitive function." Neurosci Biobehav Rev 33(3): 355-366.
McCoy, M. K. and M. G. Tansey (2008). "TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease." J Neuroinflammation 5: 45.
McNally, L., Z. Bhagwagar, et al. (2008). "Inflammation, glutamate, and glia in depression: a literature review." CNS Spectr 13(6): 501-510.
Medvedev, A. E., A. Sundan, et al. (1994). "Involvement of the tumor necrosis factor receptor p75 in mediating cytotoxicity and gene regulating activities." Eur J Immunol 24(11): 2842-2849.
Mellor, A. L., D. Munn, et al. (2003). "Tryptophan catabolism and T cell responses." Adv Exp Med Biol 527: 27-35.
Mellor, A. L. and D. H. Munn (2004). "IDO expression by dendritic cells: tolerance and tryptophan catabolism." Nat Rev Immunol 4(10): 762-774.
Mendelsohn, A. R. and J. W. Larrick (2011). "Reversing age-related decline in working memory." Rejuvenation Res 14(5): 557-559.
Merimsky, O. and S. Chaitchik (1992). "Neurotoxicity of interferon-alpha." Anticancer Drugs 3(6): 567-570.

Merrill, J. E. (1991). "Effects of interleukin-1 and tumor necrosis factor-alpha on astrocytes, microglia, oligodendrocytes, and glial precursors in vitro." Dev Neurosci 13(3): 130-137.
Mikova, O., R. Yakimova, et al. (2001). "Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis." Eur Neuropsychopharmacol 11(3): 203-208.
Milatovic, D., S. Zaja-Milatovic, et al. (2004). "Neuronal oxidative damage and denritic degeneration following activation of CD14-dependent innate immune response in vivo." Journal of Neuroinflammation 1.
Miller, A. H., V. Maletic, et al. (2009). "Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression." Biol Psychiatry 65(9): 732-741.
Minden, S. L., J. Orav, et al. (1987). "Depression in multiple sclerosis." Gen Hosp Psychiatry 9(6): 426-434.
Mogi, M., M. Harada, et al. (1994). "Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients." Neurosci Lett 165(1-2): 208-210.
Moreau, M., C. Andre, et al. (2008). "Inoculation of Bacillus Calmette-Guerin to mice induces an acute episode of sickness behavior followed by chronic depressive-like behavior." Brain Behav Immun 22(7): 1087-1095.
Moriuchi, S., K. Shimizu, et al. (1996). "In vitro assessment for neurotoxicity of antitumor agents before local administration into central nervous system." Anticancer Res 16(1): 135-140.
Moroni, F., R. Carpenedo, et al. (2003). "Studies on the neuroprotective action of kynurenine mono-oxygenase inhibitors in post-ischemic brain damage." Adv Exp Med Biol 527: 127-136.
Mrass, P. and W. Weninger (2006). "Immune cell migration as a means to control immune privilege: lessons from the CNS and tumors." Immunol Rev 213: 195-212.
Mucke, L. and M. Eddleston (1993). "Astrocytes in infectious and immune-mediated diseases of the central nervous system." FASEB J 7(13): 1226-1232.
Muller, N. and M. J. Schwarz (2007). "The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression." Mol Psychiatry 12(11): 988-1000.
Muller, T., T. Moller, et al. (1992). "Calcium entry through kainate receptors and resulting potassium-channel blockade in Bergmann glial cells." Science 256(5063): 1563-1566.
Muller, U., U. Steinhoff, et al. (1994). "Functional role of type I and type II interferons in antiviral defense." Science 264(5167): 1918-1921.
Munn, D. H., M. Zhou, et al. (1998). "Prevention of allogeneic fetal rejection by tryptophan catabolism." Science 281(5380): 1191-1193.
Myllykangas-Luosujarvi, R. and H. Isomaki (1994). "Alzheimer's disease and rheumatoid arthritis." Br J Rheumatol 33(5): 501-502.
Nagatsu, T., M. Mogi, et al. (2000). "Cytokines in Parkinson's disease." J Neural Transm Suppl(58): 143-151.
Naude, P. J., J. A. den Boer, et al. (2011). "Tumor necrosis factor receptor cross-talk." FEBS J 278(6): 888-898.
Neumann, H., R. Schweigreiter, et al. (2002). "Tumor necrosis factor inhibits neurite outgrowth and branching of hippocampal neurons by a rho-dependent mechanism." J Neurosci 22(3): 854-862.
Neumann, H. and H. Wekerle (1998). "Neuronal control of the immune response in the central nervous system: linking brain immunity to neurodegeneration." J Neuropathol Exp Neurol 57(1): 1-9.
Nishioku, T., J. Matsumoto, et al. (2010). "Tumor necrosis factor-Î± mediates the blood-brain barrier dysfunction induced by activated microglia in mouse brain microvascular endothelial cells." Journal of Pharmacological Sciences 112(2): 251-254.
Nistico, R., D. Mango, et al. (2013). "Inflammation subverts hippocampal synaptic plasticity in experimental multiple sclerosis." PLoS One 8(1): e54666.
Nistico, R., M. Pignatelli, et al. (2012). "Targeting synaptic dysfunction in Alzheimer's disease therapy." Mol Neurobiol 46(3): 572-587.
Njenga, M. K., L. R. Pease, et al. (1997). "Interferon alpha/beta mediates early virus-induced expression of H-2D and H-2K in the central nervous system." Lab Invest 77(1): 71-84.
Norris, J. W. (2004). "Steroids may have a role in stroke therapy." Stroke 35(1): 228-229.
O'Connor, J. C., C. Andre, et al. (2009). "Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin." J Neurosci 29(13): 4200-4209.
O'Connor, J. C., M. A. Lawson, et al. (2009). "Induction of IDO by bacille Calmette-Guerin is responsible for development of murine depressive-like behavior." J Immunol 182(5): 3202-3212.
O'Connor, J. C., M. A. Lawson, et al. (2009). "Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice." Mol Psychiatry 14(5): 511-522.
Okuda, S., N. Nishiyama, et al. (1996). "Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine." Proc Natl Acad Sci U S A 93(22): 12553-12558.

Oosterink, B. J., T. Harkany, et al. (2003). "Post-lesion administration of 5-HT1A receptor agonist 8-OH-DPAT protects cholinergic nucleus basalis neurons against NMDA excitotoxicity." Neuroreport 14(1): 57-60.
Orsini, C. A., J. H. Kim, et al. (2011). "Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction." J Neurosci 31(47): 17269-17277.
Ostroveanu, A., E. A. Van der Zee, et al. (2007). "A-kinase anchoring protein 150 in the mouse brain is concentrated in areas involved in learning and memory." Brain Res 1145: 97-107.
Ostroveanu, A., E. A. van der Zee, et al. (2009). "Exchange protein activated by cyclic AMP 2 (Epac2) plays a specific and time-limited role in memory retrieval." Hippocampus.
Ostroveanu, A., E. A. van der Zee, et al. (2010). "Exchange protein activated by cyclic AMP 2 (Epac2) plays a specific and time-limited role in memory retrieval." Hippocampus 20(9): 1018-1026.
Owens, T., T. Renno, et al. (1994). "Inflammatory cytokines in the brain: does the CNS shape immune responses?" Immunol Today 15(12): 566-571.
Paganelli, R., A. Di Iorio, et al. (2002). "Proinflammatory cytokines in sera of elderly patients with dementia: levels in vascular injury are higher than those of mild-moderate Alzheimer's disease patients." Exp Gerontol 37(2-3): 257-263.
Parola, A. L., H. I. Yamamura, et al. (1993). "Peripheral-type benzodiazepine receptors." Life Sci 52(16): 1329-1342.
Patel, J. R. and G. J. Brewer (2008). "Age-related changes to tumor necrosis factor receptors affect neuron survival in the presence of beta-amyloid." J Neurosci Res 86(10): 2303-2313.
Paul, S., C. Ricour, et al. (2007). "Type I interferon response in the central nervous system." Biochimie 89(6-7): 770-778.
Paun, A. and P. M. Pitha (2007). "The IRF family, revisited." Biochimie 89(6-7): 744-753.
Perl, D. P., C. W. Olanow, et al. (1998). "Alzheimer's disease and Parkinson's disease: distinct entities or extremes of a spectrum of neurodegeneration?" Ann Neurol 44(3 Suppl 1): S19-31.
Perrin, R. J., A. M. Fagan, et al. (2009). "Multimodal techniques for diagnosis and prognosis of Alzheimer's disease." Nature 461(7266): 916-922.
Pfefferkorn, E. R. (1984). "Interferon gamma blocks the growth of Toxoplasma gondii in human fibroblasts by inducing the host cells to degrade tryptophan." Proc Natl Acad Sci U S A 81(3): 908-912.
Pickering, M., D. Cumiskey, et al. (2005). "Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system." Exp Physiol 90(5): 663-670.
Pickut, B. A., R. A. Dierckx, et al. (1999). "Validation of the cerebellum as a reference region for SPECT quantification in patients suffering from dementia of the Alzheimer type." Psychiatry Res 90(2): 103-112.
Pimentel-Muinos, F. X. and B. Seed (1999). "Regulated commitment of TNF receptor signaling: a molecular switch for death or activation." Immunity 11(6): 783-793.
Platanias, L. C. (2005). "Mechanisms of type-I- and type-II-interferon-mediated signalling." Nat Rev Immunol 5(5): 375-386.
Poungvarin, N. (2004). "Steroids have no role in stroke therapy." Stroke 35(1): 229-230.
Pruss, R. M., R. L. Akeson, et al. (1991). "Agonist-activated cobalt uptake identifies divalent cation-permeable kainate receptors on neurons and glial cells." Neuron 7(3): 509-518.
Qin, L., X. Wu, et al. (2007). "Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration." Glia 55(5): 453-462.
Quintana, A., A. Molinero, et al. (2007). "Diverging mechanisms for TNF-alpha receptors in normal mouse brains and in functional recovery after injury: From gene to behavior." J Neurosci Res 85(12): 2668-2685.
Raineki, C., P. J. Holman, et al. (2010). "Functional emergence of the hippocampus in context fear learning in infant rats." Hippocampus 20(9): 1037-1046.
Raison, C. L., R. Dantzer, et al. (2010). "CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression." Mol Psychiatry 15(4): 393-403.
Raison, C. L., R. E. Rutherford, et al. (2013). "A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers." JAMA Psychiatry 70(1): 31-41.
Raivich, G., Z. Q. Liu, et al. (2002). "Cytotoxic potential of proinflammatory cytokines: combined deletion of TNF receptors TNFR1 and TNFR2 prevents motoneuron cell death after facial axotomy in adult mouse." Exp Neurol 178(2): 186-193.
Rao, S. M. (1995). "Neuropsychology of multiple sclerosis." Curr Opin Neurol 8(3): 216-220.

Rao, S. M., G. J. Leo, et al. (1991). "Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction." Neurology 41(5): 685-691.
Richter, A. and M. Hamann (2003). "The kynurenine 3-hydroxylase inhibitor Ro 61-8048 improves dystonia in a genetic model of paroxysmal dyskinesia." Eur J Pharmacol 478(1): 47-52.
Ringheim, G. E. and K. Conant (2004). "Neurodegenerative disease and the neuroimmune axis (Alzheimer's and Parkinson's disease, and viral infections)." J Neuroimmunol 147(1-2): 43-49.
Rios, C. and A. Santamaria (1991). "Quinolinic acid is a potent lipid peroxidant in rat brain homogenates." Neurochem Res 16(10): 1139-1143.
Risch, S. C. and C. B. Nemeroff (1992). "Neurochemical alterations of serotonergic neuronal systems in depression." J Clin Psychiatry 53 Suppl: 3-7.
Rodgers, R. J., B. J. Cao, et al. (1997). "Animal models of anxiety: an ethological perspective." Braz J Med Biol Res 30(3): 289-304.
Rogawski, M. A. and G. L. Wenk (2003). "The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease." CNS Drug Rev 9(3): 275-308.
Rossi, S., T. Lo Giudice, et al. (2012). "Oral fingolimod rescues the functional deficits of synapses in experimental autoimmune encephalomyelitis." Br J Pharmacol 165(4): 861-869.
Rothe, J., W. Lesslauer, et al. (1993). "Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes." Nature 364(6440): 798-802.
Rothwell, N. J. (1999). "Annual review prize lecture cytokines - killers in the brain?" J Physiol 514 (Pt 1): 3-17.
Rovaris, M., B. Viti, et al. (2000). "Brain involvement in systemic immune mediated diseases: magnetic resonance and magnetisation transfer imaging study." J Neurol Neurosurg Psychiatry 68(2): 170-177.
Rozemuller, J. M., F. C. Stam, et al. (1990). "Acute phase proteins are present in amorphous plaques in the cerebral but not in the cerebellar cortex of patients with Alzheimer's disease." Neurosci Lett 119(1): 75-78.
Rozemuller, J. M. and F. L. van Muiswinkel (2000). "Microglia and neurodegeneration." Eur J Clin Invest 30(6): 469-470.
Rudick, R. A. and S. E. Goelz (2011). "Beta-interferon for multiple sclerosis." Exp Cell Res 317(9): 1301-1311.
Ruiz, A., C. Matute, et al. (2010). "Intracellular Ca2+ release through ryanodine receptors contributes to AMPA receptor-mediated mitochondrial dysfunction and ER stress in oligodendrocytes." Cell Death Dis 1: e54.
Russo, C. and R. Polosa (2005). "TNF-alpha as a promising therapeutic target in chronic asthma: a lesson from rheumatoid arthritis." Clin Sci (Lond) 109(2): 135-142.
Russo, S., I. P. Kema, et al. (2009). "Tryptophan as an evolutionarily conserved signal to brain serotonin: molecular evidence and psychiatric implications." World J Biol Psychiatry 10(4): 258-268.
Salazar, A., B. L. Gonzalez-Rivera, et al. (2012). "Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge." Horm Behav 62(3): 202-209.
Sama, D. M., H. Mohmmad Abdul, et al. (2012). "Inhibition of soluble tumor necrosis factor ameliorates synaptic alterations and Ca2+ dysregulation in aged rats." PLoS One 7(5): e38170.
Samadi, P., L. Gregoire, et al. (2005). "Effect of kynurenine 3-hydroxylase inhibition on the dyskinetic and antiparkinsonian responses to levodopa in Parkinsonian monkeys." Mov Disord 20(7): 792-802.
Schall, T. J., M. Lewis, et al. (1990). "Molecular cloning and expression of a receptor for human tumor necrosis factor." Cell 61(2): 361-370.
Scheff, S. W., D. A. Price, et al. (2007). "Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment." Neurology 68(18): 1501-1508.
Scheltens, P. (1999). "Early diagnosis of dementia: neuroimaging." J Neurol 246(1): 16-20.
Schindowski, K., A. Bretteville, et al. (2006). "Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits." Am J Pathol 169(2): 599-616.
Schwarcz, R. and R. Pellicciari (2002). "Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities." J Pharmacol Exp Ther 303(1): 1-10.
Schwarcz, R., W. O. Whetsell, Jr., et al. (1983). "Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain." Science 219(4582): 316-318.
Seidler, R. D., J. A. Bernard, et al. (2010). "Motor control and aging: links to age-related brain structural, functional, and biochemical effects." Neurosci Biobehav Rev 34(5): 721-733.
Selmaj, K. W., M. Farooq, et al. (1990). "Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor." J Immunol 144(1): 129-135.
Shemer, Y. and I. Sarov (1985). "Inhibition of growth of Chlamydia trachomatis by human gamma interferon." Infect Immun 48(2): 592-596.

Shen, H. M. and S. Pervaiz (2006). "TNF receptor superfamily-induced cell death: redox-dependent execution." FASEB J 20(10): 1589-1598.
Shohami, E., M. Novikov, et al. (1994). "Closed head injury triggers early production of TNF alpha and IL-6 by brain tissue." J Cereb Blood Flow Metab 14(4): 615-619.
Shu, H. B., M. Takeuchi, et al. (1996). "The tumor necrosis factor receptor 2 signal transducers TRAF2 and c-IAP1 are components of the tumor necrosis factor receptor 1 signaling complex." Proc Natl Acad Sci U S A 93(24): 13973-13978.
Sicotte, N. L., K. C. Kern, et al. (2008). "Regional hippocampal atrophy in multiple sclerosis." Brain 131(Pt 4): 1134-1141.
Simen, B. B., C. H. Duman, et al. (2006). "TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting." Biol Psychiatry 59(9): 775-785.
Sjogren, M., S. Folkesson, et al. (2004). "Increased intrathecal inflammatory activity in frontotemporal dementia: pathophysiological implications." J Neurol Neurosurg Psychiatry 75(8): 1107-1111.
Smith, C. J., H. C. Emsley, et al. (2004). "Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome." BMC Neurol 4: 2.
Smith, R. S. (1991). "The macrophage theory of depression." Med Hypotheses 35(4): 298-306.
Smolen, J. S., D. Aletaha, et al. (2007). "New therapies for treatment of rheumatoid arthritis." Lancet 370(9602): 1861-1874.
Srinivasan, D., J. H. Yen, et al. (2004). "Cell type-specific interleukin-1beta signaling in the CNS." J Neurosci 24(29): 6482-6488.
Sriram, K. and J. P. O'Callaghan (2007). "Divergent roles for tumor necrosis factor-alpha in the brain." J Neuroimmune Pharmacol 2(2): 140-153.
Sternberg, Z., K. Chadha, et al. (2008). "Quercetin and interferon-beta modulate immune response(s) in peripheral blood mononuclear cells isolated from multiple sclerosis patients." J Neuroimmunol 205(1-2): 142-147.
Stevens, H., C. Van de Wiele, et al. (1998). "Cobalt-57 and technetium-99m-HMPAO-labeled leukocytes for visualization of ischemic infarcts." J Nucl Med 39(3): 495-498.
Streit, W. J. (2006). "Microglial senescence: does the brain's immune system have an expiration date?" Trends Neurosci 29(9): 506-510.
Su, K. P., S. Y. Huang, et al. (2010). "Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-alpha-induced depression by regulating polyunsaturated fatty acids levels." Biol Psychiatry 67(6): 550-557.
Suh, H. S., M. L. Zhao, et al. (2007). "Astrocyte indoleamine 2,3-dioxygenase is induced by the TLR3 ligand poly(I:C): mechanism of induction and role in antiviral response." J Virol 81(18): 9838-9850.
Suzuki, S., S. Tone, et al. (2001). "Expression of indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase in early concepti." Biochem J 355(Pt 2): 425-429.
Suzuki, T., I. Hide, et al. (2004). "Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia." J Neurosci 24(1): 1-7.
Swartz, K. J., M. J. During, et al. (1990). "Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors." J Neurosci 10(9): 2965-2973.
Syed, G. M., S. Eagger, et al. (1992). "Quantification of regional cerebral blood flow (rCBF) using 99Tcm-HMPAO and SPECT: choice of the reference region." Nucl Med Commun 13(11): 811-816.
Sze, G. and R. D. Zimmerman (1988). "The magnetic resonance imaging of infections and inflammatory diseases." Radiol Clin North Am 26(4): 839-859.
Szelenyi, J. and Z. Selmeczy (2002). "Immunomodulatory effect of antidepressants." Curr Opin Pharmacol 2(4): 428-432.
Takikawa, O. (2005). "Biochemical and medical aspects of the indoleamine 2,3-dioxygenase-initiated L-tryptophan metabolism." Biochem Biophys Res Commun 338(1): 12-19.
Takikawa, O., T. Kuroiwa, et al. (1988). "Mechanism of interferon-gamma action. Characterization of indoleamine 2,3-dioxygenase in cultured human cells induced by interferon-gamma and evaluation of the enzyme-mediated tryptophan degradation in its anticellular activity." J Biol Chem 263(4): 2041-2048.
Talbot, P. R., J. J. Lloyd, et al. (1994). "Choice of reference region in the quantification of single-photon emission tomography in primary degenerative dementia." Eur J Nucl Med 21(6): 503-508.
Tancredi, V., G. D'Arcangelo, et al. (1992). "Tumor necrosis factor alters synaptic transmission in rat hippocampal slices." Neurosci Lett 146(2): 176-178.

Tartaglia, L. A., T. M. Ayres, et al. (1993). "A novel domain within the 55 kd TNF receptor signals cell death." Cell 74(5): 845-853.
Tartaglia, L. A. and D. V. Goeddel (1992). "Two TNF receptors." Immunol Today 13(5): 151-153.
Tavares, R. G., C. I. Tasca, et al. (2002). "Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes." Neurochem Int 40(7): 621-627.
Ting, K. K., B. Brew, et al. (2007). "The involvement of astrocytes and kynurenine pathway in Alzheimer's disease." Neurotox Res 12(4): 247-262.
Tobinick, E. (2007). "Perispinal etanercept for treatment of Alzheimer's disease." Curr Alzheimer Res 4(5): 550-552.
Tobinick, E. (2009). "Tumour necrosis factor modulation for treatment of Alzheimer's disease: rationale and current evidence." CNS Drugs 23(9): 713-725.
Tobinick, E. L. and H. Gross (2008). "Rapid improvement in verbal fluency and aphasia following perispinal etanercept in Alzheimer's disease." BMC Neurol 8: 27.
Trenova, A. G., M. G. Manova, et al. (2011). "Clinical and laboratory study of pro-inflammatory and antiinflammatory cytokines in women with multiple sclerosis." Folia Med (Plovdiv) 53(2): 29-35.
Tuglu, C., S. H. Kara, et al. (2003). "Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder." Psychopharmacology (Berl) 170(4): 429-433.
Ulrich-Lai, Y. M. and J. P. Herman (2009). "Neural regulation of endocrine and autonomic stress responses." Nat Rev Neurosci 10(6): 397-409.
Uysal, K. T., S. M. Wiesbrock, et al. (1997). "Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function." Nature 389(6651): 610-614.
Uyttenhove, C., L. Pilotte, et al. (2003). "Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase." Nat Med 9(10): 1269-1274.
Valentine, A. D., C. A. Meyers, et al. (1998). "Mood and cognitive side effects of interferon-alpha therapy." Semin Oncol 25(1 Suppl 1): 39-47.
Vaucher, E., I. Reymond, et al. (2002). "Estrogen effects on object memory and cholinergic receptors in young and old female mice." Neurobiol Aging 23(1): 87-95.
Venneti, S., B. J. Lopresti, et al. (2006). "The peripheral benzodiazepine receptor (Translocator protein 18kDa) in microglia: from pathology to imaging." Prog Neurobiol 80(6): 308-322.
Venneti, S., C. A. Wiley, et al. (2009). "Imaging microglial activation during neuroinflammation and Alzheimer's disease." J Neuroimmune Pharmacol 4(2): 227-243.
Versijpt, J., K. Van Laere, et al. (2003). "Scintigraphic visualization of inflammation in neurodegenerative disorders." Nucl Med Commun 24(2): 209-221.
Vila, N., J. Castillo, et al. (2000). "Proinflammatory cytokines and early neurological worsening in ischemic stroke." Stroke 31(10): 2325-2329.
Villemagne, V. L., K. E. Pike, et al. (2011). "Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease." Ann Neurol 69(1): 181-192.
Vincent, A. M., B. C. Mobley, et al. (2004). "IGF-I prevents glutamate-induced motor neuron programmed cell death." Neurobiol Dis 16(2): 407-416.
Vitkovic, L., J. Bockaert, et al. (2000). ""Inflammatory" cytokines: neuromodulators in normal brain?" J Neurochem 74(2): 457-471.
Vowinckel, E., D. Reutens, et al. (1997). "PK11195 binding to the peripheral benzodiazepine receptor as a marker of microglia activation in multiple sclerosis and experimental autoimmune encephalomyelitis." J Neurosci Res 50(2): 345-353.
Wager-Smith, K. and A. Markou (2011). "Depression: a repair response to stress-induced neuronal microdamage that can grade into a chronic neuroinflammatory condition?" Neurosci. Biobehav. Rev. 35(3): 742-764.
Wajant, H., K. Pfizenmaier, et al. (2003). "Tumor necrosis factor signaling." Cell Death Differ 10(1): 45-65.
Waldemar, G. (1995). "Functional brain imaging with SPECT in normal aging and dementia. Methodological, pathophysiological, and diagnostic aspects." Cerebrovasc Brain Metab Rev 7(2): 89-130.
Walker, A. K., D. P. Budac, et al. (2013). "NMDA Receptor Blockade by Ketamine Abrogates Lipopolysaccharide-Induced Depressive-Like Behavior in C57BL/6J Mice." Neuropsychopharmacology.
Wang, J., V. C. Asensio, et al. (2002). "Cytokines and chemokines as mediators of protection and injury in the central nervous system assessed in transgenic mice." Curr Top Microbiol Immunol 265: 23-48.
Wang, M., N. J. Gamo, et al. (2011). "Neuronal basis of age-related working memory decline." Nature 476(7359): 210-213.

Wang, X. and G. Z. Feuerstein (2004). "The Janus face of inflammation in ischemic brain injury." Acta Neurochir Suppl 89: 49-54.
Wang, X., G. Z. Feuerstein, et al. (2004). "Inhibition of tumor necrosis factor-alpha-converting enzyme by a selective antagonist protects brain from focal ischemic injury in rats." Mol Pharmacol 65(4): 890-896.
Wei, Y., K. Chen, et al. (2008). "Skeletal muscle insulin resistance: role of inflammatory cytokines and reactive oxygen species." Am J Physiol Regul Integr Comp Physiol 294(3): R673-680.
Willenborg, D. O., S. Fordham, et al. (1996). "IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis." J Immunol 157(8): 3223-3227.
Williams, L. R., J. F. Pregenzer, et al. (1992). "Induction of cobalt accumulation by excitatory amino acids within neurons of the hippocampal slice." Brain Res 581(2): 181-189.
Williams, M. T., N. R. Herring, et al. (2007). "Alterations in body temperature, corticosterone, and behavior following the administration of 5-methoxy-diisopropyltryptamine ('foxy') to adult rats: a new drug of abuse." Neuropsychopharmacology 32(6): 1404-1420.
Willuweit, A., G. Sass, et al. (2001). "Chronic inflammation and protection from acute hepatitis in transgenic mice expressing TNF in endothelial cells." J Immunol 167(7): 3944-3952.
Winston, B. W., C. A. Lange-Carter, et al. (1995). "Tumor necrosis factor alpha rapidly activates the mitogen-activated protein kinase (MAPK) cascade in a MAPK kinase kinase-dependent, c-Raf-1-independent fashion in mouse macrophages." Proc Natl Acad Sci U S A 92(5): 1614-1618.
Wu, X., D. Zhu, et al. (2004). "AMPA protects cultured neurons against glutamate excitotoxicity through a phosphatidylinositol 3-kinase-dependent activation in extracellular signal-regulated kinase to upregulate BDNF gene expression." J Neurochem 90(4): 807-818.
Yamada, Y., I. Kirillova, et al. (1997). "Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor." Proc Natl Acad Sci U S A 94(4): 1441-1446.
Yamamoto, S. and O. Hayaishi (1967). "Tryptophan pyrrolase of rabbit intestine. D- and L-tryptophan-cleaving enzyme or enzymes." J Biol Chem 242(22): 5260-5266.
Yamazaki, F., T. Kuroiwa, et al. (1985). "Human indolylamine 2,3-dioxygenase. Its tissue distribution, and characterization of the placental enzyme." Biochem J 230(3): 635-638.
Yang, G. Y., C. Gong, et al. (1999). "Tumor necrosis factor alpha expression produces increased blood-brain barrier permeability following temporary focal cerebral ischemia in mice." Brain Res Mol Brain Res 69(1): 135-143.
Yang, H., G. Shaw, et al. (2002). "ANG II stimulation of neuritogenesis involves protein kinase B in brain neurons." Am J Physiol Regul Integr Comp Physiol 283(1): R107-114.
Yang, Z. Z., O. Tschopp, et al. (2004). "Physiological functions of protein kinase B/Akt." Biochem Soc Trans 32(Pt 2): 350-354.
Yen, J. H. and D. Ganea (2009). "Interferon beta induces mature dendritic cell apoptosis through caspase-11/caspase-3 activation." Blood 114(7): 1344-1354.
Zavala, F., V. Taupin, et al. (1990). "In vivo treatment with benzodiazepines inhibits murine phagocyte oxidative metabolism and production of interleukin 1, tumor necrosis factor and interleukin-6." J Pharmacol Exp Ther 255(2): 442-450.
Zhang, G., J. Li, et al. (2013). "Hypothalamic programming of systemic ageing involving IKK-beta, NF-kappaB and GnRH." Nature 497(7448): 211-216.
Zhang, Y., G. Pilon, et al. (2000). "Cytokines and endotoxin induce cytokine receptors in skeletal muscle." Am J Physiol Endocrinol Metab 279(1): E196-205.
Zheng, W. H., S. Kar, et al. (2000). "Insulin-like growth factor-1 (IGF-1): a neuroprotective trophic factor acting via the Akt kinase pathway." J Neural Transm Suppl(60): 261-272.
Zisterer, D. M. and D. C. Williams (1997). "Peripheral-type benzodiazepine receptors." Gen Pharmacol 29(3): 305-314.

CHAPTER 7

English summary

Growing body of evidence supports the notion that disturbances in both, the peripheral and central immune system contribute to the development of major depression (MD).
Chronic inflammatory diseases, e.g. Crohn's disease, rheumatoid arthritis, chronic obstructive pulmonary disease or immune therapies, like interferon alpha or interleukin-2 treatment in cancer patients are all associated with frequent occurrance of MD. Moreover, neurodegenerative diseases, such as Alzheimer's disease, Multiple sclerosis, which are also considered as inflammatory diseases have been revealed to be accompanied with depression.
In the last few decades, depression research became one of the hottest research topics mainly due to the fact, that major depression is the leading cause of disability, more than 350 million people are affected worldwide. Moreover, lost productivity and rising costs of healthcare of MD patients leads to vast financial damage. Although there are known effective therapies for MD, remarkable proportion of the patients is resistant to any kind of antidepressant therapies, thus, the better understanding of the neurobiology of MD is crucial in the development of novel therapeutic strategies. Therefore, the rationale of the present thesis was to investigate the obvious role of neuroinflammation in depression.
In particular, our research has been based on the macrophage hypothesis, which emphasizes the role of the cytokine signaling in the development of depression.

As mentioned above, multiple inflammatory processes play a detrimental role in the development of depression as well as in neurodegenerative diseases. However, previously the brain was irrefutably believed to be an immune privilege organ, this concept has been disproved by many studies. Continuous migration of immune cells through the blood brain barrier contributes to the homeostasis of the periphery and the brain. Cerebral inflammation is associated with the activation of microglia, the resident immune cells of the central nervous system (CNS). Upon immune activation, microglia become activated and start producing and releasing pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) or interferon γ (IFNγ), subsequently initiating an inflammatory cascade. Under experimental conditions, i*n vitro* or *ex vivo*, monitoring neuroinflammation is widely used. Although *in vivo* visualization of neuroinflammation has developed rapidly in the last years, the refinement, optimalization and synchronization of structural and functional techniques must be further investigated. Visualization of microglia activation is one of the most conventional procedures in the detection of neuroinflammation. In the present thesis, Chapter 2 reviews basic neuroinflammatory mechanisms and particularly, the role of TNFα in neuroprotection and neurodegenerative diseases. Furthermore, general neuroimaging techniques are also described.

Recently, immunmodulatory cytokine-therapies or targeting the specific cytokine-receptors have gained mounting interest in research. For instance, the biological effects of TNFα and its two main receptors, tumor necrosis factor alpha receptor 1 (TNFR1) and tumor necrosis factor alpha receptor 2 (TNFR2) have been extensively investigated in neuropsychiatric disorders. However, some promising results bring us closer to the therapeutic use of receptor agonists or antagonists, due to the complexity of the receptor crosstalk and the important physiological functions of TNFα, further examinations are necessary. Additionally, aging has also been shown to influence the cytokine signaling and receptor function. Therefore, in Chapter 3 we intended to study the physiological role of TNFR1 and TNFR2 on cognition, behavioral and neuromuscular functioning in young and aged mice

using wildtype, TNFR1 and TNFR2 knock out mice. The most important finding of this chapter was, that TNFR2 has a fundamental role in hippocampus dependent learning and memory and muscle functioning.
It has been shown that neuroinflammation and pro-inflammatory cytokines are able to activate an enzyme, indoleamine 2,3-dioxygenase (IDO) that catalyzes the first and rate-limiting step of tryptophan catabolism, along the kynurenine pathway. According to the well-known monoamine hypothesis of depression, the disturbance of the serotonin system is responsible for the depressive symptoms. Nevertheless, this hypothesis is not able to exclusively characterize the neurobiology of depression, rather should be combined with others, like the cytokine-hypothesis and focus on a more complex concept. In this approach, since serotonin is synthetised from tryptophan, and IDO is activated upon immune challenges, the question is given, whether and how IDO is involved in the pathophysiology of depression.
In Chapter 4 we investigated in our experimental animal model, how cerebral inflammation is involved in the expression and activation of IDO and whether the activation of IDO is responsible for the depressive-like behavior. Neuroinflammation was monitored by small animal positron emission tomography using an activated microglia marker and the behavior of the mice was observed. We could demonstrate that inflammation induced depressive-like behavior is dependent on the activation of IDO, as it was proven in a group of mice treated with a competitive IDO-inhibitor. Interestingly, IDO was found to be upregulated in the brain stem of the animals, the most important serotonin producing area of the brain.

As indicated before, interferon therapies are often accompanied by depressive-symptoms. Therefore, the mechanisms behind the effects of interferons are in major focus of research. Interferons are immune modulators and potent activators of IDO, thus, consequently might be responsible for depression. Indeed, research studies have reported positive correlation between the activity of IDO, amount of tryptophan catabolites, and the severity of the depressive symptoms. Fortunately, Prof. Dr. Marco Prinz and Dr. Thomas Blank kindly offered to test a newly developed interferon α/β receptor knock out mouse line and our collaboration aimed to unravel the action of interferon β (IFNβ) in concern with neuropsychiatric dysfunctions. In Chapter 5 we established, that 7 consecutive days of IFNβ injection is sufficient to provoke the depressive-like behavior and cognitive impairment in mice. Moreover, the use of the IFNα/β receptor knock out strain we were able to show that IFNβ acts directly through the IFNα/β receptors resulting in the abovementioned behavioral changes. Furthermore, in accordance with previous studies and the findings of Chapter 4, we found elevated IDO expression in the brain stem in wildtype animals treated with IFNβ, allowing us to hypothesize that IFNβ induced IDO activation is responsible for the development of depressive-like behavior and cognitive impairment.

Overall, the novel findings of this thesis further support the cytokine-hypothesis and provide evidence for the involvement of the kynurenine pathway and the upregulation of IDO in major depression. As further hypothesized and discussed in Chapter 6, the characterization of the signaling mechanisms underlying the regulation and activation of the kynurenine pathway could open a new avenue in the development of novel antidepressant therapies.

CHAPTER 8

Nederlandse samenvatting

Steeds meer bewijs ondersteunt het idee dat verstoringen in zowel het perifere immuunsysteem als neuroimmuunsysteem in de hersenen bijdragen aan de ontwikkeling van depressie, meestal aangeduid als 'major depression' (MD).
Chronische ontstekingen, bijvoorbeeld bij de ziekte van Crohn, reumatoïde arthritis, chronische obstructieve longziekte, maar ook immuuntherapieën zoals interferon alfa of interleukine-2 behandeling van kankerpatiënten, worden frequent geassocieerd met depressieve klachten. Ook treden bij neurodegeneratieve ziekten zoals de ziekte van Alzheimer en multiple sclerose, die ook kunnen worden beschouwd als ontstekingsziekten, vaak depressieve klachten op bij deze patiënten.
In het afgelopen decennium heeft het onderzoek van depressie een opvallende ontwikkeling ondergaan wat vooral het gevolg is van het feit dat MD een van de belangrijkste oorzaken is van gedragsstoornissen waardoor meer dan 350 miljoen mensen wereldwijd zijn getroffen. MD leidt niet alleen tot verlies van arbeidsproductiviteit maar ook tot alsmaar stijgende kosten van gezondheidszorg. Hoewel er effectieve therapieën voor MD beschikbaar zijn, is een opmerkelijk deel van de patiënten resistent tegen elke vorm van antidepressie therapie, en dus is een beter begrip van de neurobiologie van MD van kritsiche betekenis voor de ontwikkeling van nieuwe therapeutische strategieën. Het doel van dit proefschrift was om de rol van ontstekingsmechanismen in de hersenen bij depressie nader te onderzoeken. Dit onderzoek was met name gebaseerd op de zg. macrofaag hypothese, een hypothese die zich richt op de rol van de cytokines als signaalbron bij de ontwikkeling van depressie.

Zoals hierboven vermeld, spelen verschillende ontstekings processen een belangrijke rol in de ontwikkeling van MD en in neurodegeneratieve ziekten in het algemeen. Echter, aanvankelijk werd verondersteld dat de hersenen een orgaan was dat niet toegankelijk was voor het algemene perifere immuunsysteem. Dit concept is echter inmiddels verlaten nu we weten dat ook de hersenen toegankelijk kunnen zijn voor de cellen en boodschappers van het perifere immuunsysteem. Migratie van immuuncellen kan onder bepaalde omstandigheden altijd optreden waarbij deze immuuncellen de bloed-hersenbarrière passeren wat bijdraagt tot de homeostase van de periferie en de hersen. Ontstekingsprocessen in de hersenen leiden tot activering van microglia, die algemeen worden gezien als de intrinsieke immuuncellen van het centrale zenuwstelsel (CZS). Na immuunactivatie en activering van microglia beginnen deze cellen met het produceren en het vrijgeven van pro-inflammatoire boodschapper moleculen: de cytokines zoals tumor necrose factor α (TNFα) en interferon-γ (IFNy), die vervolgens een ontstekingscascade in gang zetten. Onder experimentele omstandigheden, kan zowel *in vitro* als *ex vivo* het neuroinflammatie worden gemonitored. Hoewel de *in vivo* visualisatie van neuroinflammatie zich snel ontwikkeld heeft in de afgelopen jaren, is het van groot belang voor het begrijpen van dit proces dat voor er onderzoek plaatsvindt aan de verfijning, optimalisatie en synchronisatie van structurele en functionele technieken. Visualisatie van microglia activatie is een van de meest gebruikelijke procedures voor de detectie van ontstekings processen in het centrale zenuwstelsel. Hoofdstuk 2 van dit proefschrift, handelt met name over de basale neuroinflammatoire mechanismen en over de rol van het cytokine TNFα in het bijzonder bij neuroprotectie en neurodegeneratieve ziekten. Verder worden algemene neuroimaging technieken beschreven.

De afgelopen jaren zijn immuunmodulerende cytokine therapieën, die zijn gericht op specifieke cytokine receptoren, in toenemde mate onderwerp van onderzoek en klinische toepassing geweest. Zo zijn de biologische effecten van TNFα en de twee receptoren voor dit cytokine, tumor necrosis factor alpha receptor 1 (TNFR1) en tumor necrosis factor alpha receptor 2 (TNFR2) uitgebreid onderzocht in neuropsychiatrische aandoeningen. Sommige veelbelovende resultaten brengen ons dichter bij het therapeutische gebruik van agonisten of antagonisten voor deze receptoren. Door de grote complexiteit van deze receptoren en de manier waarop zij elkaar beinvloeden, en de omvangrijke fysiologische functies van TNFα, is verder onderzoek noodzakelijk. Bovendien wordt signaaloverdracht door cytokines en haar receptorfuncties beinvloedt door het verouderingsproces. Om die reden wilden we in hoofdstuk 3 de fysiologische rol van TNFR1 en TNFR2 bij cognitie, gedrag en neuromusculaire functioneren bij jonge en oude muizen bestuderen met behulp van wildtype, TNFR1 en TNFR2 knock-out muizen. De belangrijkste bevinding van dit onderzoek was dat TNFR2 een fundamentele rol speelt bij leer- en geheugenprocessen die afhankelijk zijn van een intacte.

Het is ook aangetoond dat neuroinflammatie en pro-inflammatoire cytokines een enzyme kunnen activeren, het indoleamine 2,3 dioxygenase (IDO), dat de eerste en snelheidsbepalende stap vormt van het tryptofaan katabolisme, langs de zg. kynurenine route. Volgens de bekende monoamine hypothese van depressie, wordt verstoring van het serotonine systeem verantwoordelijk geacht voor het ontstaan van de depressieve symptomen. Het is echter in toenemende mate duidelijk dat deze monoamine hypothese niet een volledige verklaring kan bieden voor de neurobiologie van depressie, en moeten combinaties met andere factoren zoals weergegeven in de cytokine-hypothese een beter en meer compleet begrip opleveren voor deze complex emotionele stoornis.
Omdat serotonine wordt gesynthetiseerd uit tryptofaan en het eerder genoemde IDO wordt geactiveerd bij activering van het neuroimmuunsysteem, hebben we de vraag gesteld, of en hoe IDO betrokken is bij de pathofysiologie van depressie.
In hoofdstuk 4 hebben we in deze vraag onderzocht in een experimenteel diermodel, en met name hoe herseninflammatie bij de expressie en activering van IDO betrokken is, en of de activering van IDO verantwoordelijk is voor de depressief gedrag. Het ontstekingsproces werd gevolgd door middel van scanning met positron emissie tomografie. Deze techniek maakt het mogelijk om geactiveerde microglia te volgen in het levende organisme en tegelijkertijd het depressieve gedrag van muizen waar te nemen. Wij konden aantonen dat ontsteking leidt tot depressief gedrag, dat afhankelijk is van de activatie van IDO, zoals wordt bewezen in een groep van muizen behandeld met een remmer van het enzyme IDO. Belangwekkend is verder dat IDO is toegenomen in de hersenstam van de dieren, en met name in de belangrijkste serotonine producerende gebieden van de hersen.

Zoals eerder aangegeven, worden interferon therapieën vaak geassocieerd met depressieve-symptomen. Daarom zijn de mechanismen achter de effecten van interferon een belangrijk focus van huidig onderzoek. Interferonen zijn immuun modulatoren en krachtige activatoren van IDO, die derhalve verantwoordelijk voor depressie kunnen zijn. Inderdaad hebben recente studies aangetoond dat er een positieve correlatie bestaat tussen de activiteit van IDO, de hoeveelheid tryptofaan katabolieten en de ernst van de depressieve symptomen. Uit een samenwerking met de duitse onderzoekers Marco Prinz en Thomas Blank zijn een nieuw

ontwikkelde interferon α/β-receptor knock-out muislijn voor onderzoek beschikbaar gekomen voor de ontrafeling. Deze samenwerking is met name gericht op ontrafeling van interferon β (IFNβ) bij in de behandeling van neuropsychiatrische stoornissen. In hoofdstuk 5 hebben we vastgesteld dat 7 IFNβ injectie in opeenvolgende dagen voldoende is om depressief gedrag en cognitieve stoornissen bij muizen te veroorzaken. Door gebruik te maken van de IFN α/β receptor knockout-stam konden we aantonen dat IFNβ rechtstreeks werkt via de IFN a/β receptoren en op die wijze resulteert in voornoemde gedragsveranderingen. In overeenstemming met eerdere studies en de bevindingen van hoofdstuk 4, hebben we verhoogde IDO-expressie in de hersenstam in wildtype dieren behandeld met IFNβ gevonden, zodat we veronderstellen dat IFNβ geïnduceerde IDO activering is verantwoordelijk voor de ontwikkeling van depressief gedrag en cognitieve stoornissen.

Samengenomen ondersteunen de nieuwe bevindingen van dit proefschrift de cytokine-hypothese, en leveren meer bewijs voor de betrokkenheid van de kynurenine route en de upregulatie van IDO bij depressie. Zoals verder werd verondersteld en besproken in hoofdstuk 6, zou de karakterisering van de onderliggende signalerings mechanismen van de regulering en activering van de kynurenine route een nieuwe weg in de ontwikkeling van nieuwe antidepressant-behandelingen kunnen openen.

CHAPTER 9

Magyar nyelvű összefoglaló

Egyre több bizonyíték támasztja alá azt a megfigyelést, miszerint mind perifériális, mind központi immunrendszeri folyamatok is vezethetnek major depresszióhoz (MD).Krónikus gyulladásos megbetegedések, mint a Crohn betegség, a rheumatoid arthritis, a krónikus obstruktív tüdőbetegség vagy immunterápiák, mint pl. rákos betegek esetében az interferon alfa vagy az interleukin-2 kezelés gyakran járnak együtt az MD előfordulásával. Bizonyos neurodegeneratív kórképek pedig, mint az Alzheimer-kór vagy a Szklerózis multiplex, melyek egyfajta gyulladásos betegségeknek tekinthetőek, szintén társulhatnak depresszióval.
Az elmúlt néhány évtizedben, a depressziós megbetegedések kutatása egyre nagyobb méreteket öltött, melyhez az is hozzájárult, hogy a major depresszió a munkaképtelenség egyik vezető oka, mely több, mint 350 millió embert érint világszerte. Ráadásul, a csökkent termelőképesség és a major depresszióban szenvedő betegek egészségügyi ellátásának egyre növekvő költségei súlyos gazdasági problémákat okoznak. Az ismert és esetenként hatékonynak tekinthető antidepresszáns terápiák mellett a betegek számottevő része mégis rezisztens ezekkel a kezelésekkel szemben, ezért a depresszió neurobiológiájának pontosabb ismeretei elengedhetetlenek az új terápiás stratégiák kifejlesztéséhez.
Ezért disszertációm céljául azt tűztük ki, hogy megvizsgáljuk hogy a különböző gyulladásos folyamatok milyen szerepet tölthetnek be a major depresszió kialakulásában. Kutatásaink alapjául a makrofág hipotézis szolgált, mely a citokinek jelentőségét hangsúlyozza a betegség hátterében állható neurobiológiai tényezőként.

Számos gyulladásos folyamat játszik szerepet mind a MD, mind a neurodegenratív betegségek kialakulásában. Jóllehet, korábban az agy immunprivilégiuma megcáfolhatatlannak tűnt, ez a felfogás mára már megdőlt. Az immunsejtek folyamatos vándorlása a vér-agy-gáton keresztül nagyban hozzájárul a perifériás és a központi homeosztázis fenntartásához. A központi idegrendszer rezidens immunsejtjei, a mikrogliák aktiválódása egyenes velejárója az agyi gyulladásoknak. Immunaktiváció hatására az aktivált mikrogliák többek között gyulladásos citokineket, pl. tumor nekrózis faktor alfát (TNFα) és interferon gammát kezdenek el termelni és környezetükbe üríteni, ezáltal egy gyulladásos kaszkádot iniciálva. A neuroinflammáció követése kísérleti körülmények között, *in vitro* és *ex vivo* is igen elterjedt. Habár az *in vivo* technikák is gyors ütemben fejlődtek az elmúlt időben, a strukturális és funkcionális módszerek finomításához, optimalizációjához és szinkronizációjához további vizsgálatok szükségesek. Az idegi gyulladás megfigyelésének egyik konvencionális módszere az aktivált mikrogliák vizualizációja. A jelen tézis második fejezete alapvető neuroinflammációs mechanizmusokkal foglalkozik, illetve részletesen a TNFα szerepével a neuroprotekcióban és neurodegenerációban, valamint neurológiai képalkotó eljárásokat ismertet.

Az immunmoduláló citokin-terápiák és a specifikus citokin-receptorok, mint cél molekulák kutatását egyre nagyobb érdeklődés kíséri. Példaként, a TNFα és a két fő receptora, a tumor nekrózis faktor receptor 1 (TNFR1) és tumor nekrózis faktor receptor 2 (TNFR2) szerepének neuropszichiátriai kutatása egyre elterjedtebb. Jóllehet számos ígéretes eredmény áll rendelkezésünkre a receptor agonisták és antagonisták használatával kapcsolatban, a receptor crosstalk és a TNFα alapvető fiziológiai funkciójának komplexitása miatt további vizsgálatok szükségesek. Ráadásul, az öregedés hatása a citokin signaling illetve a receptor funkciókban sem elhanyagolható. Épp ezért, a harmadik fejezetben kutatásaink célja az volt, hogy megvizsgáljuk a TNFR1 és TNFR2 fiziológiai szerepét a kognitív, viselkedésbeli és

neuromuszkuláris funkciókban fiatal és idős, vad típusú, TNFR1 és TNFR2 knock out egereken. Kísérleteink egyik legfontosabb eredményeként elmondhatjuk, hogy a TNFR2 elengedhetetlen szerepet játszik a hippokampuszhoz kötött tanulási és memóriafolyamatokban, valamint az izomrendszer működésében.

A neuroinflammáció és a gyulladásos citokinek képesek egy enzimet, az indolamin 2,3 dioxigenázt (IDO) aktiválni, mely a triptofán katabolizmus első és egyben sebesség meghatározó lépését szabályozza a kinurenin útvonalon keresztül. A depresszió monoamin hipotézise alapján részben a szerotonin rendszer zavara felelős a depresszív tünetek kialakulásáért. A legújabb ismereteink szerint azonban a depresszió neurobiológiai alapjait nem lehet kizárólagosan ezzel a hipotézissel leírni, sokkal inkább egyesíteni kell más elméletekkel is, mint pl. a citokin-hipotézissel, ezáltal a betegség megközelítéséhez egy összetettebb koncepcióra kell összpontosítani. Ebben a vonatkozásban, mivel a szerotonin szintézisében kulcsfontosságú a triptofán, és az IDO immunaktiváció hatására aktiválódik, a kérdés szinte magától értetődő: részt vesz-e az IDO a depresszió patofiziológiájában, és ha igen, vajon hogyan.

A negyedik fejezetben kísérletes állatmodellünkben azt vizsgáltuk, hogy a központi idegrendszeri gyulladás hogyan járul hozzá az IDO expressziójához és aktivációjához, és vajon az IDO aktivációja felelős-e a depresszív tünetekért. A neuroinflammációt kisállat pozitron emissziós tomográfiával követtük nyomon, egy mikroglia marker segítségével, mely szelektíven jelöli az aktivált mikrogliákat. Az állatok viselkedését viselkedéstesztekkel vizsgáltuk. Kimutattuk, hogy a gyulladás okozta depressziós tünetek az IDO aktivációjához kötöttek, melyet egy kompetitív IDO-gátló használatával bizonyítottunk be. Eredményeink alapján elmondhatjuk továbbá azt is, hogy az IDO a fő szerotonin termelésért felelős agyterületen, az agytörzsben upregulálódik..

A már említett interferon kezeléseknek is gyakori velejárója a depresszió. Ezért az interferonok hatásmechanizmusának vizsgálata kulcsfontosságú. Az interferonok immunmodulátorok és potenciális IDO aktivátorok, így ezzel együtt felelősek lehetnek a depresszió kialakulásáért. Mint ahogy azt több vizsgálat is bebizonyította, pozitív korreláció van az IDO aktivitása és a triptofán katabolitok mennyisége, valamint a depressziós tünetek súlyossága között. Kutatásaink során lehetőségünk nyílt egy interferon α/β receptor knock out egér törzs tesztelésére, melyet Prof. Dr. Marco Prinz és Dr. Thomas Blank laboratóriumában fejlesztettek ki. A kollaboráció célja az interferon β neuropszichiátriai viselkedésre gyakorolt hatásának feltérképezése volt. Az ötödik fejezet vizsgálataival kimutattuk, hogy már egy 7 napos interferon β kezelés is elegendő ahhoz, hogy depressziós tünetekhez és kognitív diszfunkcióhoz vezessen. Interferon α/β receptor knock out egér törzs használatával azt is bebizonyítottuk, hogy az interferon β közvetlenül az interferon α/β receptoron keresztül fejti ki a viselkedésbeli hatásait. Korábbi saját, és a szakirodalmi ismeretekkel azonosan, a vad típusú, interferon β-val kezelt egerekben az IDO expressziója az agytörzsben emelkedett volt, mely szintén arra enged következtetni, hogy az interferon β indukált IDO aktiváció felelős a depresszív tünetekért és kognitív károsodásért.

Összegezve, a jelen tézis eredményei új bizonyítékokkal támasztják alá a citokin-hipotézist, és bebizonyítják a kinurenin-útvonal jelentőségét és az IDO upregulációját major depresszióban. Továbbá, a hatodik fejezetben feltételezzük és részletesen megvitatjuk az IDO regulációjáért és aktivációjáért felelős szignalizációs mechanizmusokat, melyek pontos leírása új utakat nyithat meg az antidepresszáns terápia fejlesztésében.

ACKNOWLEDGEMENTS

Well…These words are closing this thesis and a remarkable part of my life. The completion of my PhD took longer than expected and was full of experiences, emotions, excitements and tensions. When I started my PhD in 2007, I could not have imagined where I would be now. There was a great project given and only four years to accomplish. And now here we are.
During the last years, I met a lot of people who guided me and changed my mind, attitude, carreer and life. I am very grateful for this. Here, I would like to thank all the people for their contributions in the realization of this thesis.

First, I would like to express my deepest gratitude to my promoters, Paul Luiten, Uli Eisel, Hans den Boer and Erik de Vries. I am mostly and in the first place grateful to Paul and Uli. Thank you for giving me the possibility to join your group and for your continuous guidance, support and constructive criticism. Under your supervision I started to develop from a verdant student to a more independent researcher. Hans and Erik, despite your busy schedule, you always had time to help me with your unquestionable expertise and excellent ideas concerning the behavioral and PET experiments, thank you!

Ingrid, you were my mentor. I am very grateful for your patience and supervision, the brainstormings and woman-stuff we could discuss. I remember how shocked and unspeakably sad I was when you accepted a position in Zwolle. Anyway, I have learnt everything about behavioral experiments from you and I am encouraged by your advices and tuition. Thank you!

I would like to thank the members of the reading committee, Prof. dr. R. Oudevoshaar, Prof. dr. R.A.J.O. Dierckx and Prof. dr. B. Olivier, for their interest, time and effort spent reviewing this thesis.

Hálával tartozom Nyakas Csaba professzor úrnak a hosszú, tartalmas diszkussziókért és a rengeteg gyakorlati segítségért!

I am grateful for the help of our secretaries, Joke, Pleunie and Marlies. Special thanks to Henk! Without your help, not just me, but all the bursaal students would have been lost in the deepest jungle of the Dutch bureaucracy. You saved our lives!

I really appreciate the dedicated work of our technicians! Jan, even that sometimes it was not a pleasure to work in the mysterious western blot lab, and everything went wrong and we were all frustrated, I was happy to spend at least half of my PhD in your territory! Also, thanks for reanimating my computer after fatal viruses! Definitely, without your optimism and great solutions this work would not have been done. Wanda, thanks for taking care of the organizations and managements, for the Sinterklaas and BBQ parties, and lab outjes! Bert and Folkert, I am grateful for your guidance in the immuno lab and Bert, for the baby-chats! Jaap, Auke and Linda, I am thankful for your support in the animal facility.

Thanks to all the PhDs being around and having fun together! Special thanks to my roomies in Haren and Zernike!

And the AD/PD dream-team: Ivi, Marcelo and Pieter!
Ivi, I really appreciate your very nice welcome in Groningen and friendship! We both knew the "eastern-country" feeling and of course we spoke the same language from the very beginning so it was not a surprise, that you became one of my best friends in Groningen! The BBQs, the dinners, the Hoge Veluwe, Prague, Bremen, they are all those nice memories which always make me smile! Lotte, thank you for sharing all your how-to-become-a-mom-experiences with me! Ivi and Lotte, I hope our friendship will last forever!
Dear Mr. Dobos, Marcelo, I lack words to let you know my appreciation…I miss you so much, you are always in my thoughts! I am grateful for your friendship, help, crazy mind, excellent ideas, dance-classes, nirvana, salsa, merengue…for everything! Without you my Dutch life would have been so empty and this thesis would have never been accomplished. A huge thank you!!!
Pieter, we shared a lot of ups and downs during our PhD-life! I really enjoyed our scientific brainstormings and also non-scientific chats! Thanks for the great lunch-times, I am sure, Paul will always remember the smell of the "strange" South-African and the "unhealthy" Hungarian dishes in our office! Your delicious, famous South-African pumpkin-cookies are unforgettable!

Federica, my little Italien friend! A very special thank to you for the great moments we spent together, like rolling on each other on Marcelo's dance class!
Girste, it is a great pleasure to get to know you, your infinite optimism and energy always flooded the lab! Thank you and Massi for the dinners, cocktails, BBQs and lots of fun!

My dear master students, Jyothi, Marloes, Tjitske, Kristine, Anuka and Kostas and all the bachelors! I must acknowledge your excellent laboratory skills, special views, and great ideas! Our discussions and your critical comments helped me a lot to complete this thesis. Without your support this project would have been done in ten years, I believe. Thank you so much!

Kristine and Marcelo, my paranimfen…I can not express enough my gratitude to you to take the responsibility!

And last, but not least, without mentioning names, thanks to all the colleagues of the Molecular Neurobiology group, the Department of Animal Physiology, Department of Nuclear Medicine and Molecular Imaging and of course special thanks to BCN and CBN for the workshops, courses and invaluable support!

Eszti és Roland, szem nem marad szárazon, megígértem…Nélkületek ez a tézis borító nélkül, formázatlanul jelent volna meg…El sem tudom mondani, mennyire hálás vagyok a segítségetekért! Köszönök mindent! Tibor, Laci, Melinda, Petra, Juci és Orsi, valamint a molekuláris neurobiológia csoport minden tagja! Hálásan köszönöm a támogatást, együttműködést és segítséget, amivel hozzájárultatok a tézisem megírásához!

És végül a legfontosabb, köszönöm családomnak szeretetüket és támogatásukat!

CURRICULUM VITAE

Nikoletta Dobos was born 15th January 1981 in Debrecen, Hungary. She finished secondary school in 1999 in Kecskemét, where she grew up. In the same year she was admitted to the University of Szeged, Hungary, where she graduated as a biologist in 2005, majoring in microbiology and molecular biology. In her master project, Nikoletta performed the molecular analysis of the mitochondrial DNA of the *Cryptococcus neoformans* species complex. As the outcome of this project, the gene map of the mtDNA of *Cryptococcus neoformans* was created. After her graduation, her previous boss at the Department of Microbiology offered her a junior research associate position.

At the end of that project Nikoletta joined the Research Group of Alzheimer's disease at the Department of Psychiatry at the University Medical Center Szeged in 2006. This group focuses its research activities on the different kinds of dementia. Within the area they put special emphasis on Alzheimer's Disease. She investigated the gene expression and regulation of amyloid β precursor protein in rat brain.

In the meantime, in 2007, she successfully applied for a PhD position about neuroinflammation, depression and neurodegenerative diseases in the group of Prof. U.L.M. Eisel and Prof. P.G.M. Luiten at the Department of Molecular Neurobiology, University of Groningen, The Netherlands. The completion of this project led to the present dissertation titled *'Neuroinflammation in depression'*.

Currently, Nikoletta is working as a junior research fellow in the laboratory of Dr. T. Hajszán, Molecular Neurobiology Group; Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary. The research group investigates the molecular basis of major depression, the synaptogenic effect of antidepressant treatment and the reason of antidepressant resistance.

LIST OF PUBLICATIONS

Ulrich L.M. Eisel, **Nikoletta Dobos**, Rudi Dierckx, Paul Luiten and Jakob Korf. Tumor Necrosis Factor as a Neuroinflammatory Mediator in Alzheimer's Disease and Stroke: Molecular Mechanisms and Neuroinflammatory Imaging. Book chapter in Neuroimmune Biology. The Brain and Host Defense, Volume 9, 2010.

Nikoletta Dobos, Jakob Korf, Paul G.M. Luiten, Ulrich L.M. Eisel. Neuroinflammation in Alzheimer's disease and major depression. Biol Psychiatry. 2010 Mar 15;67(6):503-4.

Nikoletta Dobos, Erik F.J. de Vries, Ido P. Kema, Konstantinos Patas, Marloes Prins, Ingrid M. Nijholt, Rudi A.J.O. Dierckx, Jakob Korf, Johan A. den Boer, Paul G.M. Luiten and Ulrich L.M. Eisel. The role of indoleamine 2,3-dioxygenase in a mouse model of neuroinflammation induced depression. J Alzheimers Dis. 2012;28(4):905-15.

Petrus J. W. Naude, **Nikoletta Dobos**, Dennis van der Meer, Cornelis Mulder, Kim G.D. Pawironadi, Johan A. den Boer, Eddy A. van der Zee, Paul G.M. Luiten, Ulrich L. M. Eisel. Analysis of cognition, motor performance and anxiety in young and aged tumor necrosis factor alpha receptor 1 and 2 deficient mice. Behav Brain Res. 2013 Oct 14;258C:43-51.