**Aus dem Insititut für Immunologie**
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**Des Fachbereichs Medizin der Philipps-Universität Marburg**

# Effects of Macrophage Migration Inhibitory Factors' (MIF) inhibition on chronic neuroinflammation

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***Dedicated to***

*To those that I adore,*

*My mother Johanna*

*My Brother Sam,*

*Thanks for standing by my side, not because you had to*

*But because you decided to.*

# Table of Contents

# Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia. Considering the aging population, AD will be one of the challenging problems of the public health in the near future. Current medication used for AD can only alleviate the symptoms temporarily; therefore it is imperative to identify new therapeutic targets to prevent the progression of the disease. Being one of the early key events in early AD pathology, chronic inflammation precedes the cognitive decline and an increasing interest has been focused on identifying potential targets within inflammatory cascades.

Macrophage Migration Inhibitory Factor (MIF) is a multi-potent pro-inflammatory cytokine, which promotes production of other proinflammtory mediators. MIF is synthesized by several cell types in the brain such as microglia, astrocytes and hippocampal neurons, and is secreted in early asymptomatic stage of the disease, which might contribute to the persistent activation of glial cells and perpetuating neuroinflammatory responses leading to neurodegeneration. In this project we hypothesized that inhibition of MIF can attenuate the inflammatory milieu in the brain and improve the cognitive deficits as a result of chronic cytokine production.

We used Intracerebroventricular Streptozotocin Injections (STZ-ICV) to test our hypothesis.

In *in vitro* experiments, ISO-1 was used to inhibit the MIF molecule in primary astrocyte, neuron and microglia treated with proper concentrations of STZ molecule as stimulus. The cytokine response was documented in protein and mRNA level. The collected *in vitro* data suggests that MIF inhibition can alleviate inflammation by down-regulating production of proinflammtory cytokines (such as IL-6 and IL-12p40).

*In vitro* experiments were followed up by *in vivo* behavioral assessment of cognitive deficits and cytokine production in streptozotocin induced model of neurodegeneration. For this purpose, MIF-KO and wild type mice were intracerebroventricularly injected with streptozotocin (STZ-ICV) or vehicle (Veh-ICV).

We confirmed that by triggering an ongoing and chronic immune response, STZ interferes with learning via disrupting the spatial learning in C57BL/6

mice.

We observed significant relationships between cognitive improvement (in terms of contextual memory) and MIF inhibition in STZ-ICV model for neuroinflammation. The inhibition of MIF (by novel Intraperitoneal (IP) application of ISO-1), tended to improve the spatial learning and memory in the context of clockmaze and fear conditioning in wild type animal.

Similar trend as *in vitro* has been observed in down regulation of cytokines (IL-6 and IL-12p40) as a result of MIF inhibition.

In contrast to wild type STZ-ICV, we observed no significant upregulation in inflammatory mediator or glial cell markers in mRNA levels of MIF-KO animals.

In conclusion, the results from my thesis confirms the role of MIF as an upstream cytokine in regulating secretion of other inflammatory mediators and shows the potential of this molecule as a therapeutic target to attenuate cytokine induced cognitive deficits.

# List of figures

# List of Tables

# Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| Aβ | Beta-amyloid fragments |
| APP | Amyloid precursor protein |
| ANOVA | Analysis of variance |
| APC | Antigen presenting cell |
| bp | Base pair |
| BACE1 | β-site APP cleaving enzyme |
| BBB | Blood brain barrier |
| BSA | Bovine serum albumin |
| CAA | Cerebral amyloid angiopathy |
| cAMP | Cyclic adenosine monophosphate |
| ChAT | Choline acetyltransferase |
| CNS | Central nervous system |
| COX | Cyclo oxygenase |
| DNA | deoxyribonucleic acid |
| DDT | D-dopamo tautomerase |
| DMSO | Dimethysulfoxide |
| EDTA | Ethylene diamine tetra acetic acid |
| ELISA | Enzyme-linked immunosorbant assays |
| ER | Endoplasmic reticulum |
| ERK | Extracellular signal-regulated kinase |
| FAD | Familial Alzheimer's disease |
| FC | Fear Conditioning |
| GAPDH | Glyceraldehyde - 3 - phosphate dehydrogenase |
| GFAP | Glial fibrillary acidic protein |
| H | Histidine |
| HR | Homologous Recombination |
| HFIP | Hexa-Fluor-2-Propanol |
| InDel | insertion/deletion |
| IR | Insulin receptor |
| ICV | Intracerebroventricular |
| IFN | Interferon |
| IL | Interleukin |

| | |
|---|---|
| IP | Intraperitoneal |
| IRS 1 | Insulin Receptor Substrate 1 |
| KO | Knock Out |
| LPS | Lipopolysaccharide |
| LDH | Lactate dehydrogenase |
| mRNA | messenger ribonucleic acid |
| mtDNA | mitochondrial DNA |
| MIF | Macrophage Migration inhibitory factor |
| MAPKAPK | Mitogens activated protein kinase activated protein kinase |
| MHC | Major histocompatibility complex |
| MIP | Macrophage inflammatory protein |
| MKP | MAP kinase phosphatase |
| MyD88 | Myeloid differentiation factor 88 |
| NADH | Nicotinamide adenine dinucleotide hydrogen |
| NF-kB | Nuclear factor-kB |
| NFT | Neurofibrillary tangles |
| NIRegs | neuro-immuno-regulatory proteins |
| NK | Natural Killer |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| NSAID | Non steroidal anti-inflammatory drug |
| NHEJ | Non-homologous end joining |
| PAMPs | Pathogen-associated molecular patterns |
| PFA | Paraformaldehyde |
| PCR | Polymerase chain reaction |
| PD | Parkinson’s disease |
| PS-1 | Presenilin 1 |
| PS-2 | Presenilin 2 |
| PBS | phosphate buffered saline |
| PAM | Protospacer Adjacent Motive |
| PRRs | pattern recognition receptors |
| qPCR | quantitative polymerase chain reaction |
| R | Arginine |
| RNA | ribonucleic acid |
| ROS | reactive oxygen species |

| | |
|---|---|
| SEM | Standard Error of the Mean |
| STZ | Streptozotocin |
| STZ-ICV | Intracerebroventricularly injected Streptozotocin |
| SP | senile plaque |
| sgRNA | Short guided RNA |
| Veh-ICV | Intracerebroventricularly injected Vehicle |

# 1. Introduction

Several neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD), share common features that lead to a loss of function followed by neuronal death within the central nervous system (CNS). Remarkably, researchers have identified familial forms of these neurodegenerative disorders that are strongly linked to rare genetic mutations that, invariably, produce an early onset and faster course of the disease. However, the more common and sporadic forms of neurodegeneration occur as a result of a combination of factors, such as protein misfolding and oxidative stress. It is somewhat surprising that until recently these causative factors have been less studied than the genetic mutations.

Brain inflammation, or neuroinflammation, has emerged as a critical factor to consider when studying the origin of neurodegenerative disorders. In fact, several findings indicate that the activation of glial cells and ongoing neuroinflammation in the CNS may play a central role in many neurodegenerative diseases, such as AD and PD. The exaggerated inflammatory responses to immune stimulation in the brain have been shown to lead to impaired microglial and astrocyte function. It is also believed that disease progression, particularly at the pre-neurodegenerative stages, can result in behavioral deficits, such as cognitive impairments. Furthermore, when patients use anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), they appear to be at lower risk of developing neurodegenerative diseases.

## *1.1. Glia in the central nervous system*

Proper brain function depends on the precise interaction of different types of the cells in the CNS, especially neurons and glia (Kolodziejczyk et al. 2010). Glial cells can alter their shape and function in response to internal and external signals to serve different purposes in the CNS, such as regulating the formation of brain regions during early brain development. These cells also play a key part in the proper functioning of the adult CNS and are crucial for supplying the neurons with the appropriate nutrients and signals (Fields, Stevens-Graham 2002). For instance, myelinated axons in the CNS are en-sheathed by oligodendrocytes, which support the axon and

modulate signal transmission (Russell, 1990). It is clear that neurons are highly dependent on glial cells, which support them by supplying nutrients, by modulating the synaptic communication between neurons, and by providing homeostatic factors that are crucial for the well-being of neurons. Another key aspect is that glial cells are responsible for the clearance of extracellular molecules (Araque, Navarrete 2010). In recent years, it has become apparent that yet another key role for glial cells is the regulation of inflammatory response in the brain (Czeh et al. 2011).

### *1.1.1. Oligodendrocytes:*

Oligodendrocytes are the myelin producing cells within the CNS, which are differentiated directly from progenitor cells. Myelin is produced as a spiral structure from extensions of the plasma membrane (Simons, Nave 2016), and it functions as a physical barrier for protecting the axons of neurons and for increasing the speed of the axonal conductance (Kandel 2000). Nodes of Ranvier are the spacers along a myelinated axon, wherein the axon is exposed to the extracellular environment (Baumann and Pham-Dinh, 2001). Demyelinating diseases such as multiple sclerosis manifest as acute focal demyelination and loss of axons. Other resident cells of the CNS such as astrocytes and microglia express modulatory factors, which affect the course of neurodegenerative diseases by influencing the maturation of the oligodendrocytes.

### *1.1.2. Microglia:*

These cells comprise up to 12% of the cells in the murine CNS (Dri and Gordon, 1990). They are considered the macrophage-like resident cells in the CNS because they control innate immunity regulation, which they attain by monitoring the local brain environment for any sign of pathogen intrusion (Kandel 2000). Interestingly, the developmental origin of microglia is recently been delineated with the use if genetic mapping techniques. It is clear that primitive macrophages from the yolk sac, a group of progenitor cells, migrate from the mesoderm into the neuroectoderm early in development and differentiate into immature microglia (Chan et al. 2007), which then migrate to CNS areas named fountains of microglia and proliferate into mature cells (Mouton et al. 2002). Resting microglia play a key role in supporting the neurons and clearing extracellular debris (van Rossum, Hanisch 2004), as they also constantly sample their local area for danger signals (Raivich 2005).

Also during brain development, microglia are necessary for neural refinement. The overall number of microglia seems to be constant in the brain. Tissue injury leads to their proliferation, but this is normally followed by apoptosis, which controls the total number of these cells (Gehrmann, Banati 1995). The density of microglia differs in distinct brain regions; the highest numbers are found in the hippocampus, basal ganglia, and substantia nigra, while the numbers are low in fiber tracts, the cerebellum, and brain stem, (Block et al. 2007). In addition, the size of microglia varies in relation to different brain regions; in fiber tracts, they are highly branched and occupy a territory with a cross-section of ~200 $\mu m^2$, whereas hippocampal microglia occupy a territory of ~550 $\mu m^2$ and are far less branched (Lawson et al. 1990).The activation of microglia leads to morphological changes, secretion of cytokines, phagocytosis, and presentation of antigens (Garden & Moller, 2006). Importantly, microglia return to their resting state once the problem is resolved. MHCII, CD86, CD68, scavenger receptors, and integrins are used as markers of microglial reactivity. Activation of microglial cells upstream of CNS inflammatory responses is necessary for cytokine production by other glial cells and is also a critical step in inflammation-mediated neurotoxicity (Block et al. 2007). Recent findings show that the older classification of microglia into M1 (pro-inflammatory) vs. M2 (anti-inflammatory) appears too simplistic, because these cells are involved in a wider range of processes, including neurodegeneration and synaptic function. The latter, for example, can be regulated indirectly by microglia that engulf synapses and through the production of chemokine and cytokines modulate neuronal synapses (Parkhurst et al. 2013).

Postmortem studies of brains from AD patients have shown that amyloid and tangle pathologies correlate with the expression of specific microglial markers that control the activation of microglia, particularly neuroimmunoregulatory proteins (NIRegs), which are listed in **Table 1.** NIRegs are a group of proteins expressed in different brain cells that act on microglia through specific interacting receptors (**Table 1**). Notably, each NIReg may represent a potential therapeutic target for controlling the progression of inflammation.

| NIRegs | Interacting receptors |
|---|---|
| CD200 | CD200R |
| CD22 | CD45 |
| CD74 | SIRPα |
| semaphorin | Plexin B1/CD72 |
| fractalkine | Fractalkine receptor |

***Table 1:*** *Neuroimmunoregulatory proteins (NIRegs) acting on microglia*

Microglia are “primed” as a result of aging. The primed cells show a prolonged and amplified inflammatory response to stimulation, which might lead to exaggerated neuroinflammation and potentially long-term cognitive deficit. Priming can occur as a result of normal aging in humans and other mammals, such as mice and rats (Streit, Sparks 1997). Microglial priming also leads to increased oxidative stress (Godbout et al. 2005). Markers that are used generally for activated microglia cannot be used in the context of microglial reactivity with aging. Although increased oxidative stress is thought to play the dominant role in age-related priming, the presence of certain proteins (or DNA) is also considered a causative factor in aging (Sohal, Weindruch 1996). In rodents, aging is accompanied by increased glial activity, increased scavenger receptors (Godbout et al. 2005) as well as increased MHC II expression in microglia (Frank et al. 2006).

### *1.1.3. Astrocytes:*

Astrocytes are the most abundant glial cells in the CNS (80% of all glia). These star shaped cells participate in many functions that ensure the homeostasis of brain neurons, such as providing nutrients and being associated with synapses and axonal terminals, in which they clear the excess of neurotransmitters and potassium ions after the occurrence of action potentials (Kandel 2000). Astrocytes are part of the blood brain barrier (BBB) and play a highly specialized role in innate immunity by cooperating with microglia. It is also increasingly clear that astrocytes are involved in a variety of CNS neurodegenerative disorders (Watkins et al. 2008), such as AD and PD (Sofroniew, Vinters 2010). Depending on their morphology and location, astrocytes can have different functions and can be classified into protoplasmic (type I) and fibrillary (type II) groups (Bond et al. 2006). Importantly, these two classes of astrocytes differ in their electrical properties (**Table. 2**).

| | **Type I** | **Type II** |
|---|---|---|
| *Main location* | gray matter | white matter |
| *Input resistance* | low | high |
| *Potassium currents* | voltage and time-independent | voltage-dependent |
| *uptake* | + | low |
| *Membrane potential* | Extremely negative | negative |
| *Recognition Marker* | GFAP | S100b |
| *Morphology* | Extremely branched | tripartite |

***Table 2:*** *different type of astrocytes*

Protoplasmic (type I) astrocytes constitute the vast majority of glial cells, and show irregular morphology and dense branching. These cells normally populate the grey matter and areas around blood vessels, with their processes surrounding synapses and neuronal cell bodies (Volterra, Meldolesi 2005). Type I astrocytes are immunoreactive for GFAP, a marker that is widely used to label them in brain samples, especially in the hippocampus, cerebellum and cortex. Interestingly, GFAP is upregulated following inflammation (Farina et al. 2007) and during neurodegenerative diseases (Thal et al. 1999). GFAP is also strongly expressed in neurogenic areas of the hippocampus.

Fibrillary (type II) astrocytes express the calcium binding protein S100β, have a simple morphology, and are found in white matter in cooperation with nodes of Ranvier. This population of astrocytes is much less immunoreactive than type I astrocytes. Other markers expressed on the membrane of astrocytes include Glt-1 and GLAST, which are markers for glutamate transporters. Given that Glt-1 and S100β are also expressed in oligodendrocytes, they are not considered exclusive astrocytic markers (Nishiyama et al. 1999).

Additionally, Bergmann glial cells are a separate category of astrocytes, which are present only in the cerebellum where they modulate the function of local neurons (Hirrlinger et al. 2004). Apart from glial cells, perivascular macrophages are located near the vascular endothelia, whereas meningeal macrophages are found at the basement membrane surrounding the blood vessels and in the subarachnoid space (Kennedy, Abkowitz 1997).

These two types of brain-residing macrophages function similarly to peripheral macrophages. These cells express CD45 receptor at a much higher level in comparison to microglia cells.

### *1.2. Cross-talk between astrocytes and microglia*

The communication between astrocytes and microglia plays an important role in the pathology of the inflammatory response. It is also clear that astrocytes and microglia are activated at different stages of the neurodegenerative diseases (Jebelli et al. 2014). In general, the activation of astrocytes is subsequent to local microglial activation, but it then facilitates and amplifies the immune response by microglia (Lokensgard et al. 2016). This leads to the production of different substances, such as growth factors that can promote microglial growth and activation and modulate its cytotoxic activity (Loane, Byrnes 2010). Cytosolic calcium increases as a result of astrocyte activation, and it controls the release of excitatory neurotransmitter and extracellular glutamate (Fellin, Carmignoto 2004).

Astrocytes decrease the production of microglial NO, ROS and tumor-necrosis-factor-α (TNFα), and inhibit the expression of co-stimulatory molecules responsible for the stimulation of dendritic cells (Acevedo et al. 2013). These cells can also suppress the phagocytic activity of microglia through the production of transforming growth factor β (TGF-β), thus hindering the efficient clearance of senile plaques (DeWitt et al. 2008).

### *1.3. Neuroinflammation*

Inflammation is an active process that occurs in response to danger signals, with the purpose of removing or inactivating potentially damaging agents or damaged tissue. The brain is almost isolated from systemic circulation by the BBB, and therefore the inflammatory signals are primarily resolved by brain-residing microglia. These cells are responsible for monitoring the micro-environment to sense injury or pathogen invasion. When such stimuli are detected, microglia become activated and release a cocktail of inflammatory mediators (cytokines, chemokines, etc.), which interact with neighboring astrocytes and neurons (Glass et al. 2010) and indicate the onset of the local inflammatory response (Gonzalez-Scarano, Baltuch 1999).

Indeed, local inflammation is initiated by an interaction between pathogen associated molecular patterns (PAMPs), present in the pathogenic organism, and pattern recognition receptors (PRRs) located on the membrane or intracellularly in microglial cells. NOD-like-receptors, RIG-1-like-receptors, Toll-like-receptors (TLRs), and C-type-lectin-receptors are four PRRs that are involved in these interactions (Takeuchi, Akira 2010). Up to twelve different types of TLRs have been characterized in murine and human cells. These membrane molecules, which are widely expressed on astrocytes and microglia, are rarely expressed on neurons (Konat et al. 2006). Signaling via PRRs can activate downstream signaling pathways, such as IκB and mitogen-activated protein kinase (MAPK), leading to the activation of transcription factors such as nuclear factor κB (NF-κB) and interferon regulatory factors (Smale et al., 2010). Eventually, this leads to the release of pro-inflammatory cytokines such as TNF-α, interleukin-β (IL-1β), and interleukin-6 (IL-6) to enhance the response. The microglia-released mediators can also engage systemic immune cells to facilitate the inflammatory processes (Glass et al. 2010).Since the brain is very sensitive to tissue damage, several counter-regulating mechanisms are used to tightly regulate and terminate the inflammation within the CNS. For instance,up-regulation of anti-inflammatory mediators such as IL-10 counter-regulates the cytokine production from microglia and astrocytes.

### *1.4. Inflammation and neurodegenerative diseases*

An increasing body of evidence indicates that inflammatory processes are involved in the pathophysiology of many neurodegenerative diseases, such as AD, PD, amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS). All of these disorders can be characterized by massive activation of microglia and astrocytes (Glass et al. 2010), leading to prolonged secretion of inflammatory mediators including chemokines, cytokines and complement factors. Activated microglia release pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, and TNF-α (Rivest 2009).

The innate immune response results in local inflammation, which is critical for eliminating the invasive elements but also for causing detrimental tissue damage as a bystander injury (Rivest et al., 2009). When local inflammation is not resolved in a timely fashion, it leads to the activation of distal microglia

because they also react to pro-inflammatory cytokines, and this seems to be a critical event for propagating the inflammation (Rosenberg et al. 2006). In fact, histological studies have shown that early, focal activation of glial cells can lead to a global increase in the expression of IL-1β, IL-6, and up-regulated activity of BACE1 (Heras-Sandoval et al. 2016). Also, the release of pro-inflammatory cytokines lead to the inhibition of hippocampal neurogenesis, as well as neuronal dysfunction or death by a variety of mechanisms, including enhancement of glutamate-induced excitotoxicity (Marambaud et al. 2009). Interestingly, high serum levels of systemic inflammation markers can be used to predict cognitive decline or dementia in subjects with normal cognition (Trollor et al. 2012), indicating that inflammatory processes precede mild cognitive impairment (MCI) in patients (Albert et al. 2011).

### *1.4.1. Cytokines:*

Cytokines are humoral proteins with low molecular weight and various activities depending on their target cells. These immune response-related molecules and their receptors are expressed in all CNS cell types (Allan, Rothwell 2001). The levels of multiple pro- and anti-inflammatory cytokines are elevated in the CNS in response to pathological stimuli. TNF-α and IL-1β are quickly upregulated in cells as the first wave of cytokines, in response to insults (Dinarello et al. 2000). This is followed by expression of the second wave of inflammatory mediators, including IL-6 and chemokines, such as CXC and fractalkine (Geisterfer et al. 1995). Aging can also lead to the progressive increase in expression of certain cytokines such as interleukin (IL)-1 and IL-6 (Godbout, Johnson 2004). In rat models, aging leads to dramatic increase in TNF-α gene expression (Gemma et al. 2002). In AD-affected brains, both complement factors and cytokines are upregulated in damaged neurons (Akiyama et al. 2000). Primarily, this upregulation is supposed to remove the detritus from pathological processes, however over time it causes damages to CNS. This effect can be partially inhibited after administration of non-steroid anti-inflammatory drugs to AD patients (Etminan et al. 2003), indicating the injurious effect of chronic increase of the inflammatory mediators over an extended period of time.

Expression of pro-inflammatory cytokines is inhibited by many feedback mechanisms, many of which include upregulation of anti-inflammatory mediators such as IL-10. However a concomitant increase of these proteins is

missing in neurodegenerative conditions, implying that the balance between pro- and anti-inflammatory members of the innate immunity is disrupted. Interestingly, chronic inflammation leads to ongoing expression of the pro inflammatory cytokines (Caraci et al. 2011), meanwhile it results in decrease of hippocampal neurogenesis and down regulation of anti-inflammatory cytokines (Butters et al. 2008). Of interest in this thesis is the involvement of inflammatory cytokines in chronic inflammation. Specificly, we investigate MIF release as a result of streptozotocin (STZ) stimulation from astrocytes and microglia, and determine the degree by which STZ-induced neuroinflammatory cytokines are released. The rationale behind choosing STZ for *in vitro* experiments will be discussed in details in the next section. To explain it briefly, after ICV injection of this molecule in the hippocampus, numerous inflammatory mediators including cytokines, chemokines and nitric oxide are released from glial cells. STZ induced changes lead to a self-propelled and maintained inflammation in the CNS even 6 weeks after surgery, which is similar to the “hit and run phenomenon” explained by Nath and colleagues in 1999. The cytokine upregulation is followed by behavioral changes including cognitive deficits in the animals, which makes this model proper for the investigation of neuroinflammation driven cognitive deficits and dementia.

The role of some key cytokines in neurodegenerative diseases is described in the following section.

#### *1.4.1.1. IL-1beta:*

IL-1β is one of the cytokines involved in inflammatory response in the brain (Shaftel et al. 2008). This cytokine is constitutively produced by neuronal and glial cells (Veerhuis et al. 1999) and is released in response to activation of innate immunity. This cytokine resembles many neurotoxic characteristics of TNF-α (Yadav, Collman 2009); both molecules play a determining role in inducing neuronal death in chronic inflammation (Brabers, Nottet, 2006). Although the mRNA expression of IL-1β remains unchanged (Sheng et al. 2001, Zhao et al. 2001a) upon stimulation, the inactive precursor form is cleaved (which activity is increased by normal aging) by caspase-1 to generate the active form of the enzyme (Gemma et al. 2005), which binds with high affinity to its receptor, IL-1RI (Dinarello 1998). IL-1RI is highly expressed in certain areas of the brain such as the hippocampus (Friedman 2001).

IL-1β expression in CNS derives mostly from activated microglia (Zhao et al. 2001b), and it regulates the proliferation of cells by activating the NF-κB/Iκκ pathway (Koo, Duman, 2008). It directly affects the survival of newborn neurons by inducing apoptosis via phosphorylation of the stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK) pathway in vitro (Zhang et al. 2013). This mediator increases the differentiation of astrocytes, which can be determined by measuring GFAP marker (Wang et al. 2007), resulting in subsequent production of TNF-α and iNOS (Zhao et al. 2001a, Jana et al. 2005).

#### *1.4.1.2. IL-6:*

IL-6 is a multifunctional cytokine secreted upon stimulation, and is produced by a variety of cells within the CNS, such as astrocytes, microglia and neurons (Erta et al. 2012). The expression of this protein is extremely altered by aging as a result of age-related changes in the normal regulation of the cytokine, thus this protein is referred to as “a cytokine for gerontologist” (Daynes et al. 1993). IL-6 is elevated in association with senile plaques and neurofibrillary tangles in the AD (Wang et al. 2015), and is also elevated in the blood of the patients with cognitive decline, post-operative delirium (Capri M. et al. 2014).

IL-6 is produced in the second wave of cytokines in response to a stimulus, and it induces the synthesis of other inflammatory substances, including acute phase proteins (Gabay C, Kushner 1999). It is a key regulatory for the acute phase of inflammation; at this stage IL-6 can also exert anti-inflammatory properties that counteract the induced pathology by TNF-α, by triggering the production of IL-10 (Tilg et al. 1994). In animal studies IL-6 is shown to both inhibit and promote neurodegeneration depending on the context (Allan et al. 2001). Increased plasma levels of IL-6 are a marker for the cognitive decline in elderly (M.T. Heneka et al. 2014)

#### *1.4.1.3. TNF-α:*

TNF-α is a pro-inflammatory cytokine involved in many neurodegenerative diseases, for instance AD, PD, stroke and multiple sclerosis. This cytokine is a type II transmembrane protein. The active form is 26 kDA homo-trimer, which is processed by TNF-α converting enzyme (TACE/ADAM17) to a 17 kDa active protein. Two different trans-membrane receptors exist for this cytokine: TNF receptor type I (TNFR1), which is

expressed on different cells, and TNF receptor type II (TNFR2) expressed on immune cells in low levels (Dembic et al. 1990, Cabal-Hierro, Lazo 2012). Stimulating TNFR1 leads to activation of apoptotic pathways (Beyaert, R et al. 1995), whereas TNFR2-mediated signaling pathway provokes cell survival and proliferation (Okamoto et al. 2009), it also can activate NF-kβ via PI3K-PKB/Akt signaling pathway (Marchetti et al. 2004). A balance between activation of these two receptors, plays a pivotal role in determining whether TNF-α acts in a neuroprotective or neurodegenerative way. As mentioned before, neuroinflammation has been shown to play a significant role in the progression of neurodegenerative diseases. TNF-α is one of the important mediators released by glial cells in this process (McAlpine, Tansey 2008). High TNF-α levels promote progressive pro-inflammatory processes, which later may contribute to degeneration of neurons in the brain (Melinda E Lull. et al. 2010). Multiple polymorphisms in the TNF-α gene are associated with higher risk of developing AD (Di Bona et al. 2009). This is also confirmed by the observation that higher levels of TNF-α are detected in AD brain tissues, which is thought to have a microglial source (Benzing et al. 1999, Zhao et al. 2003). TNF-α and its receptors contribute to neurodegenerative conditions in murine models as well. It has been shown that TNFR1 signaling exacerbates cognitive decline in a mouse model of traumatic brain injury (TBI-Longhi et al. 2013), whereas activation of TNFR2 attenuates it. Targeting these molecules and the signaling pathways they initiate, can contribute to the development of potential therapeutic strategies.

#### *1.4.1.4. IL-12:*

Members of IL-1.2 family play a critical role in the innate and adaptive arms of the immune system. IL-12 is one of the cytokines increased during early and acute infections (Nilsson et al. 2007), whereas the levels for this cytokine are normally reduced in chronic infections, such as HIV (Marshall et al., 1999). This cytokine controls the secretion of IFNγ by Th1 cells (Trinchieri 2007), and it inhibits Th2-dependant IL-10 production (Marshall et al. 1999). The active form of IL-12 is a heterodimer, composed of two subunits, p35 and p40. It exerts its activity through interacting with IL-12 cell surface receptors and promotes the polarization of the CD4+ T cells to Th1 phenotype (Takuma Kato et al. 2000, Hoshino et al. 2013). IL-12 receptor consists of two subunits, IL-12Rg1 and g2 (Chua et al. 1995, Presky et al. 1996).

#### *1.4.1.5. IL-10:*

Whereas most pro-inflammatory cytokines are accepted as predominantly neurodegenerative, anti-inflammatory mediators are viewed as rather neuro-protective (Ross et al. 2003). Under normal conditions anti-inflammatory cytokines are released in order to regulate the expression of the pro-inflammatory cytokines (Hurley, Tizabi 2013). Being one of the best known anti-inflammatory mediator, IL-10 expression is upregulated in activated microglia (Koscso et al. 2012). This upregulation is thought to inhibit the transcription factor NF-κB, thus resulting in prevention of antigen presentation on immune cells. This is followed by attenuated expression of IL-6. Also, expression of IL-1, TNF-α and chemokines are downregulated by IL-10, which is followed by reduced antigen presenting in brain monocytes (Konsman et al. 2002). IL-10 can inhibit the aggressive Th1 response (Leonard, Myint, 2006). Also, when injected intravenously, IL-10 inhibits the production of TNF-α in the brain (Di Santo et al. 1997). Recombinant IL-10 also improves neurological recovery in hippocampus and cortex (Knoblach, Faden 1998).

## 1.5. Macrophage Migration Inhibitory Factor (MIF)

Although macrophage migration inhibitory factor (MIF) was the first cytokine to be discovered (Bloom and Bennett 1966), it was not well studied until its human cDNA was cloned in 1989 (Weiser et al. 1989). MIF is secreted in response to LPS by various cell types, such as lymphocytes (Bacher et al. 1996), neutrophils (Daryadel et al. 2006), and eosinophils (Rossi et al. 1998). In contrast to most pro-inflammatory cytokines, MIF is stored as a preformed mediator that is packed into vesicles (Nishino et al. 1995). Since the structure of MIF does not include a N-terminal signal sequence, it is not translocated into the endoplasmic reticulum (ER), which would be otherwise necessary for a classical pathway of secretion (Flieger et al. 2003). Secretion occurs with the use of ATP-binding cassette transporters (ABCA1) (Flieger et al. 2003).

The *MIF* gene consists of 2 introns and 3 exons (205bp, 173bp and 183bp, Weiser et al., 1989), and contains several DNA-binding sequences for transcription factors such as AP-1, NF-κB, GATA and cyclic adenosine 3',5'-monophosphate (cAMP)-responsive element binding (CREB) protein (Roger

et al. 2003). There is an 89% sequence homology between human and murine *MIF* gene sequences (Donn et al. 2004). , In humans, it is located on chromosome 22q11.2 (**Fig. 1-1**; Budarf et al. 1997), which encodes for a nonglycosylated 12.5 kDa protein of 115-amino acids. The promoter region of the *MIF* gene contains two functional polymorphisms (see **Fig.1-1. Bottom**).

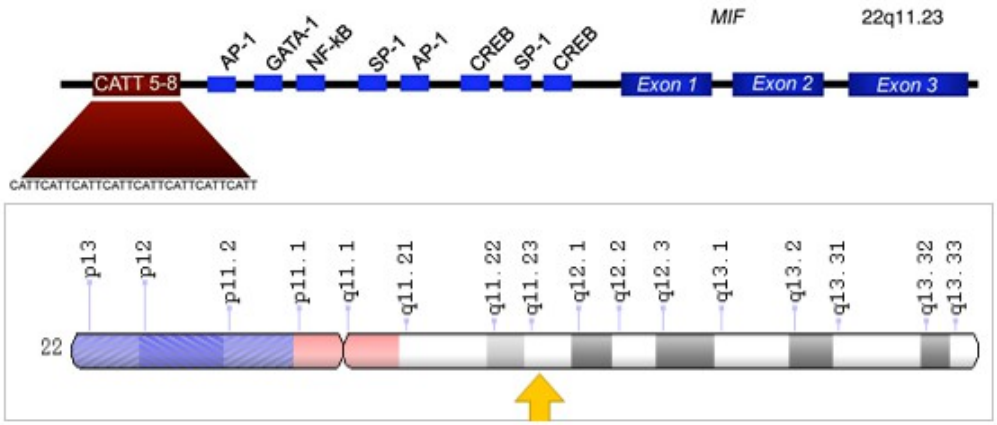


***Figure 1.1.. Structure of the MIF gene:***

***TOP:*** *Schematic representation of the human MIF gene. Several consensus DNA-binding sequences for transcription factors are present at 5' regulatory region followed by exons one to three are shown as blue boxes (respectively 107, 172 and 66 base pairs).*

***Bottom:*** *Cytogenetic location 22q11.23, which is the long (q) arm of chromosome 22 at position 11.23. Molecular location: base pairs 23,894,378 to 23,895,222 on chromosome 22 (Homo sapiens, Annotation Release 107, GRCh38.p2). Credit: Genome Decoration Page/NCBI, Transcription factor ICBP90 regulates the MIF promoter and immune susceptibility locus, Jie Yao, Patty Lee, Richard Bucala Published February 1, 2016*

The MIF protein forms a homo-trimer barrel structure with a channel at its center (Sugimuto et al. 1996). Each monomer consists of six β-strands and 2 anti-parallel α-helices. Both the monomer and trimer forms can exert activity depending upon the concentration of the protein (Mischke et al. 1998). **Figure 1-2** represents the crystal structure of the MIF molecule. MIF exists in two different trimeric states; the ratio in their mixture is modulated by the binding of small molecules to the active site of the MIF.

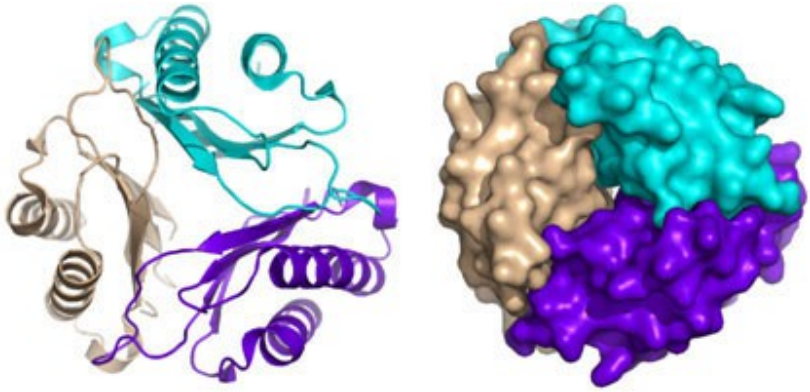

***Figure 1.2.: Crystal structure of MIF protein molecule***

***Left:*** *Ribbon diagram of MIF molecules, composed of three identical subunits (colored in green, purple and brown) each of which contains a four-stranded beta-sheets with two anti parallel alpha helices.*

***Right:*** *the MIF trimmer, the same orientation as in presented in the left picture. Credit: Giardia lamblia. Buchko GW, Abendroth J, Robinson H, Zhang Y, Hewitt SN, Edwards TE, Van Voorhis WC, and Myler PJ. J Struct. Funct. Genomics (2013) 14:47-57.*

D-dopachrome tautomerase (D-DT), referred to as MIF-2, is the other member of the MIF super family, which functions as a homolog to MIF and shares a similar protein structure. The *D-DT* gene, like MIF, is located on chromosome 22 (Merk M. et al. 2012). MIF also shares a similarity in protein structure and functional activity with human D-DT and some prokaryotic enzymes (e.g. the 4- oxalocrotonate tautomerase).

### *1.5.1. Mechanisms of action of MIF:*

The MIF protein can function as an enzyme, cytokine, chemokine and also a hormone.

As an enzyme, MIF possesses two enzymatic activities,by either acting as a tautomerase or as an oxidoreductase. However, the exact physiological role of the enzymatic activity of this molecule is still being elucidated. MIF's enzymatic activities are listed in **Table 3.**

| Enzymatic activity | Dependence | Catalytic activity |
|---|---|---|
| **Tautomerase activity** | Proline-Proline | Catalyzes D-dopachrome or L-dopachrome to its indole derivatives, Mediated by proline residue present at the N terminal |
| **Thiol-Protein oxidoreductase activity** | CALC dependent | Catalyzes reduction of insulin and HED by GSH and DHL |

***Table 3: Enzymatic activities of MIF***

*(based on Rosengren and Bucala, 1996; Rosengren and Bucala 1997; Lubetsky and Swope, 1999)*

As a pro-inflammatory cytokine, MIF serves as a mediator to maintain the inflammatory response. For example, exogenous MIF can up-regulate p-selectin expression, which results in TNF-$\alpha$ induced leukocyte rolling and adhesion (Simons et al. 2011). MIF also functions by activating MAPK, leading to the secretion of other cytokines and chemokines such as IL-6 and TNF$\alpha$ (Lue et al. 2006). In cell cultures, it can directly induce TNF-$\alpha$ secretion from monocytes and macrophages (Dios A et al. 2002). MIF induces the proliferation of T lymphocytes (Bernhagen et al. 1998). It binds directly to tow cysteines in p35 (Cys242 and Cys238); this association leads to inhibition of p35 and consequently suppresses apoptosis. MIF acts as a mediator for hypothalamic-pituitary-adrenal (HPA) axis in rats (Bay Richter C. et al. 2015) as it is released from hypothalamus, pituitary gland and adrenal gland in response to stressful stimuli (Bacher et al. 1998). However, the baseline level of this cytokine (0.1–100 ng/ml) in healthy serum is much higher than other cytokines. MIF levels can rise as a response to hormones such as adrenocorticoid hormone (ACTH) (Nishino et al. 1995).

In human studies, MIF is secreted in a diurnal rhythm, with a plasma concentration of 0.1-100 ng/ml, which can override the hydrocortisone induced-inhibition of NF-κB activation (Daun et al. 2000). Regardless of MIF levels being released in hormone-like manner, it apparently functions either through catalytic activity or as a cytokine. It is also involved in cellular

migration. MIF-induced CCL2 and MAPK activation has been shown in vitro to increase migration of mouse neutrophils and macrophages (Edwards KM. et al. 2011). MIF-KO mice show reduced leukocyte endothelial cell interactions.

Classical chemokines including CXCL8 are directly induced by MIF. Additionally, MIF itself has “chemokine-like function” (CLF) that places it in a similar group as thioredoxin (TRX) and complement factor 5a (C5a) (Bernhagen et al. 2007, Gregory et al. 2006). MIF exerts its chemotactic properties on neutrophils, monocytes and T-cells by binding to cell receptors CD74/CXCR2/CXCR4 (Bernhagen et al. 2007). Other catalytic activities of MIF include functioning as phenylpyruvate keto-enol isomerase (Rosengren et al. 1996), D-DT and a thiol-mediated oxidoreductase. Moreover, MIF possesses a CXXC motif, similar to thiol-mediated oxidoreductase enzymes such as thioredoxin (TRX), which catalyzes the antioxidant activity of these molecules. This can be observed in the plasma of sepsis patients, in which there is a positive correlation between the anti-oxidant TRX and MIF. The amino-terminal proline (Pro-1) residue has been shown to be essential for MIF catalytic isomerase activity (Bendrat et al. 1997).

### *1.5.2. Receptors for MIF:*

CD74 is the cell surface binding protein for MIF, which is a form of the major histocompatibility class II-associated invariant chain, but lacks signal transduction ability on its own. CD74 is paired with its co-receptor, CD44, as a signal transduction molecule (Meyer-Siegler et al. 2004). CD74–CD44 activation leads to the induction of pro-survival signals in B-lymphocytes (Gore et al. 2008) and MAPK/ERK pathways (Lue et al. 2006), and modulation of JNK (Lue et al. 2011). Activation of the CD74–CD44 cascade can result both in pro-inflammatory and anti-apoptotic functions. CD74 can also complex with CXCR2, which leads to increase of adhesion in monocytes and neutrophils by PI3K activation and T-lymphocytes , leading to their recruitment and arrest (Bernhagen et al. 2007).

CD74 can also bind to CXCR4, which is expressed on immune cells including T-lymphocytes, monocytes and structural fibroblasts (Schmidt-Supprian et al. 2000). This leads to the activation of JNK MAPK, via the Src/PI3K/JNK/AP1 pathway, and the induction of CXCL8 production (Lue et al. 2011). At low concentration, MIF signals through CD74, which as mentioned before needs the CD44 for signal transduction, and functions in a pro-inflammatory manner

by controlling the transcription of NF-κB. This modulates adhesion proteins and therefore indirectly affects the adhesion of leucocytes (Lee et al. 2012). At higher concentrations, however, MIF acts via the non-receptor based c-Jun activation domain binding protein-1 (JAB1), which mediates a signaling pathway that leads to a negative effect on inflammation and cell growth (Lolis et al. 2003).

Extracellular MIF binds to CXCR4 and CXCR2 without the presence of CD74 (Bernhagen et al. 2007). CXCR7 is the most recently identified extracellular receptor for MIF (Tarnowski et al. 2010). CD74 has also been shown to make complex with chemokine receptors. CXCR2 and CD74 binding on monocytes, increases the MIF-induced adhesion of monocytes (Bernhagen et al. 2007). MIF/CXCR4 activates AKT signaling in monocytes and fibroblasts. Interestingly, the C-terminal region of MIF is crucial for stabilizing structure of MIF, but it does not play a role in binding to CD74 (Farah El-Turk et al. 2008).

Intracellular MIF (endogenous intracellular MIF and exogenous MIF after endocytosis) interacts with JAB1 (Kleemann et al. 2000). JAB1 can promote cell degradation by binding to p27Kip1, which leads to cell cycle arrest.

### *1.5.3. Role of MIF in the immune response:*

Being discovered as a cytokine secreted by T lymphocytes, MIF is thought to have an important role in innate immunity, by acting as a mediator for induction of other cytokines (Bacher et al. 1996, Bozza et al. 1999). However, its role in adaptive immunity is not completely defined and most reports are from mouse cells or models.

MIF is immediately released from different cell types as a result of exposure to PARPs (Calandra et al. 1998), and acts as an early stage cytokine by initiating the inflammatory response (Bernhagen et al. 1993). As already mentioned, it can regulate the synthesis of many inflammatory mediators (Bacher et al.1996, Mitchell et al., 2002). Inhibition of MIF in murine models results in down-regulation of the cytokine storm that follows LPS-induced toxicity (Bernhagen et al. 1993). MIF-KO mice are also known to be resistant to lethal endotoxic shock (Bozza et al. 1999).

Some of the chemokine like functions of MIF, which are relevant for its role in the immune response, are listed in the **Table 4.**

| Initiating signaling via: | Resulting in |
|---|---|
| Endocytosis | • Induced after glucocorticoids overriding<br>• Interacts with JAB-1 (intracellular receptor)<br>• Leads to down-regulating MAPK signals<br>• modulating cellular redox home- ostasis |
| CD74 receptor | • mediates signaling via CD44 to induce activation of Src-family kinase and MAPK/extracellular signal-regulated kinase (ERK)<br>• leads to PI3K/Akt pathway activation or p53-dependent inhibition of apoptosis |
| G protein-coupled chemokine receptors CXCR2 and CXCR4 | • binds and signals through alone<br>• Complex formation of CXCR2 with CD74, enabling accessory binding, appears to facilitate GPCR activation and formation of a GPCR-RTK–like signaling complex to trigger calcium influx and rapid integrin activation<br>• triggers direct activation of the respective integrin receptors (eg, LFA-1 and VLA-4)<br>• Triggers a calcium influx, which induces a rapid activation of the integrin that can subsequently mediate the Gi- and integrin-dependent arrest and the chemotaxis of monocytes and T cells. |

***Table 4:*** *Chemokine like function (CLF) of the MIF molecule*

### *1.5.4. Association of MIF in different diseases:*

Elevated expression levels of MIF have been shown in several diseases, such as asthma (Rossi et al. 1998), rheumatic arthritis (Leech et al. 1999), atherosclerosis (Burger-Kentischer et al. 2002), and systemic lupus erythematosus (Rovensky et al. 1975). However, the exact role of MIF in these diseases remains to be determined. Many pathogenic events may be

mediated or induced by the release of MIF. Of interest in this thesis is to investigate whether MIF is associated with behavioral decline in a sporadic mouse model of AD.

### *1.5.5. Inhibition of MIF:*

Different compounds have been used to demonstrate the anti-inflammatory effects of MIF inhibition, albeit at differing potencies and acting via different mechanisms. Inhibition can be achieved by neutralizing anti-MIF antibodies, but the efficiency of these antibodies decays over time. Therefore, small molecules inhibitors are generally preferred.

MIF can be inhibited irreversibly by acetaminophen metabolites (Senter et al. 2002), benzisothiazolones (Jorgensen et al. 2011), and 4-iodo-6-phenylpyrimidine (4-IPP) (Winner et al. 2008). In particular, the inhibition of the tautomerase activity of MIF can occur via at least 5 different mechanisms: [1] binding to the active site, [2] allosteric inhibition, [3] covalent modification of active site residues, [4] disruption of the active site through compound-induced dissociation of the active trimer, and [5] stabilization of the MIF monomer and prevention of its re-association to form the active trimer.

Recently, ISO-1 has been introduced as a potent inhibitor for the tautomerase activity of MIF. ISO-1 refers to (S,R)3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (**Fig. 1-3**), and has been called the ‘golden inhibitor’ for MIF because it neutralizes the pro-inflammatory activity of MIF in vivo and in vitro (Lubetsky et al. 2002). ISO-1 treated mice show enhanced survival in the sepsis model of cecal ligation and puncture (Al Abed et al. 2005).

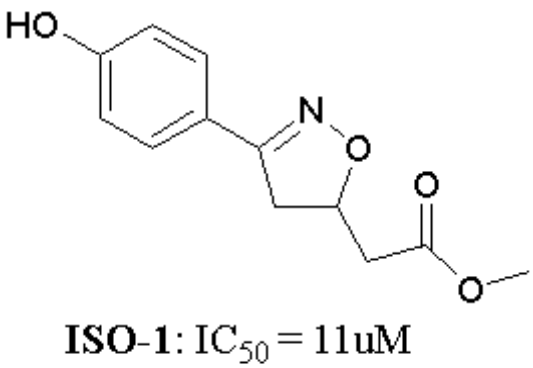


***Figure 1.3.: Molecular structure of ISO-1***

*Cell-permeable, a macrophage migration inhibitory factor (MIF) antagonist: ISO-1 inhibits MIF pro-inflammatory activities by targeting MIF tautomerase activity. Also inhibits tumor necrosis factor (TNFα ) release from macrophages isolated from LPS- treated wild type mice but has no effect on cytokine release from MIF deficient macrophages (source, Kai Fan Cheng)*

## 1.6. Streptozotocin

We used intracerebroventricular Streptozotocin injection (STZ-ICV) as our model to investigate our hypothesis. Briefly, STZ in the brain leads to the development of insulin-resistant brain state, causing cognitive deficit, specifically causing problems with spatial memory, which precedes Aβ pathology and suggests that this model is appropriate for sporadic AD. To stay consistent with the *in vivo* part of the study, STZ was used to stimulate the glial cells during the *in vitro* studies as well. Streptozotocin (deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose (C8H15N3O7)-STZ) is a naturally occurring antibiotic synthesized by *Streptomycetes achromogenes* (**Fig.1-4**), discovered in 1959.

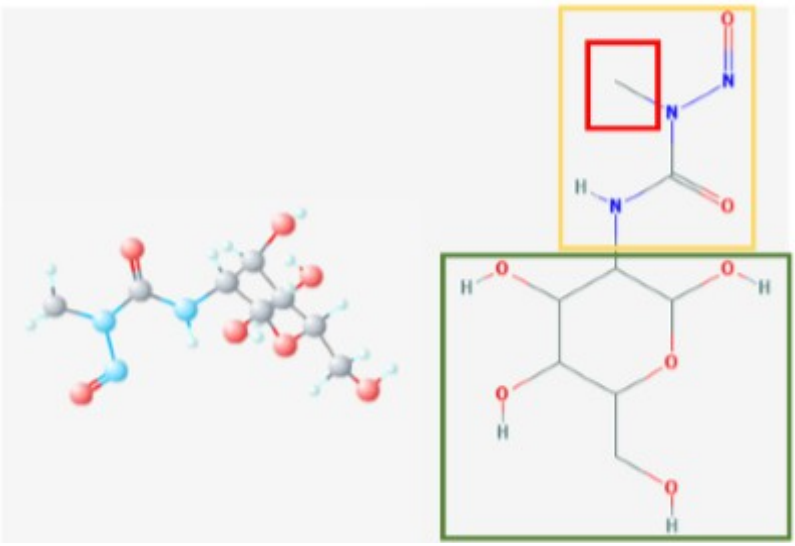

***Figure 1.4.: 2D and 3D structure of STZ molecule.***

*The molecule has got the molecular weight (MW) of 265 g/mol and is composed of a nitrosourea moiety (yellow outline) with a methyl group attached at one end (red) and a glucose molecule at the other (green) Pub-chem: open chemistery Data base.*

This molecule shows selective cytotoxicity to pancreatic β cells, and thus it is used to induce permanent diabetes in murine models. Recent studies suggest that STZ can induce cytotoxicity by producing reactive oxygen species, such as superoxide ($O2^{\bullet-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($HO^{\bullet}$) (Uttara et al. 2009). ISO-1 is highly soluble in water, which results in a slow yellow color, which is a mixture of two different anomers. However, the maximum stability is at pH 4 and thus citrate or acetate buffers are used to prepare the solutions for STZ. Even freshly prepared solutions should be used immediately for different research purposes (Wold et al. 2006). For our experiments, we used Zanosar, which is the drug form of the STZ and the powder can be dissolved directly in water.

### *1.6.1. STZ toxicity mechanisms:*

The following mechanisms has been suggested to explain the induction of diabetes by this molecule:

**Alkylation of cellular components:** After entering the cell, STZ can be metabolized and form an isocyanate molecule and a methyldiazohydroxide molecule (Weiss 1982). The earlier part can affect the intracellular proteins and enzymes via carbamolyation, whereas the CH3+ section of methyldiazohydroxide causes DNA double strand breaks via alkylation that

leads to cell death (Eleazu et al. 2013). This double break may be repaired if NAD-dependent enzyme poly (ADP-ribose) synthetase (PARP) is present (Lenzen 2008). However, in the pancreatic β cells, the cytoplasm is depleted of NAD+, that over time leads to loss of ATP, which ultimately leads to pancreatic cell death (Cnop et al. 2005) and permanent diabetes in murine models (Piepper et al. 1999). PARP deficient mice are shown to be resistance to STZ cytotoxicity (Masutani M et al.1999).

**Nitric oxide (NO) release**: As mentioned before, the STZ molecule is a nitrosureas, which means it can release NO molecule. NO molecule causes DNA damage (Wada, Yagihashi 2004). The NO detected in the cell after STZ stimulation mainly is released from the STZ molecule and not as a result of catalytic processes in the cell, since it is not inhibited following application of NOS inhibitors (Kronche et al. 1995).

**Generation of free radicals leading to oxidative stress:** Free radicals such as superoxide radical ($O2^{\circ-}$), hydroxyl radical ($OH^{\circ-}$) are also produced after STZ injection (Qinna, Badwan 2015). Auto oxidation of the glucose, and glycation of the proteins as a result of the presence of free radicals, is followed by oxidative stress (Matough et al. 2011).

**O-GlcNAcase inhibition:** STZ can produce extreme damage to the islet β-cells by inhibiting O-GlcNAcase (OGA) (Ischiropoulos al-Mehdi 1995). The STZ molecule has a selective cytotoxicity for pancreatic β cells, thus is frequently used to induce diabetes in murine models. However in sub-diabetogenic doses it can induce damages to insulin receptors (IR) followed by insulin resistance. In this case also cognitive deficits has been observed as well as tau and Aβ pathology, suggesting that this molecule is linked to sporadic AD (Watson GS et al. 2003).

## 1.7. Neuroinflammation as a risk factor for cognitive deficit

Despite the efforts to elucidate specific mechanisms underlying neurodegenerative diseases, the ethiopathogenesis is yet not completely understood. It seems that a combination of many central and peripheral factors might play a role in the progression of these conditions. Although neuroinflammation is unlikely to be the triggering factor, it likely contributes to the progression of neurodegenerative diseases (Migliore, Coppede 2002). Studies indicate that chronic activation of glial cells in the CNS, which is followed by constant elevated level of cytokines in many neurodegenerative disorders such as AD (Cartier et al. 2005), occurs in advance to the neuronal-loss stage (Frank-Cannon et al. 2009), however in humans symptoms of cognitive decline will be manifested when already massive neuronal loss took place. Therefore it is necessary to investigate the possible mechanisms that are involved in progression of neuroinflammation. Chronic inflammation is one of the characteristics of neurodegenerative diseases. Inflammatory processes in different sites of the brain such as hippocampus and cortex leads to loss of function or death of the cells in those areas, resulting in significant behavioral deficits. Increase in hippocampal expression of certain cytokines (IL1-β, IL6, TNF-α) is paralleled by cognitive impairments such as spatial memory problems. Different studies demonstrate that almost 40% of elderly (above 65 year) can be diagnosed with some extend of memory impairment (Peters 2006). It has been shown that infusion of D-APV (NMDAR inhibitor) disrupts many cognitive responses, such as the acquisition of contextual fear (Matus-Amat et al. 2007) and spatial memory. *In vivo* experiments in rats showed that ICV injection of IL-1β leads to reduction of TNF expression and impairment of hippocampal-dependent memory (Barrientos et al. 2004). Of interest to this thesis is the regulatory effect of the small cytokine MIF in controlling the expression of the inflammatory mediators and how it could contribute to neurodegeneration and cognitive deficit. In our model, STZ is injected into the hippocampus, which leads to chronic inflammation and neurodegeneration in the area followed by memory deficits. The spatial cognitive impairments is tested in the clockmaze task, and the expression levels of different cytokines are tested in the hippocampus.

### *1.7.1. The hippocampus and spatial memory:*

Memory is the mental representation of “where”, “when” and “what”, of the events that have been experienced in the past. It can be divided in many types and many specific parts of brain function together to make this mental time travel possible. The information of “where” in episodic memory, referred to as “spatial memory”, is provided by the neurons in the hippocampus. Spatial memory, which is defined as the ability to gain and retain information about the position or location of places and objects, declines during many neurodegenerative conditions in the brain. The hippocampus is the major area of the brain associated with spatial memory function (Ryan et al. 2010). This bilateral and symmetrical structure is located in medial temporal lobe of the brain and is a part of the limbic system, and is critical for encoding spatial memory. Hippocampal place cells, first discovered by O’Keefe and Dostrovsky in 1971, are involved in the spatial mapping experience in many animals. Place cells, which are considered memory cells are necessary for encoding the spatial information in mice (Cabral et al. 2014), chimps (Matsumura et al. 1999) and humans (Ekstrom et al. 2003). Plasticity is another mechanism involved in learning and memory. Spatial learning can be disrupted by blocking the N-methyl-D-aspartate receptors (NMDARs) (Caramanos, Shapiro 1994; Steele, Morris 1999), which results in failure of encoding new memories.

### *1.7.2. Anatomy of the hippocampus:*

Hippocampus is located in the medial temporal lobe, and is divided into two “U” shaped parts. The hippocampal formation is made up of five structures (Amaral et al. 1995): [1] hippocampus proper, [2] subiculum, [3] presubiculum, [4] parasubiculum, and [5] entorhinal cortex. The hippocampal formation, that was thought to be a part of olfactory section for a long time, plays an important role in spatial memory and learning. The hippocampus proper can be subdivided into three areas named CA1, CA2, CA3 followed by dentate gyrus (DG).

Numerous afferent and efferent neurons in this structure cooperate to integrate data on environment, emotions and motivations into spatial memory. The main cells are organized into a single and densely packed layer, which makes them an interesting target for electrophysiological studies. Indeed, in 1971, electrophysiological recordings by Doskrovsky et al., showed that specific cells start firing as a rat enters a restricted area from a familiar

environment, which lead to publishing "The Hippocampus as a Cognitive Map" by John O'Keefe and Lynn Nadel in 1978. The structure of hippocampus is well conserved between humans and rodents, and rodents can experience similar deficits in spatial memory, which has made them a highly used model for age-related memory decline.

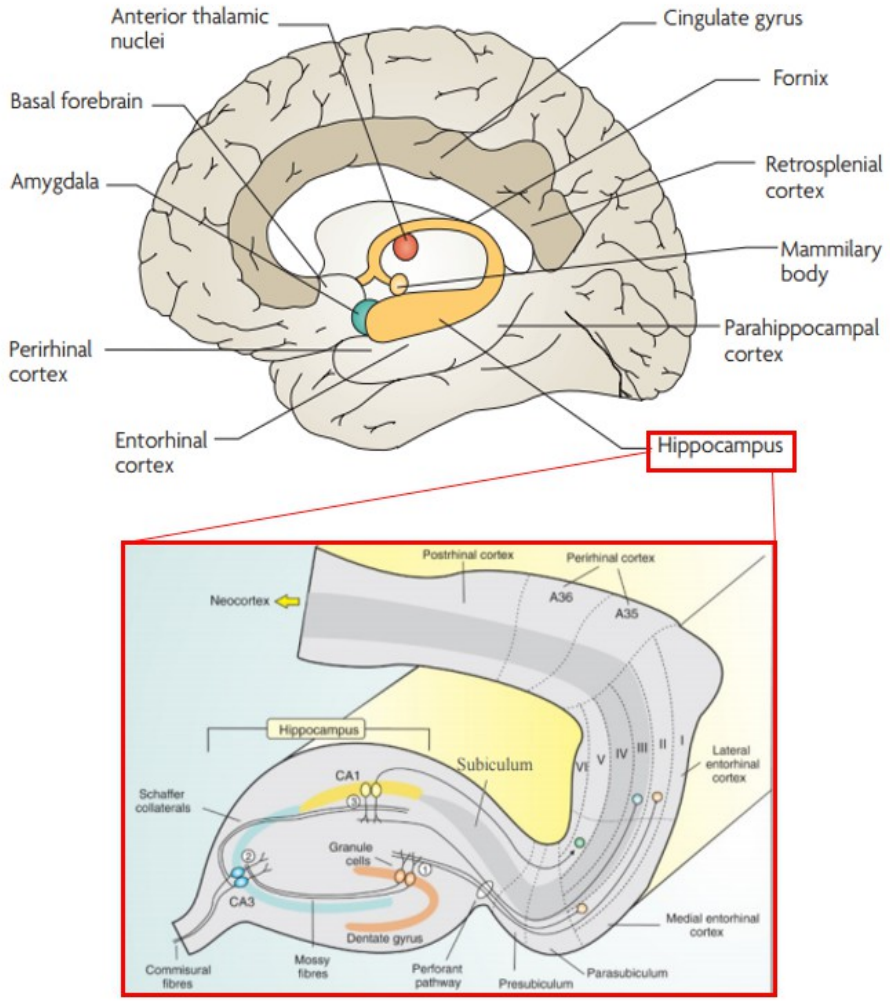


***Figure 1.5.: Structure of hippocampus***

***Top:*** *The hippocampus is placed medial temporal lobes, this part of brain is necessary for forming new memories (Bird and Burgess, 2008).*

***Bottom:*** *The neural circuitry in hippocampus (Berridge et al., 2012, modified).*

### *1.7.3. Fear and context memory*

Fear can be innate and genetically encoded, or it can be acquired through unpleasant experiences, which leads to the activation of autonomic nervous system, associated with higher adrenaline levels and “flight or fight response” (Kozlowska et al. 2015). Fear memories are created as a result of interaction between different neural systems in the brain. The hippocampus and the amygdala are two independent structures placed in temporal lobe that cooperate in fear dependent memory formation. The hippocampus is responsible for acquiring the information about the environmental contexts, which function as cues that allow subjects to recover the information associated with the experiences. These signals are processed and turned into a conditioned response (CR) by amygdala (Phelps et al. 2004). Some experiences carry emotional burden to a level that the subject would evoke the whole emotional experience (Smith, Bulkin et al. 2014).

Contextual fear conditioning is defined as demonstration of fear in a context that has previously provoked fear in the subject (Baas et al. 2004). Context itself is defined as a set of cues surrounding a specific situation.

Classical (Pavlovian) fear conditioning is often used to investigate this emotional experience. The experiment consists of the combination of a non-emotional stimulus (conditioned stimulus: CS, in our case a tone) and aversive stimulus (Unconditioned stimulus, US, we used foot shock). Animals are exposed to this pairing, and over time a conditioned response is formed (CR), where the animal reacts to CS without the presence of US, which indicated the existence of an association between CS and US. In murine models the word “Fear” doesn’t necessarily mean “being afraid”, but refers to changes in behavioral performance such as suppression of appetite and freezing (Blanchard et al. 1989), which can be objectively measured. Reaction happens as a result of re-experiencing the same context (being in the same chamber, Goosens et al. 2003; Wang et al. 2012).

The amygdala plays the central role in this reaction, however undeniable evidence indicates that proper function of the hippocampus is also involved in developing this emotional reaction in response to contextual information (Bouton, Moody 2004; Hobin et al. 2003). The interaction between the amygdala and the hippocampus reinforces the memories of emotional events (Phelps et al. 2004). It seems that stress hormones released by amygdala following a fearful situation, modulate the consolidation of the contextual memory (Cai et al. 2006, McGaugh, Roozendaal 2002). For this reason, the

hippocampus has been the target of several studies investigating the contextual acquisition and extinction of fear conditioning, but also the retrieval of the fearful memories. There is also enough evidence to confirm that the hippocampus is also involved in extinction of fear conditioning (Bouton et al. 2006). It is interesting to note that, the proper CS differs for different species. For examples, in murine models, animals are capable of associating a tone with shock, whereas they fail to associate taste as CS with shock (Tracy et al. 2004). Therefore, fear conditioning is considered a reasonable model to examine the neuronal circuitry involved in the processes that control an animal's response to aversive stimulus combined with a cue. With these experiments we aim to examine the fear conditioning when the hippocampus bears chronic inflammation, hypothesizing that the amygdala is not affected.

### *1.7.4. Fear pathways in the brain*:

The neural circuitry that is critical for acquiring, processing and responding to fear requires the involvement of key areas of the brain including the amygdala, the prefrontal cortex and the hippocampus. Briefly, the hippocampus records the incoming contextual cues, it is the memory center that stores information from different senses along with their emotional weight, and then signals to the amygdala, which is the emotional core of the brain, and has the primary role in triggering the fear response.

The amygdala is a small structure of grey matter, located in the medial temporal lobe. The structure was first described in relation to studies of Klüver-Bucy syndrome (Gaul et al. 2007) and is involved in processing emotions. Weiskrantz's (1956) study demonstrated that this structure plays a pivotal role in avoidance behavior in response to fear in monkeys (Weiskrantz 1956). In other words, the emotional load of an event can be interpreted into fear with the help of the amygdala. It plays a central role in the circuitry for the expression of fear (Ledoux 2015, Rauch et al. 2006). It is also involved in the synaptic connections that are critical for both acquisition and storage of the fear-related memories (Melia et al. 1992; Debiec, Ledoux 2004).

The amygdala plays a role in influencing hippocampal-dependent memory (Bass et al. 2014). The hippocampus is the brain center in which emotion overlaps with memory. The hippocampus is mainly involved in processing episodic memory. It is responsible for generating declarative representations of the emotional events that are stored by amygdala as fear memories

(Phelps, et al., 2004). However, memory after a certain period of time is stored in neocortex and becomes independent of the hippocampus (Young et al., 1994). In the case of contextual memory, the hippocampus plays a role in processes that underlie the acquisition and consolidation of contextual fear. This process most likely involves NMDA receptors (Sanders et al., 2003). Despite all evidence indicating that different hippocampal ensembles seem to be involved in encoding these memories (Smith & Bulkin, 2014), it has been difficult to establish a direct causal link between the neural firing and memory formation up to now. Foot-shock is the golden standard aversive stimulus used for contextual fear conditioning since 1980. A single exposure to this stimulus is followed by immediate freezing reaction in the animals. As mentioned earlier, different areas of the brain, such as the amygdala and the hippocampus, are involved in shock-induced fear conditioning. The hippocampus, the perirhinal cortex and the basolateral and central nucleus of the amygdala (BLA, CeA) seem to play a role in the foot shock reaction (Sacchetti et al. 1999). The neurobiology of the footshock is explained in **Figure 1-6.**

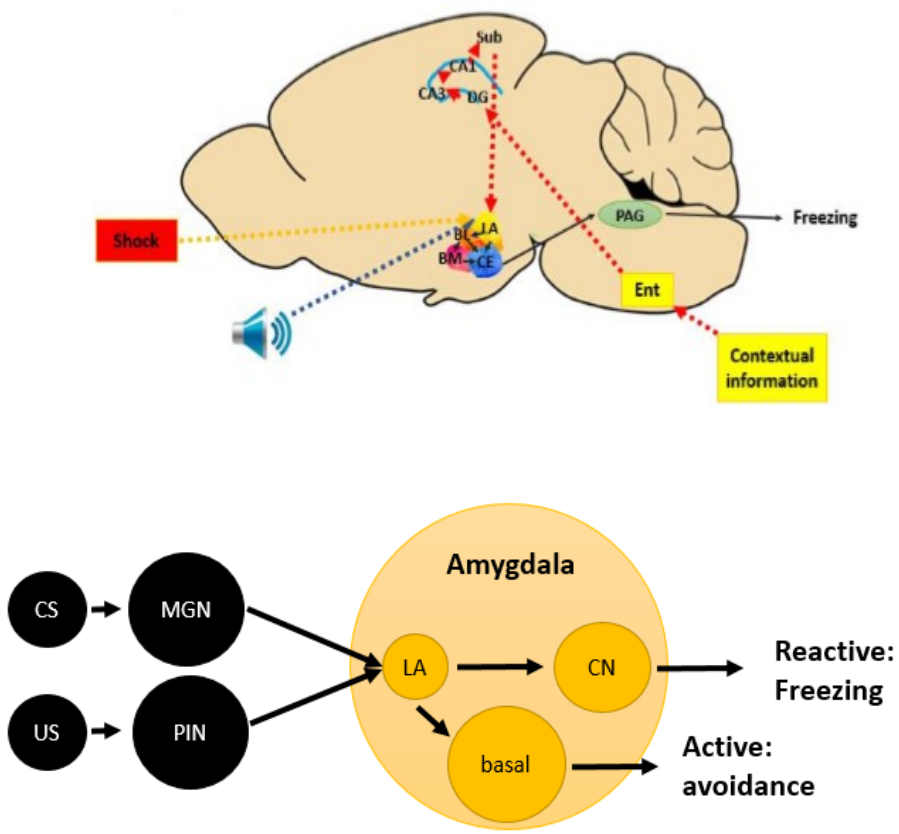


***Figure 1.6.: Fear conditioning pathways***.

***Top:*** *Scheme of the fear conditioning circuit involved in contextual fear conditioning: hippocampus plays the central role in acquisition of the information. Amygdala: LA, Lateral, BL, basolateral, BM, baso-medial, CE, central nucleus. Hippocampus: CA1, CA3 regions, DG, Dentate Gyrus. Ent: Entorhinal cortex. PAG: Periaqueductal Gray*

***Bottom:*** *In this task, the animal learns to pair the tone (CS) with the foot-shock (US), US-CS combinations are paired in the lateral amygdala (LA). To be specific, tone information is delivered to LA by passing medial geniculate body (MGN) and the foot shock data is delivered by posterior intralaminar nucleus (PIN), resulting in neuronal plasticity. These neurons are activated in response to subsequent presentation of the tone, by signaling central nucleus (CN), which mediates the a reactive response (freezing) or an active one (escape or avoidance)*

## 1.8.Systemic factors involved in cognitive impairment:

For the purpose of explaining the role of systemic factors in causing memory deficits, the focus have mainly been on AD, which is the most common form of dementia. Mutations in certain genes may explain the onset of some of neurodegenerative conditions such as the familial form of AD, but factors triggering the late onset AD are still not fully understood. Adults with normal cognition may have markers of neurodegenerative disease but appear to be healthy (Kirk-Sanchez, McGough 2014). This is referred to as Suspected Non-Alzheimer Pathophysiology (SNAP) and is not considered a pre-clinical stage (Graff-Radford et al. 2016). Based on documentation on SNAP cases up to ¼ of cognitively normal adults exhibit neurodegeneration. Markers in PET scans and MRI of hippocampus (Leung et al. 2015). However, certain systemic manifestations are considered to be associated with, or instigate AD and vascular dementia or accelerate their progression. Obesity, diabetes mellitus and hypertension as well as pathogenic mechanism such as inflammation can increase the risk of cognitive deficits. These processes can lead to hippocampal damage or increase in CSF tau, which acts as a risk factor to trigger the neuroinflammation leading to neurodegeneration.

### *1.8.1 Systemic inflammation*

It has been shown that peripheral immune cells can contribute to neuroinflammation. Once in the brain these peripheral cells activate microglia and astrocytes, leading to cytokine production, which can disrupt normal processes in the brain, for instance triggering APP synthesis and Aβ formation in the case of AD. This results in further production of cytokines followed by neurotoxicity. Inflammation associated pathologies happen proximately in the site of activated glial cells (Saijo et al. 2010), but are not limited only to the brain. Measurement of chemokines and cytokines in the serum show elevated levels of certain biomarkers in the periphery especially in cerebrospinal fluid (CSF) (Humpel, Hochstrasser 2011).

The expression of adhesion molecules on BBB is increased in response to neuroinflammation or as a result of aging, also the expression of macrophage inflammatory protein-1α (MIP-1α) is increased in peripheral T cells, which in return leads to over expression of CCR5 on brain endothelial cells. The interaction between these two facilitates brain infiltration for the T cells (Mildner et al. 2007).

#### *1.8.1.1 Effect of cytokines on cognition:*

Cytokines such as IL-6, IL-1β and TNFα are released from microglia and astrocytes at a significantly higher level during inflammation (Zhang, An 2007), which as a part of normal response may play an essential role as a part of behavioral processes, however in chronic form it leads to deficits in different behavioral and performance abilities. It has been reported that the sustained increase in TNF-α is associated with decrease in cognitive performance of AD patients (Holmes et al. 2009). Transgenic mice that overexpress IL-6 exhibit disturbances in cognition (Akiyama et al. 2000), and there is a sustained elevation in IL-6 levels in the nigrostriatal region and cerebrospinal fluid (CSF) of PD patients (Kunze et al. 2008). Chronic cytokine elevation can lead to behavioral deficits such as impairment in cognition, which is observed in preclinical stage of AD (Guerreiro et al. 2007), suggesting the possible correlation between cytokine levels and prevention in proper function of normal cognitive processes to the extent of dire consequences. Based in these observations, many groups have tried to establish a cognition deficit rodent model by inducing and sustaining the cytokine production on CNS. Cognitive impairments have been observed upon intracerebroventricular (ICV) injection of IL-6 into hypothalamus. In 1993, Otzil and his group injected IL-1β intracerebroventricularly (ICV) to induce memory deficit that was studied by Morris Water Maze Task (MWM). All these cytokines, however, cross-induce each other and are expressed in an overlapping spectrum. Therefore, ICV injection of only one cytokine is good for investigating the effects of that specific cytokine, but is inappropriate for inducing neuroinflammation as a neurodegenerative disease model. Alternatively, lipopolysaccharide (LPS) is often used as central inflammatory stimuli to affect dopaminergic neurons, but the effects are transient and do not induce neurodegeneration (Glass et al. 2010). ICV injection of STZ, on the other hand, can induce cognitive deficit through inflammatory mechanisms that affect the cell survival and neuronal properties in the hippocampal area and recapitulate the cognitive deficits that are common in neurodegenerative diseases, such as dementia in AD. STZ-ICV is shown to induce neuroinflammation, mimicking many aspects of sporadic AD, and also exhibiting some behavioral deficits such as impaired learning and spatial memory (Kaushal et al. 2013). STZ ICV in the brain leads to upregulation of GFAP and Iba-1 markers, which label for respectively activated astrocytes and microglia in the brain.

Additional studies are necessary to determine the factors responsible for shifting the inflammatory response from being neuroprotective to neurotoxic.
Several paradigms have been designed to show and evaluate spatial memory capacities and deficits in murine models. In 1982, Morris et al, used Morris Water Maze (MWM) as an experimental paradigm in animals suffering from hippocampal lesion to show their impaired ability in spatial learning The 8 arm radial maze by Olton and Samuelson is another frequently used paradigm to test reference and the working memory. However, for the purpose of testing spatial memory in this thesis, we decided to use the clockmaze paradigm. Clockmaze is similar to MWM for using water as an stimulus for animals to escape the maze by using their spatial navigation capacities as they attend to several visual cues (external to the maze) to solve the paradigm. The clockmaze avoids the hostile condition of deep water, so the element of aversion is not used as motivation. Parameters such as “latency”, “strategy used to escape the maze” and “number of errors” are measured by this experiment.

## 1.9. Effects of STZ-ICV related neuroinflammation on context memory

Impaired glucose metabolism in patients with type 2 diabetes, in mid and late life, can be a risk factor for cognitive decline, as shown by twin-population based studies (Barbara Caracciolo et al. 2013). Recent epidemiological studies suggest that diabetes mellitus is a strong risk factor for memory impairment. Investigations are ongoing to study the interacting mechanisms between these conditions. Serious loss of cognitive ability and spatial disorientation are the common characteristics of many forms of dementia including AD, which is the most common form of dementia.Repetitive intraperitoneal injection of STZ is used to cause permanent diabetes mellitus in murine models. It was recognized after a while that these injections are followed by impaired neuronal plasticity and learning deficits (Stranahan et al. 2008). When administered at low sub-diabetogenic doses, STZ could still cause insulin resistance in the brain (Kamat et al., 2015) by playing a role in decrement of brain glucose/energy metabolism (Shah et al. 2012) and oxidative stress induction (Anwar et al. 2003). It also induced cognitive and brain cholinergic deficits (Pawel Grieb, 2015). Intracerebroventricular (ICV) application of the streptozotocin (STZ) is now a widely accepted model of neuroinflammation and neurodegeneration (Chen et

al. 2012). The purpose for the STZ-ICV model in this study is to investigate the effects of chronic inflammation on neurodegeneration, and cognitive deficits, which is critical for putting the novel therapeutic approaches against these problems to the test.

## 2. Specific Aims

The symptomatology of neurodegenerative diseases such as Alzheimer and Parkinson has been extensively characterized, but the search for a cure has not been successful yet. These conditions are classically characterized by protein aggregations (plaques and tangles), which have become targets for the current therapeutic approaches. Sadly, these strategies have failed to slow disease progression. These failures have led researchers to consider other pathological mechanisms leading to neurodegeneration. Among these, chronic inflammation of brain cells has received increasing attention as a strong candidate for novel therapies.

In this thesis we interrogate the role of macrophage migration inhibitory factor (MIF) in neuroinflammation, and the effects of obstructing this molecule as a putative anti-inflammatory strategy. The specific aims include:

1. To investigate *in vitro* whether the inhibition of MIF can halt the secretion of pro-inflammatory cytokines by inflamed astrocytes and microglia. We study murine cells in culture, challenge them with an inflammatory signal, and determine their cytokine profile (using ELISA and Western blots) in control conditions vs. MIF inhibition.

2. To investigate *in vivo* whether the inhibition of MIF can ameliorate the innate immune response, as well as cognitive and behavioral decline, which occur during brain inflammation. We use the mouse model of intra-cerebro-ventricular injection of streptozotocin (ICV-STZ) to induce neuroinflammation. We inhibit MIF with intra-peritoneal application of ISO-1. We measure behavioral performance, pro-inflammatory cytokines (using real time quantitative PCR, qPCR), and the expression level of astrocytic and glial activation markers, GFAP and Iba1 by immunohistochemistry.

3. To investigate whether MIF-KO mice, with complete MIF deficiency, represent a reasonable model to complement the studies of brain inflammation and MIF. We study, *in vivo* and *in vitro*, the responses of MIF-KO mice to the STZ inflammatory agent.

# 3. Materials

## 3.1. Buffers and solutions

The following buffers and solutions are used in this study

| Buffer | Composition |
|---|---|
| 0.5 M EDTA | 186,1 gr EDTA (with Na)<br>800 ml $H_2O$<br>20 gr NaOH<br>,pH 8, ad 1L with $H_2O$ |
| 50X TAE (electrophoresis buffer for DNA) | Tris Base 242gr<br>HCl 75,1 ml<br>0,5 EDTA (pH 8) 100ml |
| Radioimmunoprecipitation assay buffer (RIPA) Lysis Buffer | 1% NP-40 detergent<br>0,1% SDS<br>50mM Tris-HCl pH 7.4<br>150mM NaCl<br>0,5% Sodium deoxylate<br>1mM EDTA |
| Reaction buffer (cDNA synthesis) | Produced by thermo scientific |
| Denaturing solution (solution D)<br>- trizol protocol | 4 M Guanidinium thiocyanate<br>25 mM Natriumcitrat pH 7,0<br>0,5% (wt/vol) Sarkosyl<br>0,1 M 2-Mercaptoethanol |
| 2M Sodium Acetate pH 4,0<br>- trizol protocol | 16,42g Sodium Acetate (water free) to<br>40 ml DEPC-$H_20$<br>+35 ml Acetic Acid, set pH value on<br>4.0 (using Acetic Acid)<br>Fill up to 100mL using DEPC-$H_20$ |
| Water saturated Phenol<br>- trizol protocol | Solve 100 g of Phenol cristals in aqua dest at 65°C<br>discard the flow |

| | |
|---|---|
| Chloroform:Isoamyl alcohol (49:1)<br>- trizol protocol | 49 ml Chloroform + 1 ml Isoamyl-alcohol vermischen |
| Diethylpyrocarbonate DEPC-$H_2O$<br>- trizol protocol | Add 1 ml DEPC to 1l $H_20$<br>Mix<br>2x Autoclave |
| 75% Ethanol | 75 ml absolute Ethanol + 25 ml DEPC-$H_20$ |
| wash buffer (murine MIF Elisa) | PBS containing 0.05% Tween-20, pH 7.4 |
| reagent diluent (murine MIF Elisa) | 1% BSA in PBS pH 7.4 |
| Substrate buffer (murine MIF Elisa) | 7.3 g citric acid x $H_2O$ ($C_6H_8O_7$)<br>11.87g $NA_2HPO_4$ X $2H_2O$<br>Ad 1L $H_2O$, pH value of 5.0 |
| sterile Tris buffered saline | 20 mM Trizma base<br>150 mM NaCl<br>0.1% bovine serum albumin |
| Gel electrophoresis Stacking gel buffer | 30g Tris HCL<br>2g SDS<br>Ad 500ml water |
| Gel electrophoresis separating gel buffer | 186g Tris<br>4g SDS<br>Ad 1l water, pH 8.8 |
| Western Blot membrane stripping buffer(mild) | 15 g glycine<br>1 g SDS<br>10 ml Tween20<br>Adjust pH to 2.2<br>Bring volume up to 1 L with ultrapure water |

| | |
|---|---|
| Coomassie blue dye solution | 0.1% Coomassie brilliant Blue R-250, 50% methanol and 10% glacial acetic acid |
| FACS buffer | 1x PBS without Ca/Mg 500 ml<br>3% fetal calf serum (FCS) 15 ml<br>0.01% Azide 500 µl |
| 0,1 M citric acid | Dissolve 1.05 g of citric acid monohydrate in 50 ml ddH2O and store at room temperature. |
| 0,1 M sodium citrate | Dissolve 1.47 g of sodium citrate tribasic dehydrate in in 50 ml ddH2O and tore at room temperature. |
| Solution D | 4 M guanidinium thiocyanate<br>25 mM sodium citrate<br>pH 7.0<br>0.5% (wt/vol) N-laurosylsarcosine (Sarkosyl)<br>0.1 M 2-mercaptoethanol<br>*Prepare stock with:*<br>dissolving 250 g guanidinium thiocyanate in 293 ml water at 65 °C<br>add 17.6 ml of 0.75 M sodium citrate, pH 7.0<br>26.4 ml of 10% (wt/vol) N-laurosylsarcosine<br>(stored < 3 months at room temperature)<br>Working solution from stock:<br>add 0.36 ml of 98% 2-mercaptoethanol to 50 ml of stock solution (store <1 month at RT) |

| | |
|---|---|
| Citrate Buffer | Preparation: fresh in the morning by combining 20 ml of 0.1 M Sodium citrate with 30 ml of 0.1 M Citrate acid to produce 0.1 M Citrate buffer. Adjust the pH to 4.5 by using 1 N NaOH. Filter-sterilize the Citrate buffer and store it in a sterile conical tube on ice. |

## 3.2. Kits

Following kits have been used for this study

| **Kit** | **Function** | **Source** |
|---|---|---|
| GeneJET Plasmid Maxiprep kit | Isolation of high copy numbers of plasmid DNA | Thermo scientific #K0491, #K492 |
| QIAamp DNA Blood Mini Kit | purification of up to 12 µg genomic, mitochondrial, or viral DNA from blood and related body fluids | QIAgen Cat.No. 51104 |
| GeneJet Plasmid Miniprep kit | | Thermoscientific |
| ***NucleoBond® Xtra Midi EF*** | | *MACHEREY-NAGEL* GmbH & Co |
| *QIAamp®DNAMini* | | *QIAGEN* |
| *GeneJET PCR Purification Kit* | | Thermo Fisher Scientific #k0701 |
| *peqGOLD Gel Extraction Kit* | | PeqLab |
| *LDH Cytotoxicity Assay* | | *Thermo* Scientific |
| *XTT* | | *Life theconologies* |
| QIAamp DNA Blood Mini Kit (50) | For DNA minipreps | *Cat. No. 51104* *Qiagen* |

## 3.3. Primers

Following primers have been used for this study:

| Primer | Sequence |
|---|---|
| murine MIF F | CCATGCCTATGTTCATCGTG |
| murine MIF R | GAACAGCGGTGCAGGTAAGTG |
| murine IL-12a F | ATGATGACCCTGTGCCTTGG |
| murine IL-12a R | CACCCTGTTGATGGTCACGA |
| murine DDT F | ACCGGATCGTTATCCGCTTC |
| murine DDT R | AGTCGCAGACGTTTAGGCAA |
| Mu.IL-12b F | TCGCAGCAAAGCAAGATGTGT |
| Mu.IL-12b R | CGTGAACCGTCCGGAGTAAT |
| mIl6 F | ACAAGTCCGGAGAGGAGACT |
| mIl6 R | GTCTTGGTCCTTAGCCACTCC |
| mTNF F | CTATGGCCCAGACCCTCACA |
| mTNF R | AAGTAGACGTGCCCGGACTC |
| mIl-12aF | GGTGAAGACGGCCAGAGAAA |
| mIl-12aR | AGATGCTACCAAGGCACAGG |
| mIl-10F | CAGTACAGCCGGGAAGACAA |
| mIl-10R | CCTGGGGCATCACTTCTACC |
| m IL-1βF | CAACCAACAAGTGATATTCTCCATG |
| m IL-1βR | GATCCACACTCTCCAGCTGCA |
| mCD74 F | CGAGGCTCCACCTAAAGAGC |
| mCD74 R | CGTGTCCTGGGACGATGAAA |
| mCD44 F | CTGTCATACACTGGTCCGGG |
| mCD44 R | CTTGGCCACCACACAGAGTC |
| mCXCR2 F | TGCTACTAGCCTGCATCAGC |
| mCXCR2 R | AGACAAGGACGACAGCGAAG |
| mIL-27 p28 F | CTCTGCTTCCTCGCTACCAC |
| mIL-27 p28 R | GGGGCAGCTTCTTTTCTTCT |
| mIL-15 F | AGGTCCTCCTGCAAGTCTCT |
| mIL-15 R | TGCTTTGAAGAGCCAGAGGG |
| murine TLR4 F | AGCTTGAATCCCTGCATAGAGGTAG |

| | |
|---|---|
| murine TLR4 R | ATTTTGTCTCCACAGCCACCAG |
| murine CXCR3 F | ACAGCACCTCTCCCTACGAT |
| murine CXCR3 R | AATCTGGGAGGGCAAAGAGC |

**qPCR primers**

| | |
|---|---|
| muBACTIN | CTAAGGCCAACCGTGAAAAG |
| re_muBACTIN | ACCAGAGGCATACAGGGACA |
| mu_IL6 | GCTACCAAACTGGATATAATCAGGA |
| re_mu_IL6 | CCAGGTAGCTATGGTACTCCAGAA |
| mu_IL1b | AGTTGACGGACCCCAAAAG |
| re_mu_IL1b | AGCTGGATGCTCTCATCAGG |
| mu_TNFa | CTGTAGCCCACGTCGTAGC |
| re_mu_TNFa | TTTGAGTCCATGCCGTTG |
| muIFNb | GCACTGGGTGGAATGAGACT |
| re_muIFNb | AGTGGAGAGCAGTTGAGGACA |
| muINFa | TCTGATGCAGCAGGTGGG |
| re_muINFa | AGGGCTCTCCAGACTTCTGCTCTG |
| muIL-12p35 | CACCCTTGCCCTCCTAAACC |
| re_muIL-12p35 | CACCTGGCAGGTCCAGAGA |
| muIL-12p40 | ACAGCACCAGCTTCTTCATCAG |
| re_muIL-12p40 | TCTTCAAAGGCTTCATCTGCAA |
| muIba1 | GGATTTGCAGGGAGGAAAAG |
| re_muIba1 | TGGGATCATCGAGGAATTG |
| muGFAP | TCCTGGAACAGCAAAACAAG |
| re_muGFAP | CAGCCTCAGGTTGGTTTCAT |

## 3.4. Media and cells

The following media are used in this study:

3.4.1. Iscove's Modified Dulbecco's Medium (IMDM): 5% bovine serum, 1% L-glutamine (200 mM), 1% penicillin-streptomycin; source: Neuro-Zone.

3.4.2. Opti-MEM® I Reduced Serum Media: 10% FCS; 1% penicillin-streptomycin; 0.01% mercaptoethanol; source: Life Technologies.

3.4.3. Roswell Park Memorial Institute medium, RPMI 1640: 10% FCS, 1% penicillin-streptomycin, 1% L-glutamine, 1% Amino acid cocktail; source: Life Technologies Cat# 11875-093.

3.4.4. LB-Agar (Lennox): Trypton, 10 g/l; Hefeextrakt, 5 g/l; NaCl, 5 g/l; Agar-agar, 15 g/l; pH 7.0; source: Roth

3.4.5. LB Broth (Lennox): Tryptone, 10 g/l; yeast extract, 5 g/l; NaCl, 5 g/l; pH 7.0 ±0.2; source: Roth.

3.4.6. Hank's Balanced Salt Solution (HBSS): HBSS, calcium, magnesium, no phenol red; source: Life Technologies

3.4.7. Dulbecco's Modified Eagle Medium (DMEM): low glucose, sodium pyruvate, GlutaMAX™, Phenol Red; source: Life Technologies Cat# 10567014

The following cells are used in this study:

3.4.8. N9 microglial (IMDM)

3.4.9. Immortalized macrophage cell line (Opti-MEM® I Reduced Serum Media)

3.4.10. RAW macrophages (RPMI)

3.4.11. DH5 alpha Competent E. coli cells (LB and LB-A)

3.4.12. SH - SY5Y human neuroblastoma (DMEM)

## 3.5. Chemicals

| **Chemical** | **Source** |
|---|---|
| MIF recombinant mouse Protein | Life Technologies Cat# 50066-MNAE-25 |
| Human MIF Biotinylated Antibody | R&D Systems Cat# BAF 289 |
| Anti MIF antibody | ABcam Cat# ab7207 |

| | |
|---|---|
| RiboLock RNase Inhibitor | Thermo Scientific |
| Reverse transcriptase | Thermo Scientific |
| Complete Protease Inhibitor Cocktail Tablets | Roche Cat. No. 11 836 153 001 |
| Anti-mouse TNF-α antibody | eBioscience Cat. No. 51-26731E |
| Biotinylated anti-mouse TNF-α antibody | 51-26732E, BD Bioscience |
| Murine TNF-α | R&D, Nr. 410-MT |
| Streptavidin POD | Roche, Cat-No : 11 089 153 001 |
| Anti-mouse il-6 antibody | R&D, Cat-No: MAB 406 |
| Biotin-rat anti-mouse IL-6 | R&D, Cat-No: BAF 406 |
| Murine IL-6 | R&D, Cat-No: 406-ML |
| Rat anti-human IL-6 | Pharmingen, Cat-No 554543 |
| Biotin rat anti-human IL-6 | Pharmingen, Cat-No: 554546 |
| Human IL-6 | Immuno-tools, Cat-No: 11340064 |
| anti-mouse IL-12p40/p70 | BD#551219 |
| Recomb. mouse IL-12p40 | Biolegend # 573102 |
| Biotin anti-mouse IL-12p40/p70 | BD#554476 |
| β-amyloid (Aβ1-42) | Invitrogen, Carlsbad, CA |
| Anti CD11b antibody [M1/70] | abcam CAT. No. # ab8878 |
| Anti-ACSA-2 antibody | MACS miltenyi biotec order No. 130-102-365 |
| Bovine Serum Albumin (diagnostic grade) | Millipore Cat# 82-045-1 |
| Guanidium thiocyanat | Roth |
| Natriumcitrat | Roth |
| Sarkosyl | Sigma |
| 2-Mercaptoethanol | |

| | |
|---|---|
| Natriumacetat(water free) | Roth |
| Acetic acid | Roth |
| Phenol (nucleic acid grade) | Roth |
| Chloroform | |
| Isoamyl Alkohol | Roth |
| Isopropanol | Roth |
| Ethanol | Roth |
| DEPC | Roth |
| 100 bp DNA ladder | thermo scientific |
| 6x DNA loading 100 bp | thermo scientific |
| SuperSignal West Femto Substrate | thermo scientific Cat# 34095 |
| RapidStep™ ECL Reagent | Calbiochem Cat# 34581 |
| Sodium chloride | ROTH CAS-No. [7647-14-5] |
| SDS Pellets | ROTH |
| IGEPAL® CA-630 | Sigma, CAT# I8896-100ML |
| Sodium deoxycholate | Sigma |
| TRIS PUFFERAN® ≥99,9 %, p.a. | ROTH, CAS-Num [77-86-1] |
| 10X DreamTaq Buffer | Thermo scientific lot# 00125872 |
| Dream taq DNA polymerase | Thermo scientific lot# 00140114 |
| Nuclease Free water | Thermo scientific lot# 00143631 |
| dNTP mix | Thermo scientific lot# R0191 |
| citric acid | Sigma C1909, MW 210.14 |
| sodium citrate | Sigma C0909, MW 294.10 |

## 3.6. Devices

| Product | Source |
|---|---|
| Cell Culture Dish | BD Falcon™ Cat# 353003 |
| 6 well Culture plate | Greiner CELLSTAR® Cat# 65160 |
| Double net ruling Fuchs Rosenthal Counting Chamber | Neubauer Germany |
| Thermo-shaker TS-100 | GmbH |
| automated plate washer | Thermo Scientific |
| stir bar, VWR, 7*2mm, | Kallestad ,Cat. Nr. 58948-976 |
| Speedvac, Vacuum concentrator | |
| magnetic stirrer | |
| Protein LoBind Tubes | Eppendorf order Nr. 0030 108.116 |
| Sonicator | |
| spectrophotometer ND 1000 | Thermo Scientific |
| Strile work bench | HLC BioTech |
| ChemiDoc™ MP System | Biorad |
| $CO_2$ incubator CB210 | Binder, Tuttlingen, Germany |
| Eclipse 80i light microscope | Nikon, Langen, Germany |
| Lab scale | Kern, Balingen-Frommern, Germany |
| Magnetic stirrer | VWR, Darmstadt, Germany |
| Microcerntrifuge 5415D | Eppendorf, Hamburg, Germany |
| pH-Meter MP 220 | Mettler Toledo, Gießen, Germany |
| pH Meter | Accu Jet® pro, Brand |
| PowerPac Universal Power Supply | Bio-Rad Laboratories, Richmond CA, USA |

| | |
|---|---|
| precision balance | Satorius, Göttingen, Germany |
| Tecan M200 Multimode Reader | Tecan, Crailsheim, Germany |
| Speed Vac RC 1010 | UVP, Upland CA, USA |
| 1D-SDS PAGE System | Mini PROTEAN TetraCell Bio-Rad |
| Autoclave | Systec |
| 37°C Incubator | Binder |
| Ice machine | Amersham Biosciences |
| ELISA-Washer | Biotrak II Washer 4 Amersham Biosciences |
| ELISA reader | Multiscan EX 355 Thermo Electron Corporation |
| Heating block | MKR 13 HLC |
| -80°C freezer | Grant Instrumental Ltd |
| -20°C freezer | Sanyo |
| 4°C refrigerator | Sanyo |
| Nanodrop | TS100 Nikon Eclipse |
| Pipettes, in the range 0,1 µL-1000 µL | ND-1000 PEQLab Biotechnologie GmbH |
| Pipettes 5 mL, 10 mL, 25 mL | Eppendorf |
| Pippetor | Greiner Bio-one |
| shaker | Unimax 1010 Heidolph |
| vortex | KS12 Thermo scientific |
| Water bath | Sartorius |
| centrifuges | Biofuge stratos, ThermoScientific – 16DH, |

# 4. Experimental procedures: *In Vitro*

In order to perform the *in vitro* part of the study, astrocytes, microglia and neurons were isolated from either embryonic or 3 to 5 days old mice pups. The cell types were confirmed using FACS analysis. The cell cultures were stimulated with proper concentrations of STZ and different ISO-1 concentrations. Supernatant of the samples were used for ELISA, Western Blot and Nitric oxide assay. The cell lysate was used to extract the mRNA as well as protein to perform ELISA, PCR and Western blot.

## 4.1. Isolation of mouse embryonic Astrocytes and neurons

These cell cultures were prepared to study the properties of individual cell types. Fetuses were isolated from pregnant mouse (late pregnancy), lower abdomen was sprayed with 70% EtOH and cut medially through the skin and muscles with a pair of scissors in order to expose the uterus and embryos. Fetuses were removed and placed in a sterile 100-mm dish containing an excess of cold L 15 medium. Embryos were rinsed and transferred to the second 100 mm dish containing dissecting medium.

The hindbrain (containting astrocytes) and forebrain (or cortex, containing neurons) were separated. Dura matter and blood vessel were removed from the brain and samples were transferred to a 15ml Falcone and centrifuged to remove the media. In order to detach the cells from each other, accutase was added to the samples, followed by incubation at 36°C water-bath for 5 min. samples were filtered using a 40μM strainer to remove the larger cells and DMEM was added to stop the accutase activity followed by cetrifugation and removing of the media.

Proper media were added to each sample (Neuroblastoma and DMEM respectively for neurons and Astrocytes). Number of the cells were determined. Proper amount of cells were transferred to plates and incubated in 37°C for further use.

Alternatively glia cells were isolated from 3 to 5 day old mouse pups using CD11b Microbeads (Microglia) or ACSA-1 Microbead kit (for astrocytes) following company's instructions.

The cells were sub-cultured regularly to refresh nutrients in the media. The cultured were trypsinz d once cells reached the proper confluence and passaged into new dishes containing fresh media and were incubated at 37°C, in 7.4% $CO_2$

## 4.2. FACS staining of microglia and astrocytes

Flueroscence-activated cell sorting (FACS) was performed to confirm that the isolated and sorted cells from mouse pups and fetuses were indeed the types of the cells that we aimed for. Aliquots of samples were prepared containing at least 2 x 105 cells in each tubes. Microglial samples were blocked using Fc block (anti-CD16/CD32) and CD11b-FTIC was used to label these cells.

ACSA-PE was used as the marker to label the astrocytes. Samples were analyzed immediately or went through a fixing protocol for later use.

## 4.3. Activation of cultured cells with STZ

A total amount of $10^6$ cells were added to each well of a 6 well culture plate. (using Fuchs Rosenthal Counting Chamber) and were incubated overnight at 37°C, 7.4% $CO_2$. Proper concentrations of ISO-1 were added to appropriate wells 1 hour prior to stimulation, followed by delayed administration of STZ (after one hour) with the purpose of avoiding interactions between ISO.1 and STZ.

## 4.4. Cell proliferation assay XTT

XTT was used to measure viability of the our different cell types to external factors (ISO-1 and STZ). The experiment was performed in 96 well plates over 3 days.

On the first day, cells were passaged and diluted the to 30,000 cells per well which was incubated overnight at 37°C, 7.4% $CO_2$. On the second day, proper concentraions of staurosporin was added to the cells. Staurosporin is a kinase inhibitor that induces cellular apoptosis. Other wells were treated with different concentrations of ISO-1 or STZ and plates were incubated

overnight. On the third day,the working reagent and XTT activators were prepared and the experiment was performed based on the manufacturs protocol ( cell signaling technology; #9095). The plates were evaluated by defining the absorbency at 490nm (or 450nm), with a reference wavelength of 630-690 nm (to correct for fingerprints, smudges) in order to define the toxic levels for different chemical compounds used for the experiements.

## 4.5. Nitrite measurement (Griess reaction)

This procedure was performed in order to measure the amount of nirite as one of the primary products of the NO breakdown. The samples were com paired to an standard series, which is prepared in phosphate buffered saline (PBS) without Ca or Mg (PBSdef was used as blank). 1% Sulfanilamide Solution (in 5% phosphoric acid) was added to all experimental samples and wells containing the dilution series for the Nitrite Standard reference curve and the plate was incubated for 5 minutes at the room temperature. 50µl of the 0.1% NED (0.1 % N-1-naphtylethylendiamin-dihydrochloride in ddH2O) Solution was added to all wells followed by another 5 minutes of incubation while protected from light. A purple/magenta color was formed and the absorbance of the plate was measured immediately at 550nm .

## 4.6. Cell lysis and protein extraction

In the Radio-immunopercipitation Assay (RIPA) lysis of monolayer-cultured mammalian cells, the RIPA Buffer was used to lyse mammalian cells,enabling extraction of proteins from cytoplasm, membrane and nucleus, without degrading them. Protease inhibitor was added to buffer before using (Complete Protease Inhibitor Cocktail Tablets). The culture media was removed and the cells were washed gently by adding 1x cold PBS to remove the residual media and dead cells. RIPA buffer was added to the plate followed by 5 min incubation on ice. Cells were scraped and transferred into a 15mL falcon tube and centrifuged at 14000 x g for 15 min. Supernatant was discarded at the pellete was stored at -20°C for further investigations. In the case of Tissue samples, Tissue Protein Extraction Reagent (TPER) was used instead of RIPA to extract the protein. The cells were homogenized in TPER

and then centrifuged to remove the cell debris. Samples were stored at -20°C.

## 4.7. Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to measure the secretion of different cytokines from primary cell cultures of mouse glial or neural cells treated with different concentrations of STZ and ISO-1. Media was collected for each well and centrifuged for 5 min with 13.000rpm to remove the dead cell bodies. The supernatant of this step were transferred to new 1.5ml tubes and 50µl of Protease inhibitor (20x) was added to the samples. Tubes were stored at -20°C or used directly.

High binding ELISA plates were coated with proper concentrations of primary antibody solved in PBS and stored at 4°C overnight. Plated were *"blocked"* on the following day using 100µl reagent diluent (1% BSA in PBS pH 7.4).

Standard curves for cytokine of interest was generated using purified recombinant proteins provided by the kit and the assay was performed according to manufacturer's instructions. Absorbance was measured at 450 nm to 650 nm using SpextraMax Plus 384 microplate spectrophotometer. The cytokines were normally measured in the spectrum of 10 to 1000 pg/ml.

The represented results reflect (at least) three separate measurements obtained from different cell cultures on different days.

## 4.8. Isolation of total RNA:

The mRNA was extracted from confluent monolayers of primary cultures of mouse glia cells or neurons using Piotr Chomczynski and Nicoletta Sacchi protocol. The concentration of RNA in each sample was quantified by measuring absorbance at 260 nm using spectrophotometer.

## 4.9. Synthesis of cDNA:

Synthesis of complementary DNA (cDNA) was performed prior to qPCR, in order to synthetize DNA from mRNA, using cDNA Reverse Transcriptase Kit following manufacturer's structure. Proper primer pairs were designed using ncbi library and PCR was performed using

## 4.10. BCA protein assay:

Bicinchoninic acid assay, also called Smith assay, is a test for detecting andquantifying the amount of proteins. Protein causes reduction of $Cu^{2+}$ to $Cu^{1+}$ ion in an alkaline solution; bicinchoninic acid (BCA) reacts with the reduced ion to form violet coordination complexes. The intensity of the color and hence the absorption at 562nm is directly proportional to the amount of protein present in the sample. Bovine serum albumin (BSA) is used to create a standard series. The experiment was performed based on manufactures protocol. Absorbence of the samples were measured at 560 nm to define the concentration of the protocol in each sample

## 4.11. Western blot:

50µg of total protein measured by BSA assay, was separated according to their molecular weight by 12% SDS-PAGE polyacrilamide gels . Molecular weight marker is loaded into the first lane.

The separated proteins were transferred to Immobilon-P PVDF (Millipore) or nitrocellulose membranes using a semi-dry transfer apparatus with a current density of 1 mA / cm2 for 2 hours, following by an overnight blocking step at 4°C. Proper detection antibody (BAF289 Human MIF Biotinylated Affinity Purified PAb, Goat IgG) was used for detection of the protein. Horseradish peroxidase (HRP)-conjugated streptavidin secondary antibody was used to visualized and identify target proteins according to the manufacturer's recommended protocol. Pierce ECL western blotting substrate was used to detect HRP activity in the samples and the images were aquired in ChemiDoc MP system device.

## 4.Experimental procedures: *In Vivo*

Following *in vitro* studies to show the inflammatory potential of STZ molecule in primary brain cell cultures, we decided to test whether local inflammation induced by ICV administration of STZ in the hippocampus of wild type (C57BL/6) and MIF-KO mice can induce the expression of cytokines *in vivo* as well, and if this inflammatory response leads to impairment in learning and memory in animals.

Different groups of animals tested included:

1. Untreated control
2. Vehicle ICV injected (Veh-ICV)
3. STZ ICV injected (STZ-ICV)
4. STZ-ICV + ISO-1 treatment: the purpose of this group is to determine the effects of MIF inhibition in downregulating the inflammatory cytokines and possible effects on spatial and reference memory in C57BL/6 mice
5. MIF-KO Veh-ICV
6. MIF-KO STZ-ICV

All animals were tested by Rotorad and open-field task to show the loco-motor capability among different study groups. As mentioned before Streptozotocin (STZ) is a fungal antibiotic which was originally used for rodent model of diabetes mellitus (200mg/kg intraperitoneal; Like & Rossini 1973). This substance is also a clinically approved drug for late-stage pancreatic cancer under the name of zanosar. For the purpose of our experiments we also used Zanosar, injected intracerebroventricularly (3mg/kg, ICV-STZ). Different groups were tested for spatial and context memory 6 weeks after operation using clockmaze and classical, fear conditioing.

The animals were sacrificed after this step and sample were prepared for immunohistochemistery and qPCR analysis (**Fig. 4.1.**)

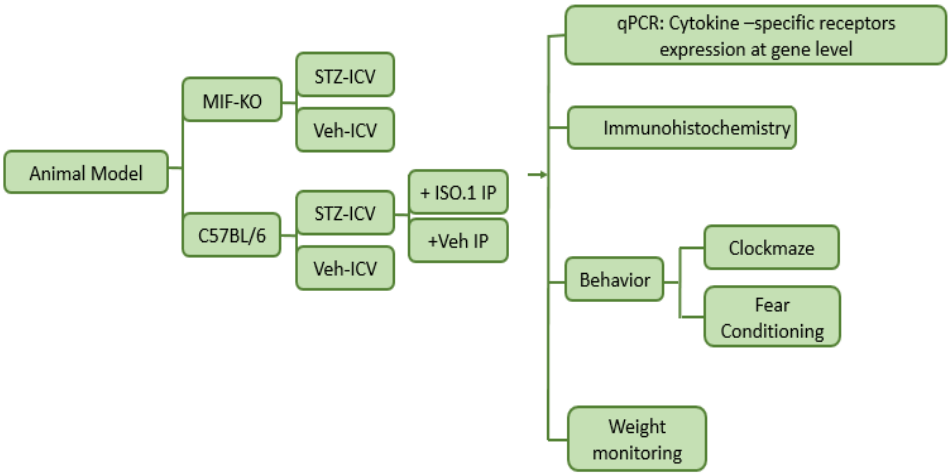


***Figure 4.1. flow chart for in-vivo experimental design:***

*the in vivo experiments were performed on wild type and MIF-KO animals, both being ICV injected with STZ molecule. STZ-ICV group of wild type was divided into two groups being treated either with and MIF inhibitor (ISO-1) or Vehicle.*

*Animal were tested for hippocampal dependent spatial memory (spatial, contextual) and after sacrificing different experiments (immunohistochemistery and qPCR) were performed to analyze the samples.*

## 4.12. Animals:

Male C57BL/6 animals were obtained from the Jackson Laboratory, USA. The MIF-KO strain was provided by the Feinstein Institute for Medical Research (FIMR). Animals were caged with *ad libitum* access to food and water, maximum 5 mice per cage in a controlled environment on 12 hour Light-Dark cycle (lights on 9 am). All animals were handled to reduce anxiety before starting the experiments. All experiments were carried out during the dark period and in a noise controlled room, in accordance with regulations in FIMR and US ethics rules. The ICV-STZ model of dementia and neurodegeneration has been used to study molecular and behavioral changes due to inhibition of MIF, as well as *in vitro* cultures of neurons, astrocytes and microglia.

## 4.13. Rotarod

Rotarod task was performed to monitor the motor coordination and learning in mice. The mouse was placed on a rotating rod and the latency to fall from this apparatus was measured. Animals normally fall very quickly in the first trials but over the trials the duration increases in normal healthy

animals. Motor learning was assessed by making a comparison between latencies in different treatment groups. The velocity of the rotating rod was increased by 0.5 cm/sec. every 5 sec and the mice was tested for 4-6 trials. Mean latency to fall in different trials was used for plotting the data.

***Figure 4.2. Rotarod apparatus***

*Rota-rod is a basic experiment performed to evaluate the motor coordination in rodent.*

*Source:*

*https://www.mouseclinic.de/screens/neurology/technologies/index.html*

## 4.14. Open field

In this task the exploration behavior and the anxiety level in animals were measured. As a normal behavior mice are hesitant to stand in the center and they only explore this area once in the while. The apparatus is a white box measured 72 x 72 cm with 36 cm walls. A central square is draw in the original box, and measure the duration the mouse spends in the center square as well as the distance the animal has walked during the experiment. The task was performed in dark room with background light, the mice was placed in the box for 5min and the behavior was recorded via a camera.

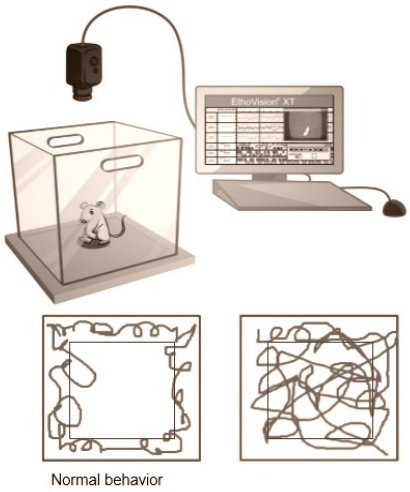


***Figure 4.3. schematic presentation of open field apparatus,***

*EthoVision®XT software is used to analyze the data, latency in center zone and near walls area are measured and plotted. Below are the representative images showing different behavior in the open field. While representing the normal behavior mice spent most of the time close to walls. In unusual conditions however (for example neophobia; one of the characteristics in APP mice), animal tends to stay within the center of the cage (Down right).*

*Source: https://mazeengineers.com/tag/mouse-behavior/*

## 4.15. Rodent stereotaxic surgery

This type of surgery is used to access a precise location in the brain, based on the idea that the features on the skull can indicate the position of different structures in the brain. In fact, the bregma is the point where the sagittal and coronal sutures joins the parietal and frontal bones, and is used as the “stereotaxic zero point”. From this point, the hippocampus is localized for injection of STZ or vehicle, to create a mouse model for neurodegenerative driven dementia.

The animal weight was measured to decide the amount of anesthetics (isoflurane, an inhalatory anesthetic 1.5% v/v supplemented with oxygen.) animal was placed in a stereotaxic head holder (David Kopf, Tununga, CA, USA). The head was scrubbed with ethanol, on the top if the head. An incision was made in the brain from posterior to anterior. The bregma was used as stereotactic zero point, stereotaxic coordinates used to target the

hippocampus for toxin delivery are: x, +1.0, y -0.3. Drill a hole in that position, avoiding drilling too deep. The Hamilton syringe (Hamilton 80100, 1ul, 26G) is filled with either STZ or Vehicle and the needle is re-positioned to be at stereotaxic zero point, and then to injection position x y z coordinates of (+1.0, -0.3, 0). And then slowly to target z: -2.6. Volume of 3 ul of toxin or vehicle was injected very slowly and at least 10 min is given for the drug to diffuse after injection. The needle was pulled slowly up, a small amount of surgical glue was used to close the cut down. As post surgical care, the animal was injected with buprenorphine and placed on a heat pad for recovery.

## 4.16. Clockmaze

The clockmaze apparatus is made of a transparent circular plastic pool with 12 possible exits. Different masks are placed around the maze as cues to assist formation of the spatial memory and the position of the objects remains unchanged throughout the experiment.

Water was added to the maze, up to 2 cm deep (shallow water), the temperature was reduced to create a stimulus for escape motivation from the maze. Cages were transferred to the experimental room, drapes were used to block the view of the maze and also the animal being tested cannot see the experimenter during the trials. A video camera was placed directly above the pool and connected to a PC running Ethovision Software. The area of the maze was defined and the 12 exits were defined as sub-zones in the program. The tracking started automatically as the animal was placed in the maze (based on the contrast between the mouse and the background).

The procedure can be broken down into following parts

A. Black Corridor, the study started in the black corridor (a separate light protected chamber) where the animal learned to use the tube exit as escape from water (4 trials on day1). The cut-off for these trials was 30 sec.

B. White corridor: a transparent chamber placed inside the clockmaze, for visual training, and to teach animal to escape from the maze via a tube (4 trials on day2)

C. The clockmaze had 11 closed exits and only one correct path to exit the maze. The animal was placed in the middle of the maze facing the tube opposite the exit (facing tube 6, if the exit is tube 12). Training

consisted of 12 trials in 3 successive days, with maximum length of 60 seconds per trial. In case of failure in finding the proper exit, the animal was manually guided towards the exit. Mice were placed under heating lamp for recovery to avoid hyperthermia. Parameters measured in this task include:

a. Latency and path length: the time and distance that the animal requires to find the exit.

b. Errors: defined as trying to enter the wrong exit

c. Strategy: they are based on the number of the errors and the latency, and defined as follows:

(c.1) Spatial: when the animal exits the maze with 3 or less errors.

(c.2) Chain: when the animal tries more than 3 decoys to find the open escape, thus number of errors is >3.

(c.3) Futile: when the animal fails to find the exit within 60 sec.

Passing by the real exit without entry frequently happened in early trials but if the animal did not show improvement, it was considered as a sign of impairment in spatial cognition.

D. Probe trial: in this trial the exit tube was blocked. We measured the time the animal spends in front of the escape exit, as an indicator for the usage of extra-maze cues and reference memory.

E. Reverse trial (or spatial reversal): the exit was relocated to the opposite tunnel and animal was tested for another set of 12 trials over 3 days.

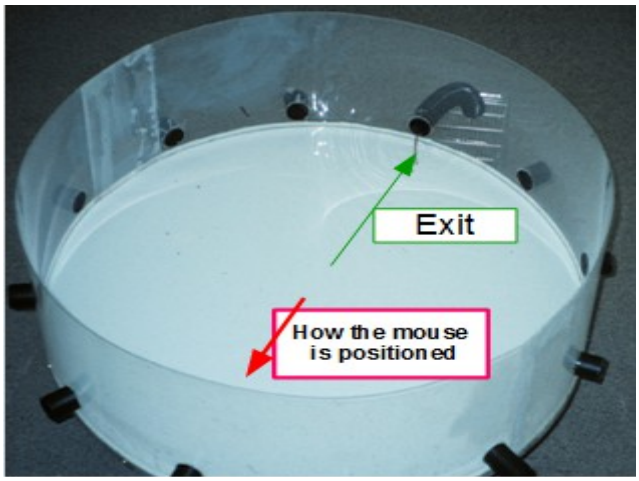


***Figure 4.4. Clock maze;*** *the round arena is surrounded by 12 exits, only one of them is an escape to the maze (represented in green arrow). The mouse is placed to face the opposite exit (the red arrow) and is given one minute to solve the task.*

*Jove: journal of visualized experiments: www.jove.com*

## 4.17. Fear conditioning

In Fear conditioning paradigm, a neutral conditional stimulus (CS), such as a pure tone (10 sec, 80 dB), occurs in conjunction with an unconditioned stimulus (US), such as a foot-shock (1 sec, 0.5 mA). The animals learn to quickly associate the CS with the US and display a conditional response, such as freezing when presented with the CS alone. Therefore we measure the duration of freezing, which is described as a complete absence of movement except for respiration, as is the index for fear memory.

The apparatus was cleaned before starting each trial to avoid an olfactory context. All trials were recorded for further analysis.

The amount of freezing and the presentation of CS and US was controlled by software (FreezeFrame). This experiment was conducted over the period of 4 days:

Day1, Habituation: the animal was familiarized to the fear-conditioning (FC) chamber for 10 min.

Day2, Fear conditioning: the animal learned to associate the US with the CS. Mice were given 5 presentations of the CS, which co-terminates with the US.

Day3-, Context memory: the mice returned to the test apparatus and observed for 10 min.

Day4-Tone Memory: the animal was placed in a differently shaped chamber and is exposed to the auditory cue alone.

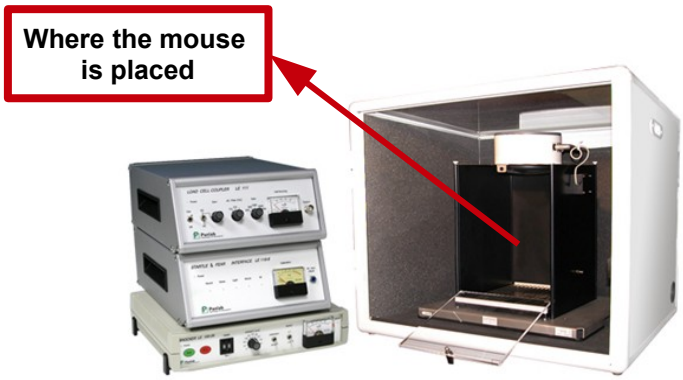


***Figure 4.5. Fear conditioning apparatus:***

*The red arrow represents where the mouse is placed, the camera placed on the top allows recording and analysis of the animals movement in terms of activity to immobility ratio (fear conditioning).*

## 4.20. Immunohistochemistry

After sacrificing the animals, the brains were excised and fixed in Paraformaldehyde (PFA, 4% PFA, bufferd with PBS) overnight at 4°C and then transferred into 30% glucose with azide for long-time storage. The samples were cut at the thickness of 50 μm slices (cryosections) and incubated in 3% $H_2O_2$ in PBS to remove endogenous peroxidase, which may create non-specific background after HRP conjugate application. Samples were blocked with 5% milk in PBS for 1h at RT and transferred to wells containing first antibody at proper concentration, and incubated overnight at 4°C. Secondary antibody was applied on the following day to the samples at RT for 2h. Samples were transferred to HRP conjugate solution for 1h at RT. Brain sections were visualized using 3,3'-diaminobenzidine (DAB). and 0.3% H2O2 was used to stop the visualization reaction. Samples were transferred to glass slides and dehydrated using 80%, 95%, 100% ethanol. Finally paramount was used to fix the slides. The slides were left overnight under the hood to complete drying and fixation and analyzed under the microscope.

## 4.21. qPCR

The hippocampus of the animal was stored in 200μl TRIZOL realgent (Invitrogen) for the purpose of RNA isolation. 50μl 1-Bomo-3-chloropropan (BCP) was added to the samples and the mRNA was isolated using QiAgen mRNA isolation kit (Rneasy mini-kit). The cDNA was prepared as described previously. The primers for this purpose were designed based on accession number from a library of primers for SYBR green and were ordered from *Fisher Scientific.* Quantitative PCR (qPCR) was performed using SYBR Green Master Mix (*SIGMA-ALDRICH)*. The quantity of target genes were normalized to house keeping gene of choice (Beta-actin) using the comparative Treshhold Cycle (CT) method (ΔΔCT). In this method, the average of the Ct values for the house-keeping gene and the target genes of interest are compared in the experimental and control conditions, returning 4 different values. ΔΔCT is calculated by subtracting differences between target and housekeeping values under control condition from differences between target and housekeeping values under experimental conditions. Value of 2^ ΔΔCT is calculated to get the expression fold change. Results were expressed as mean ± standard error of the mean (SE) of at least four different

animals per each experimental group.

## 4.22. Statistical analysis

All graphs were prepared using either ggplot2 package (R program) or excel. Data are expressed as means ± Standard Error of the Mean (SEM). Statistical analysis was performed using dplyr packages in R program or excel. For the clock maze and fear conditioning, the daily performance of the treatment groups was analyzed using One or Two-way ANOVA followed by unpaired student T test or Bonferroni`s post test. Unpaired student t test was performed on ELISA results to test the null hypothesis and Mann–Whitney test was used to asses differential expression on qPCR data.

# 5. Results

## 5.1. Characterization of the anti-inflammatory effect of MIF inhibition on glial cells undergoing STZ-induced inflammation

In the first part of this study we investigated the effect of MIF inhibition on cytokine production in glial and neural murine cultures *in vitro.* STZ was used to stimulate the microglia astrocytes or neurons *in vitro.* Release of different cytokines was measured in transcriptional and translational levels both with or without MIF inhibition in order to elucidate the role of MIF as the early cytokine in regulating the expression of other mediators of inflammation.

### *5.1.1. MIF protein is released in response to STZ stimulation by microglia and astrocytes:*

In a pilot study, MIF production was measured using ELISA kit to show that this cytokine was released in response to STZ stimulation of astrocytes and microglia. Intracellular MIF was detected by Western blotting, as well as PCR, to show mRNA expression levels of this protein within the cell (data not shown). We used primary cell cultures of astrocytes and microglia, isolated from postnatal C57BL/6 mice, for stimulation with 1mM and 0.5mM STZ (respectively for astrocytes and microglia). ELISA was used to test cell culture supernatants, after 24 h, for MIF release (**Fig.5.1**). We observed a baseline level for MIF release from the cells in both astrocytes and microglial cells (150 pg/ml). Unlike most cytokines, MIF is not produced *de novo* in an early response to a stimulus. It has been previously shown that upregulation in the mRNA levels of MIF is a delayed response (Lanahan et al. 1992) and that MIF is pre-stored intracellularly, which allow for its release as an early-phase cytokine (Atsumi et al. 2007). In the next step, we isolated the intracellular mRNA which was used for cDNA transcription followed by a PCR. In the visualization on the agarose gel we observed no influence on mRNA expression levels after STZ stimulation. It might be possible that to see an increase in the mRNA level of MIF, we should look for time points later than 24 hours. We observed that the use of different concentrations of MIF inhibitor, ISO-1, does not affect the extracellular secretion levels of the protein,which is consistent with the finding that ISO-1 blocks the tautomerase active site of MIF molecule, without affecting the amount of the protein itself

(Al-Abed et al. 2005). MIF protein was released in both cell types in response to STZ molecule

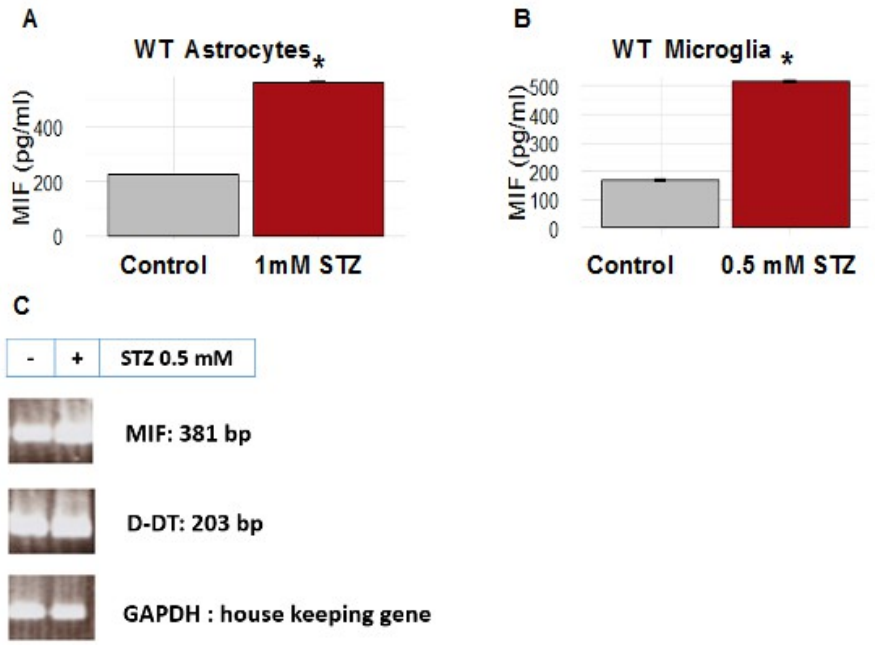


***Figure 5.1. Extra and intracellular MIF, in response to STZ stimulation,***

***A and B:*** *Significant increase in MIF secretion in supernatant of the astrocytes (A) and microglia cells (B) in response to STZ stimulation after 24h measured by ELISA . Graph represent the mean of three biological replicates, bars represent standard errors of the mean(± SEM), (*P < 0.05, unpaired Student's t test)*

***C****. Analysis of PCR products on agarose gel, the polymerase chain reaction (PCR) was performed using samples collected from astrocytes, reflecting the intracellular mRNA expression levels for MIF and D-DT genes, the expression in mRNA level remained unaffected in comparison to housekeeping gene after STZ stimulation. Similar results were observed with microglial samples (data not shown)*

### *5.1.2. Application of ISO-1 does not affect the secretion of MIF*

Following the observation that MIF is released in response to STZ, we applied the well-established MIF inhibitor ISO-1 in order to find out if the STZ dependent release of MIF can be blocked. ISO-1 was used at final concentrations of 25, 50 and 100 µM to the cell culture one hour prior to STZ stimulation, and the supernatant was tested after 24 hours for the concentration of MIF molecule. Based on our observation, ISO-1 does not affect the extracellular amount of the MIF molecule.

### *5.1.3. STZ stimulation induces IL-1beta and IL-6 protein and mRNA*

IL-1β is a member of IL-1 superfamily of cytokines (Maldonado, Andrian 2010) and one of the major neuroinflammatory signals (Drake et al. 2011) released from microglia and specially astrocytes in the hippocampus, where it plays a dual role in either supporting hippocampal-dependent memory (Pearson-Leary et al. 2015), or impairing the memory (Ross et al. 2003). Astrocytes seem to be the main source of IL-1β in the CNS (Wen Yin Wang et al. 2015).Using primary microglia and astrocytes, we assayed for IL-1β and IL-6 cytokine production, at mRNA (PCR) and protein levels (ELISA; **Fig.5.2**). MIF-KO cells exhibit decreased type 1 IL-1R expression (Toh et al. 2006), indicating that inhibition of the MIF can lead to down regulation of IL-1β secretion from the cells. In our experiment, extracellular IL-1β protein was significantly and dose dependently inhibited by blocking the MIF's activity even at the lowest concentration of ISO-1 (**A and B**).

IL-6 is classically known to be a pro-inflammatorycytokine and a marker for systemic activation, which is shown to have also regenerative activity (Scheller etal.2011). MIF regulated IL-6 cytokine expression by influencing NF-kβ (Chuang et al. 2010). Apart from being one of the major cytokines, IL-6 in the brain plays both neuroprotective and neurotropic roles (Quintana et al. 2013). Although astrocytes are known to be the main source of this cytokine (van Wagoner et al. 1999; Quintana et al. 2013), microglial expression of IL-6 increases dramatically in the brain of aged mice (Van Wagoner et al,. 1999), which is associated with cognitive decline. IL-6 was increased in response to STZ treatment; this expression was attenuated while MIF was inhibited (Fig. 5-2 C and D). Interestingly MIF inhibition at highest concentration (100µM ISO-1) leads to suffocation in release of these cytokines in mixed glial culture (astrocytes to microglia ratio, 1:5 – Data not shown).

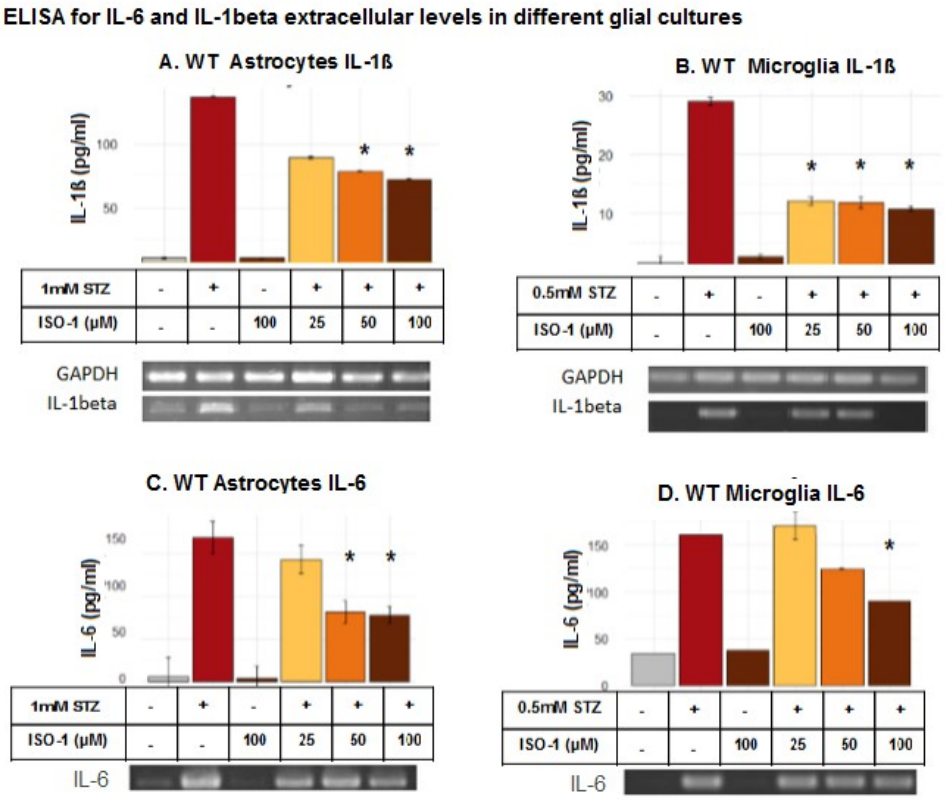


***Figure 5.2. MIF-mediated expression of IL-6 and IL-1ß from astrocytes and microglia, inhibitory effect of MIF suppression.***

*ELISA results are plotted in graphs, Data are representative of three independent biological replicates, error bars represents ± SEM (*P < 0.05, unpaired Student's t test).*

***A. B. C. & D. TOP:*** *IL-1ß and ELISA assay results for supernatant of murine astrocytes and microglia after 24h STZ stimulation (red bar), both cytokine were released in response to STZ stimulation in Microglia and astrocytes Using different concentrations of ISO-1 to inhibit the MIF molecule (yellow, orange and brown representing 25, 50 and 100µM of ISO-1 respectively) resulted in dose dependent decrease in cytokine release in both cell types.*

***A. B. C. & D. Bottom:*** *PCR showing the mRNA expression level of proinflammatory cytokines interleukin-6 and interleukin-1β. IL-1β was strongly and dose dependently in transcriptional level after MIF inhibition. Similar effect was seen in a lesser degree in the case of IL-6 .*

### *5.1.4. IL-10 is expressed by microglia in response to STZ:*

It is known that STZ can induce release of anti-inflammatory mediators (Naxin Sun, et al. 2005), to investigate this statement in our model, we investigated presence of IL-10 as an anti-inflammatory cytokine in transcriptional and translational levels.

IL-10 is one of the anti-inflammatory cytokines that is elevated during the course of chronic inflammation in the brain (Strle et al. 2001), for the purpose of promoting the survival of the neurons (Jia et al. 2009). It inhibits the production of pro-inflammatory cytokines from astrocytes and is known to be produced in different brain regions such as neural tissue, pituitary and hippocampus, where it induces a specific activated microglial phenotype concomitant with changes in hippocampal Long term potentiation (LTP) responses (Almolda et al. 2015). IL-10 is decreased by aging (Frank, Barrientos, et al. 2006), which leads to priming of microglia and increased IL-1β expression (Hermes et al., 2008). Based on our observations STZ caused 1.5 fold induction of anti-inflammatory IL-10 in microglia, which remained unaffected as MIF was blocked,even at highest concentration (100 μM) (**Fig.5.3**). It has previously been ascertained that the absence of MIF enhances IL-10 production in vivo (Madeira et al. 2012) and that MIF can down regulate IL-10 expression (Sashinami et al. 2006 ). IL-1β expression is down regulated by IL-10 (Heyen, JR et al. 2000). The synchronized presence of IL-10 and blockade of MIF can clarify the strong IL-1β inhibition in the lowest applied concentration of ISO-1 (25μM). Furthermore IL-10 is known to hinder synthesis of MIF and subsequently the NO released by MIF (MIF dependent nitric oxide synthase- Kerschbaumer et al. 2012).

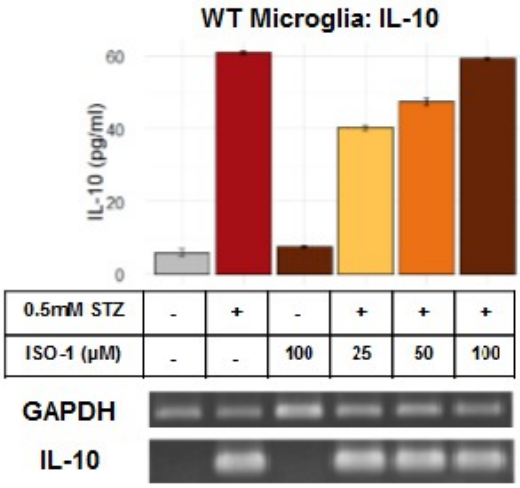


***Figure 5.3. in vitro effect of STZ stimulation on IL-10 expression in protein and mRNA level in microglia cells.***

***TOP:*** *IL-10 ELISA results plotted in graphs, representative of three independent biological replicates, error bars represents ± SEM of triplicate technical replicates. Murine microglia supernatant was checked after 24h of STZ stimulation (red bar), with or without ISO-1 inhibition MIF molecule (yellow, orange and brown representing 25, 50 and 100µM of ISO-1 respectively). IL-10 was significantly released in response to STZ stimulation in comparison to control (Grey bar, P < 0.01) and remained unaffected when MIF was inhibited.*

***Bottom:*** *Representive agarose gel electrophoresis illustrating IL-10 PCR results, inhibition of MIF didn't affect the STZ mediated IL-10 expression in transcriptional level after 24h in microglia.*

### *5.1.5. TNF-alpha is released by microglia in response to STZ*

TNF-α contributes to the pathogenesis of neurodegenerative diseases (Buko et al. 2015) and has a diverse range of functions in the CNS. TNF-α release in hippocampus results in excitotoxic neuronal damage (Bock F et al. 1996), and the induction of ICAM-1 on human fetal astrocytes (Zhou et al. 2016). Astrocytes express TNFR1 high affinity receptors for TNF-α. In our experiments, protein and mRNA levels of TNF-α in astrocytes were not affected by STZ stimulation, leading to the assumption that maybe the astrocytes react to STZ stimulation by upregulating the receptor for TNF-α and not the cytokine itself, however we did not look for the TNFR1 expression

levels in the astrocytes. However TNF-α was released from astrocytes in response to LPS (data not shown). TNF-α was detectable extracellularly in microglial culture *in vitro,* 24 h after STZ stimulation (**Fig.5.4.**), which may suggest that microglia are among the primary sources of TNF-α production in CNS inflammatory response. ISO-1 treatment effectively suppressed the expression of this cytokine in microglia cultures, yet it was not dose dependent, which can be explained by fact that IL-6 can control the TNF-α expression as well. Whereas MIF induces the production of TNF-α, IL-6 is capable of down regulating its expression (Stenvinkel et al. 2005), meaning that the concentration of TNF-α is not solely MIF dependent, as a result of which MIF inhibition affects TNF-α, but not in a dose dependent manner.

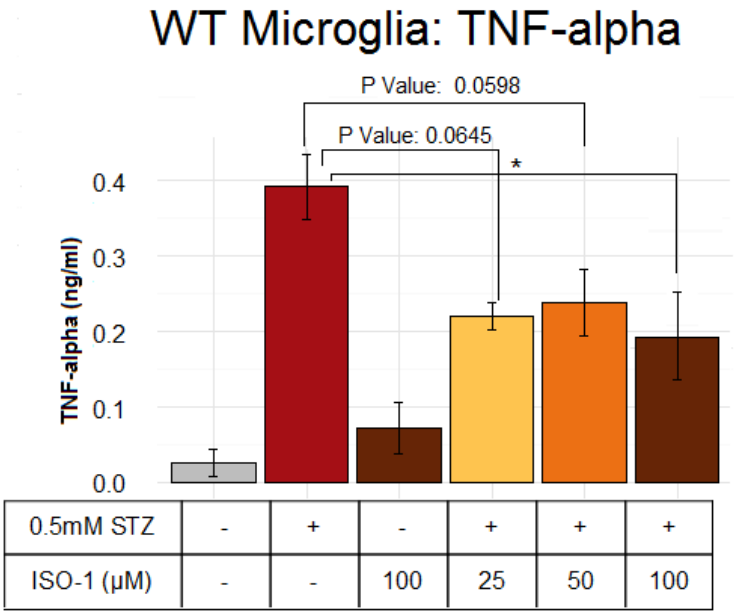


***Figure 5.4. TNF alpha expression in microglia***

***TOP:****TNF alpha released from microglia cells measured by ELISA after 24h following stimulation with 0.5 mM STZ (red bar) and 25, 50 and 100 μM ISO-1 (respectively yellow, orange and brown bars) as MIF inhibitor. Graph is representative of three independent biological replicates, error bars represents ± SEM of triplicate technical replicates. (*P < 0.05, unpaired Student's t test).TNF was released extracellularly 24h after STZ stimulation from microglia cells, and was significantly reduced in response to 100 μM ISO-1.*

### *5.1.6. Astrocyte-specific induction of IL-12p40:*

IL-12 is a pro-inflammatory cytokine that in bioactive form (IL-12p70) is a heterodimer made of two subunits: IL-12p35 and IL-12p40. In regards to our study, these two subunits are regulated differently in response to STZ stimulation. IL-12p40 is also capable of forming a homodimer, IL-12p80, which can induce the nitric oxide synthase in microglia via attachment to IL-12R beta 1 receptor on microglial cells (Jana M, et al,. 2009) and is also found in multiple sclerosis lesions (Windhagen et al. 1996). We detected no differences in mRNA and protein expression level for IL-12p35, IL-12p40 was increased in response to STZ and diminished in mRNA transcript and protein level in presence ISO-1 in astrocytes(**Fig. 5.5.**), we were unable to detect the signal in microglia.

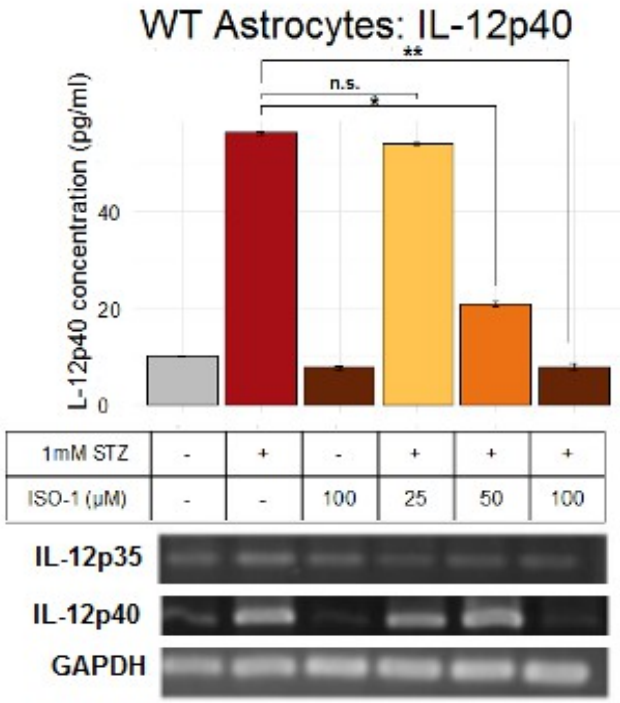


***Figure 5.5. STZ mediated astrocytic IL-12-p40 production (ELISA)***

***TOP:*** *Effect of MIF inhibition on IL-12p40 release from astrocytes in response to 1mM STZ stimulation after 24h. Graph is representative of 3 biological replicates, Data are expressed as the mean of 3 technical replicates ± SEM ( *P < 0.05 and and **P ≤ 0.01, unpaired Student's t test)*

***Bottom:*** *cDNA after 24h from astrocytes treated with 1mM STZ analyzed by agarose gel electrophores. IL-12p40 was upregulated in transcriptional level after 24 hours, which was inhibited with Inhibition of MIF (at highest concentration only). The mRNA level for IL-12p45 stayed unaffected throughout the experiment.*

### *5.1.7. Cytokine profile in MIF-KO astrocytes and microglia:*

Based on the observation that inhibiting MIF leads to attenuated cytokine response in glial cells after STZ stimulation, we hypothesized that MIF-KO cells might exhibit a decreased response to STZ stimulation. To test this hypothesis, MIF-KO astrocytes and microglia were prepared using prenatal mice pups, and STZ treatment was performed with the same protocol as WT cells, and the supernatants were tested after 24 hours (by ELISA) for the regulation of different cytokines.

### *5.1.8. MIF-KO cell cultures exhibit dampened IL-6 but not IL-1 beta response to STZ:*

We determined the expression level of IL-6 in these cells in response to STZ stimulation. No significant increase was detected in extracellular amount of the IL-6 cytokine in MIF-KO astrocytes, even when we increased the concentration of STZ to 2 mM. Also, we did not observe any increase in TNF-$\alpha$ extracellular protein level. In contrast to TNF-$\alpha$ and IL-6, IL-1$\beta$ was increased in MIF-KO astrocytes after 24 hours in response to STZ stimulation ***(Fig. 5.6.)***. This can be explained by considering the fact that IL-1$\beta$ is pre-stored in the cells as a precursor protein, and that IL-1$\beta$ can enhance its production via a positive feedback loop (Jimbo et al. 2005).

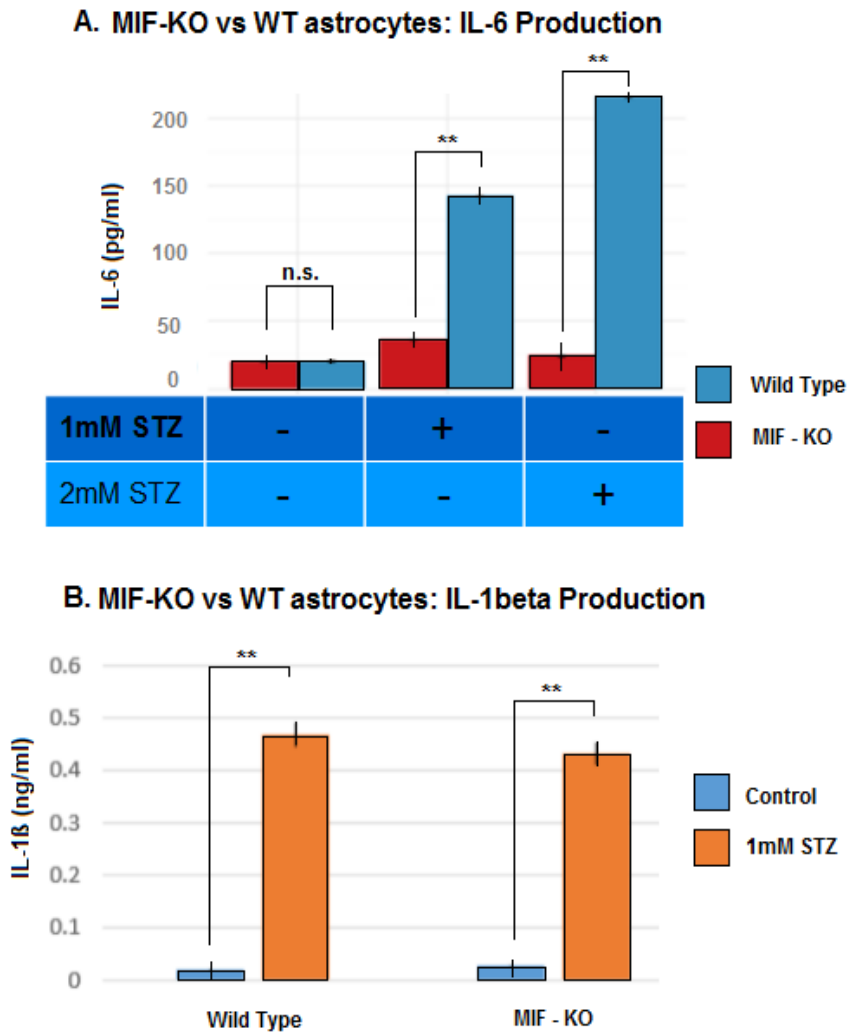


***Figure 5.6. STZ induced IL-6 and IL-1 beta production, wt vs MIF-KO***

*ELISA results for IL-6 and IL-1β of MIF-KO astrocytes in comparison to the Wild type 24h after STZ stimulation, Data are representative of three independent biological replicates, error bars represents ± SEM of triplicate technical replicates(**P < 0.01, unpaired Student's t test).*

***TOP:** Differences in IL-6 production of MIF-KO cells vs. wild type cells, Whereas IL-6 was produced in a dose-dependent manner in wild type astrocytes (blue bars), no increase in extracellular IL-6 levels was observed after STZ treatment in MIF-KO astrocytes (red bars)*

***Bottom:** Comparison in respond to 1mM STZ between MIF KO and Wild type astrocytes. No differences was observed in translational IL-1β levels between two experimental groups (wt vs MIF-KO).*

### *5.1.9. Cytokines are produced in MIF-KO neurons in response to STZ*

Neurons in the hippocampus regulate many cognitive behaviors in accordance with environmental and emotional cues, and like glial cells are susceptible to inflammatory signals. Manipulations in the homeostasis of their environment can lead to a molecular response in terms of production of inflammatory mediators as well as changes in cognition and behavior on a larger scale. It has been shown that MIF is produced by the neurons in both mRNA and protein levels (Nishino et al. 1995, Bacher et al. 1998). Hippocampal neurons from CA3 and CA4 produce MIF protein in bovine (Nishibori M, et al 1996) and rat neurons (Nishibori et al. 1996). MIF seems to play an immune regulatory role in the CNS by being involved in degeneration and regeneration of the neurons (Koda et al. 2004). It is known that STZ stimulation in WT neurons leads to activation of ROS and cytokine discharge into extracellular space. Here we tested the response in MIF-KO neurons to STZ stimulation, by controlling the cytokine production (TNF-$\alpha$, IL-6, IL-1$\beta$) after 24 hours of STZ stimulation. MIF-KO neurons were capable of producing these inflammatory mediators exposed to 1mM STZ. We observed upregulation in IL-6 and TNF-$\alpha$ extracellular protein levels (**Fig. 5.7.**). this seems to be contradictory to the findings that neuroinflammation and neurodegeneration is generally attenuated in MIF-KO mice (Leng et al. 2011), then again it has been shown that affecting inflammatory hallmarks does not happen during the first week after the insult (Inacio et al. 2011) so one might argue that results based on examining the cytokine response after 24 hours, and using *in vitro* techniques, is not completely accurate and that we should examine the cytokine production in an *in vivo* model. Generally there is not much known about IL-6 secretion in neurons, however it has been reported that membrane depolarization and excitatory amino acids can induce IL-6 production from neurons (Victoria Tarabin, et al 2002).

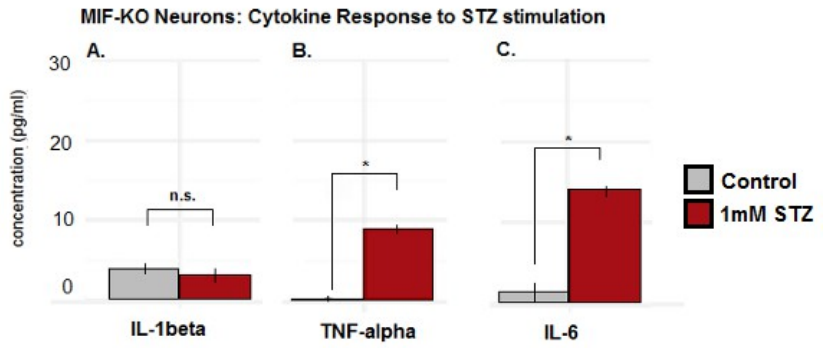


***Figure 5.7. Neuronal expression of proinflammatory cytokines in MIF-KO cells in response to STZ stimulation***

*ELISA results for IL-1β, TNF-alpha and IL-6 in MIF-KO neurons after 24h stimulation using 1mM STZ represented in Red bars (Grey bars are the unstimulated control). Represented data is the mean of two independent biological replicates ± SEM, each of which repeated in three technical replicates (*P < 0.05, unpaired Student's t test).*

***A.** unlike wild type neurons (Data not shown) no up regulation was observed in IL-1β protein levels in supernatant 24h after stimulation.*

***B. and C.** low basal levels of IL-6 and TNFa were slightly upregulated after STZ stimulation.*

### *5.1.10. IL-6 production is upregulated by extracellular MIF:*

Stimulation of microglia with MIF (10 ng/ml) leads to TNF-α and IL-6 production in these cells (**Fig. 5.8**.), indicating the possible role of this protein in amplifying the cytokine response, possibly as an upsreatem modulator for other inflammatory cytokines.

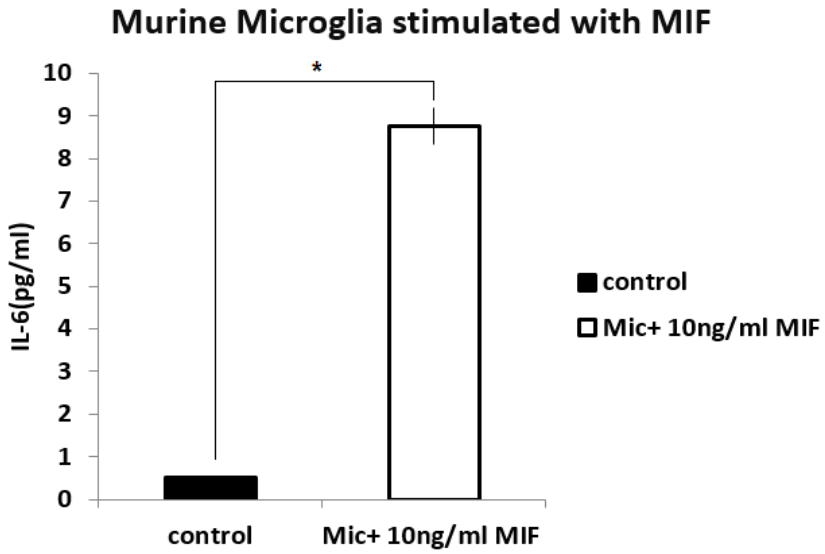


***Figure 5.8. IL-6 production in response to MIF molecule,***

*ELISA results for IL-6 production in response to stimulation with MIF molecule (10 ng/ml) in wild type microglia cells. Represented data is the mean of two independent biological replicates ± SEM, each of which repeated in three technical replicates (**P < 0.05, unpaired Student's t test). Using 10ng/ml MIF molecule resulted in increase in IL-6 extracellular protein level*

## 5.2. Neuroinflammation as a risk factor for cognitive deficit

In earlier experiments we used STZ *in vitro* to show the inflammatory potential of this molecule in primary brain cell cultures, during which the cells reacted to stimulation by expression of a wide range of cytokines including IL-6, IL-12p40, IL-1β and TNFa.

As the next step, we try to elucidate, whether systemic infection induced by ICV administration of STZ in the hippocampus of WT mice (C57BL/6) induces the expression of the above-mentioned cytokines *in vivo*, and if this inflammatory response leads to impairment in learning and memory in animals. Using qpPCR, we measure the levels of key cytokines that are involved in learning and memory. We also aim to examine the inhibition of MIF in ICV-STZ mice, suggesting the probable potential that inhibition of this molecule can disrupt the neurogenesis process and affect the alteration of learning and memory induced by neurogenesis.

Specific aims for this section includes:

- To assess the effects of ICV injection of STZ molecule in the hippocampus of the mice in terms of the upregulation of the different inflammatory cytokines and cognition deficits.
- To assess effects of inhibition of MIF in controlling the inflammation and improving the possible behavioral deficits.
- To investigate effects of the CNS chronic inflammation on weight and food intake, focusing on the possible role of MIF in this processes.

Following approached was used to achieve these goals:

Male C57BL/6 mice were obtained from Jackon Labs. The different groups of animals tested include: [1] Untreated controls, [2] Vehicle ICV injection (Veh-ICV), [3] STZ ICV injection (STZ-ICV), [4] STZ-ICV + ISO-1 treatment. The purpose of group 4 is to determine the effects of MIF inhibition in downregulating the inflammatory cytokines and possible effects on spatial and reference memory in C57BL/6 mice, each group consists of 20 mice.

As mentioned before STZ is a fungal antibiotic that was originally used for rodent model of diabetes mellitus (200mg/kg intraperitoneal; Like & Rossini 1973). This substance is also a clinically approved drug for late-stage pancreatic cancer under the name of Zanosar. For the purpose of our

experiments we used Zanosar, injected intracerebroventricularly (3mg/kg, ICV-STZ). It has been shown that ICV injection of STZ in the hippocampus is followed by tissue damage and neurodegeneration in that area (**Fig. 5. 9.**)

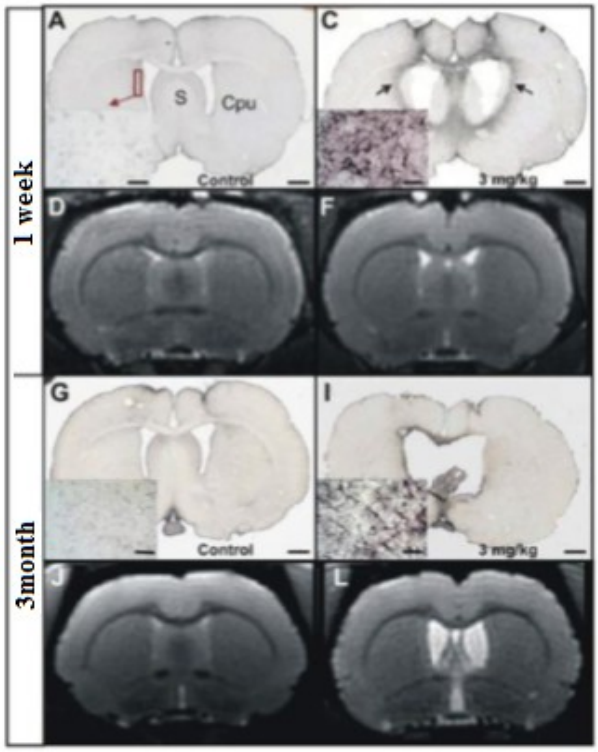


***Figure 5.9. effects of STZ molecule on hippocamus,***

*ICV injection of STZ in the hippocampus is followed by tissue damage and neurodegeneration in that area*

*Kraska et al 2012*

One of the main cognitive dysfunctions to arise as a result of neuroinflammation in hippocampus is disruption of the spatial memory. We decided to use a paradigm to assess the possible deficits in spatial memory followed by assessments of the reference memory. Morris Water Maze is a paradigm classically used to assess the hippocampal dependent behaviors such as spatial and reference memory. However the MWM is originally designed for rats that swim naturally in contrast to the mice that resent water. In this project, animal were tested for their spatial memory, using clockmaze which shares the similar theoretical basis with Morris Water Maze, however is independent of anxiety and fear component (**Fig.5.10.A**), it omits forced swimming, which is one of the main stressors in the MWM task.All animals were also handled before the experiments to reduce the anxiety level. This

memory assessment in this task is based on improvement in using the spatial strategy, reflecting hippocampus dependent learning. 6 weeks after the injections animals were tested for their hippocampal dependent spatial memory using clockmaze paradigm, which is a visuospatial learning task modified from Deacon & Rawlins 2002. The following pattern (**Fig.5.10.B**) represents the rational for the chosen strategies for each trial based on the escape path and latency. The selection of the spatial strategy by mice indicates a normally functioning hippocampus. Disruptions in the hippocampus impair spatial learning and memory.

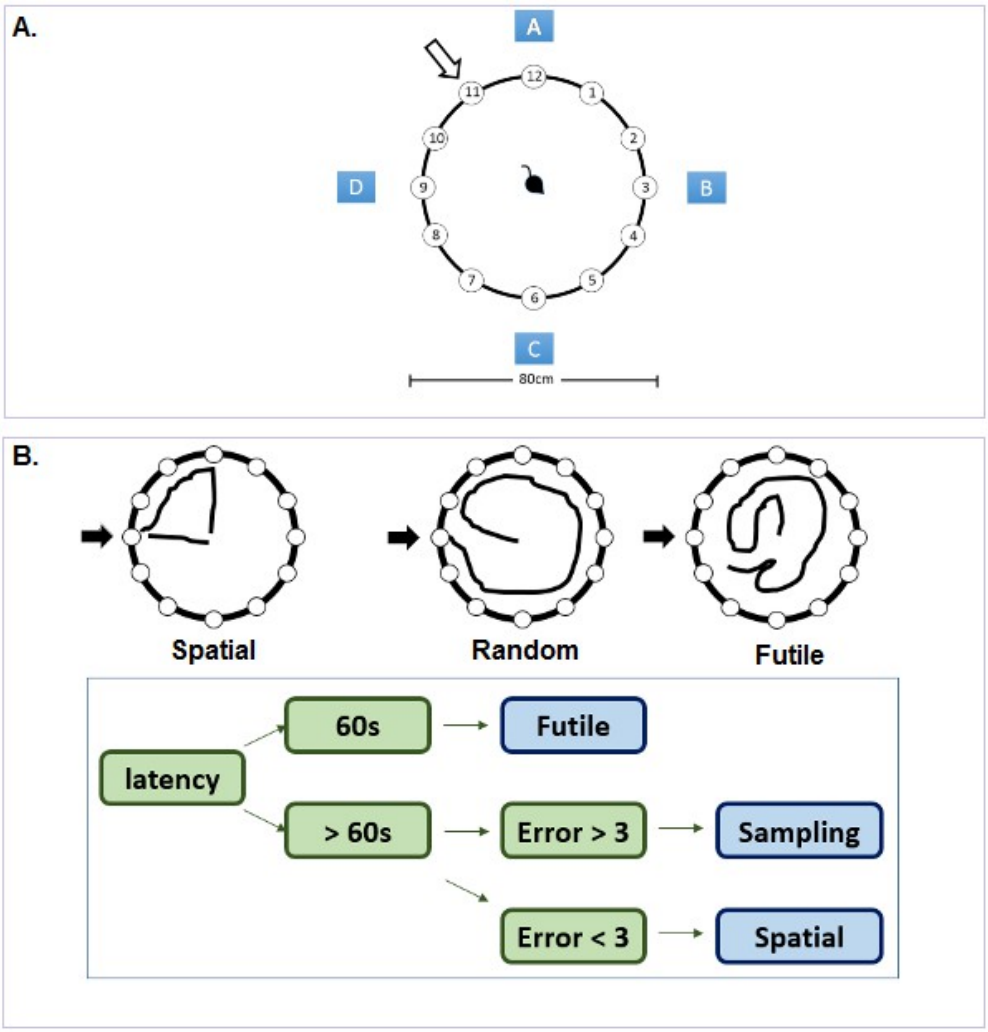


***Figure 5.10. The clockmaze, a visuo-spatial learning task:***

***A.*** *The apparatus is a water filled pool ( 80 cm diameter) with 12 exits ( represented as 1 to 12 in circles)*

- *Only one exit is open,The arrow shows where the open exit is located (exit 11)*

- *the maze is surrounded by visual cues, to help developing contextual memories ( represented as A, B, C, and D)*

- *Water wets animals' bellies, but animals can walk*

- *Animals are placed in the center with back to exit ( facing exit 4) and has 60 seconds per trial to solve the task (finding exit 11)*

***B.*** *Different strategies to solve the task:*

*Strategies can be divided in following groups:*

*1. Spatial: the most efficient, the animal immediately runs towards the exit by orienting to the extra-maze cues.*

*2. Chaining or Sampling: is less efficient, the animal runs around the maze and checks every exit until they find the target.*

*3. Futile: they fail to find the exit in 60 sec.*

### *5.2.1. Behavioral screening:*

Acute inflammation in our model was obtained by intracerebroventricular (ICV) STZ injection into hippocampus of the mice. In a primary screening, the mice were tested for muscle and spinal function, spino-cerebellar function, sensory function and autonomic function. The quick screening allowed us to look for abnormal phenotypes, but we found that no differences exist between STZ-ICV and Veh-ICV groups. Moreover, there were no differences between groups in the open field test and the rotorad test (**Fig. 5.11.**).

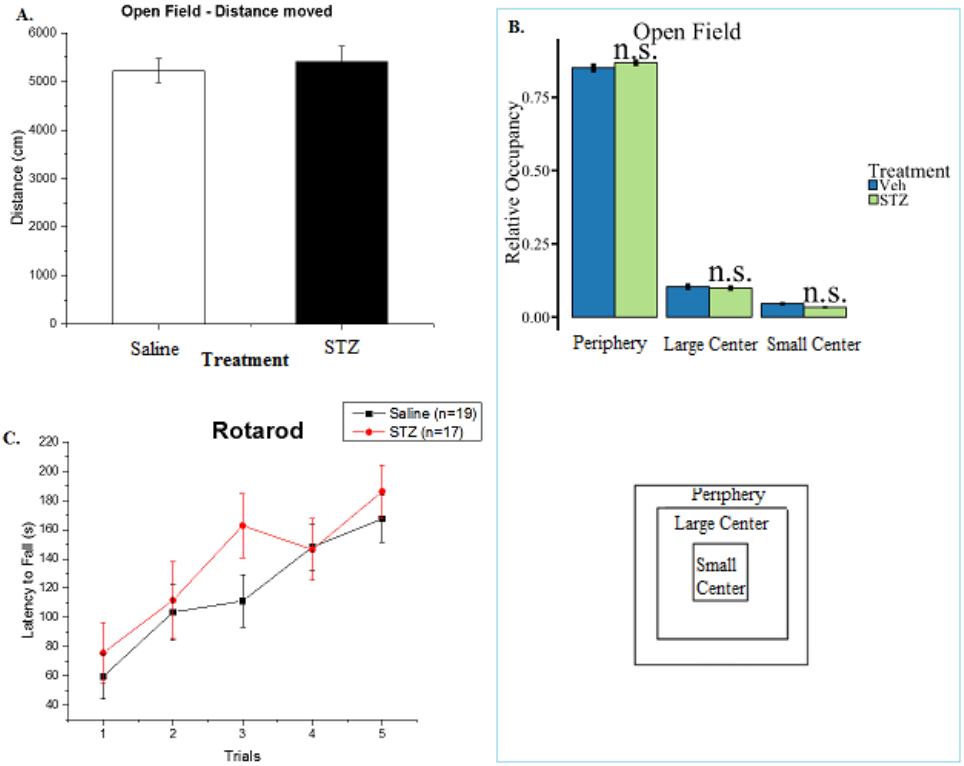


***Figure 5.11. Primary muscle and spinal screening between STZ-ICV vs Veh-ICV.***

*Open Field task and Rotarod were used to asses any probable differences in terms of motor function between two experimental groups.*

***A.*** *Traveled pathway differences between experimental groups in cm in Open field arena (mean±SEM; Saline ICV, n = 20 and STZ- ICV, n = 20 – One way ANOVA). No significant difference was observed.*

***B.*** *Different zones in Open field arena and amount of time mice spent in each zone during the task. Data are represented as group means ± SEM. The spent time in different zones the arena were similar between STZ and Saline ICV group (Saline ICV, n = 20 and STZ- ICV, n = 20 –one way ANOVA)*

***C.*** *Motor coordination expressed as time period until falling off the apparatus over 5 trials. Dots represent average activity of animals in terms seconds for STZ-ICV (in red, n=17) and Saline-ICV (in black, n=19) ± SEM for each group. Two-way ANOVA with Bonferroni`s posttest was performed to assets the statistical significant. In both groups, latency to fall increases over the trials and no significant difference was observed.*

### *5.2.2. STZ-induced neuroinflammation impair spatial learning in adult C57BL/6 mice:*

Spatial memory was investigated using clockmaze. Animals were given 4 trials per day for three successive days to locate the exit tube by using extra-maze cues. Untreated animals and the Vehicle ICV injected mice exhibit similar performance in clockmaze (data not shown). Conversely, there is a significant difference in STZ-ICV animals, which exhibit problems in learning the task (**Fig.5.12.**), whereas animals with normal functioning hippocampus are capable of finding the exit (using either spatial or sampling strategy) after the first day.

The learning curve represented by the plot for the percentage of spatial strategy over trials (**Fig.5.12.**) shows almost effective learning in both groups, but there is a significant difference in the rate of success. In the case of STZ-ICV mice, the minor improvement in the learning curve for spatial strategy does not represent a successful spatial learning process. The differences between groups of mice in choosing different strategies can be studied even closer by plotting the average number of errors made by each experimental group over 12 trails. The higher rate of errors in STZ-ICV mice implies the unsuccessful spatial learning in these animals, as they attempt to solve the task by trying every possible exit as a result of failing to solve it spatially. As it is obvious, STZ-ICV animals make significantly more errors in comparison to Veh-ICV animals (**Fig.5.13.**), indicating the incapability of this group to remember the exit. Moreover, STZ-ICV mice are less successful in solving the task than Veh-ICV animals (**Fig.5.14.**).

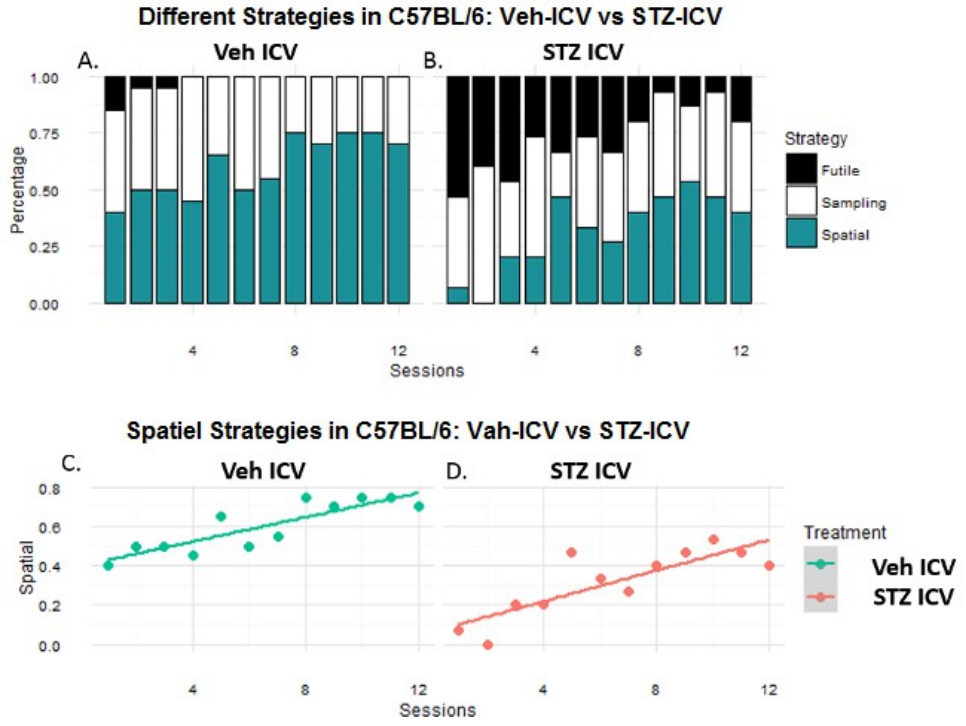


***Figure 5.12. Differences in preferred strategies by C57BL/6 mice***

***A. and B.*** *Percentage Stack bar chart representing different strategies used by animals to escape the clockmaze over 12 trials (Black; futile, failure in solving the task within one minute, white represents sampling or chaining strategy, where animal finds the open exit by testing every tube and green represent Spatial memory, when animal knows the location of the exit. Data is represents as themean±SEM; Saline ICV n = 20 and STZ- ICV n = 20). Whereas the Spatial memory is improved in Veh-ICV mice over 12 trials (A.), STZ-ICV animal showed difficulties in improving the contextual memory, resulting in either relying mainly on sampling strategy or failing the task (B.).*

***C. and D****. Average deploying of spatial strategy in C57BL/6 mice Veh-ICV and STZ-ICV. Spatial strategy significantly improves over 12 trials in Veh-ICV animals (n=20, Spearman rho= 0.85, y = 0.0311x + 0.3977, R2=0.7485, P Value (two tailed probability) = 0.000002). In contrast in STZ-ICV group, no changes were observed (n=20 Spearman rho = 0.26, y = 0.0394x + 0.0606, $R^2$ = 0.6911, P Value (two tailed probability) = 0.26). Implying the disruption of contextual memory as a result of STZ-ICV.*

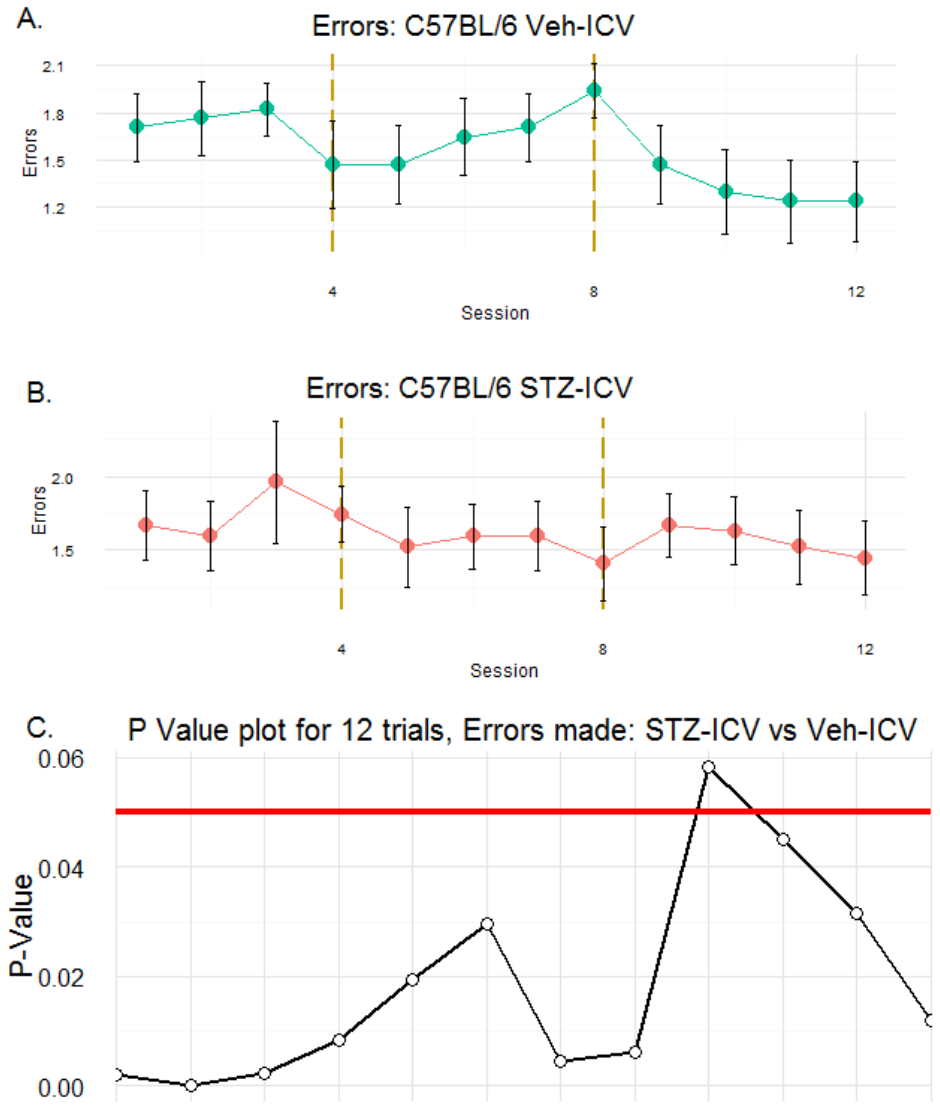


***Figure 5.13. Errors made by different experimental groups over 12 trials in the clockmaze task***

***A. and B.*** *Average number of errors made by each group over 12 trials (Errors defined average number of times animals tries a closed exit to solve the task (sampling behavior).* ***A.*** *The amount of errors made by Veh-ICV animals significantly decreased over 12 Trials (n=20, Student's T test), B. whereas no improvement was observed in STZ-ICV group (n=20)*

***C.*** *plotted P value (two tailed Student T-test), comparing the differences in errors made, between STZ-ICV and Veh-ICV over 12 Trials, red line represent P Value 0.05. Number of errors made significantly differs between test groups.*

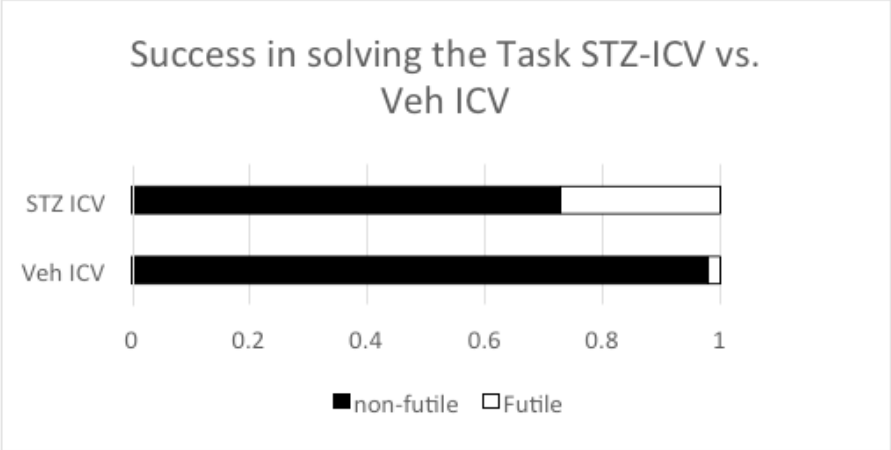


***Figure 5.14. Clockmaze Task; futile to non-futile ratio***

*The plot represent the ratio of non-Futile trials (where the task is solved using either Spatial or Sampling strategies) in comparison to Futile (failure to solve the task in 60 sec) in the last 4 trials between STZ-ICV (n=20) and Veh-ICV (n=20). Veh-ICV animal solved the task significantly more successful in comparison to STZ-ICV group (unpaired student's T test).*

Latency to finish the task is one of the primary measures that can be analyzed for the assessment of the clockmaze task. All animals initially require 60 sec or more to find the proper exit. In the clockmaze paradigm, decrease in latency order to find the escape tube from the maze is considered as a sign of acquisition of the task. Average latencies for untreated Vehicle and STZ injected animals on successive days (**Fig.5.15**) show improvements in performance for both STZ-ICV and Veh-ICV groups. Although the mean latencies decrease over the 12 trials for both groups, for Veh-ICV animals show a statistically significant decrease to find the correct exit (P value 0.01), whereas the decrease is not significant for the STZ-ICV group.

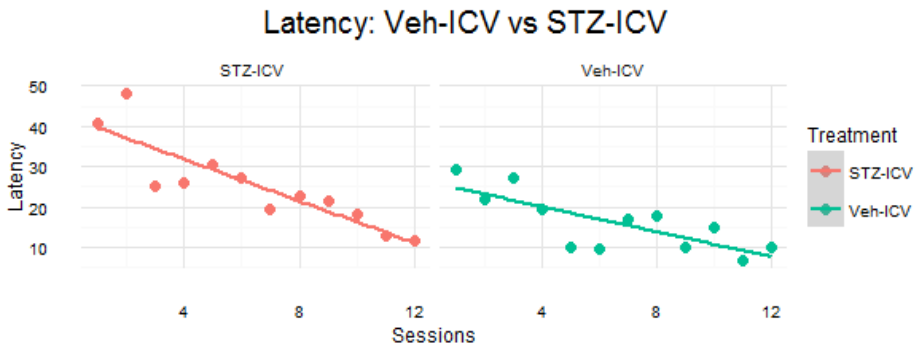


***Figure 5.15. Latency in different experimental groups***

*Amount of time needed for each group to solve the task in each trial. Data is represents as the average amount of time for each group; Saline ICV n = 20 and STZ- ICV n = 20.*

After the first day, Veh-ICV animals mostly learn to move towards the exit in a reliable manner represented by employing the spatial strategy and shorter latency time as they repeat the task in the following trials. In contrast to vehicle-ICV animals, there was no significant improvement in terms of latency and use of spatial strategy in the STZ-ICV group.

### *5.2.3. MIF inhibition in STZ-ICV mice influences spatial strategy preference*:

Inhibition of MIF in the brain by ISO-1 treatment (daily, IP, 20 mg/k) had no effect on choosing the spatial strategy in STZ-ICV animals in the 12 trials (**Fig.5.16**). However, there was an overall significant effect obvious in terms of finishing the task successfully by employing either spatial or sampling strategies (**Fig.5.17.**). Vehicle-ICV treated and untreated animals do not differ from each other, whereas the data for STZ-ICV injected animals seem to be noisier, and does not differ from the STZ-ICV mice that were treated with ISO-1.

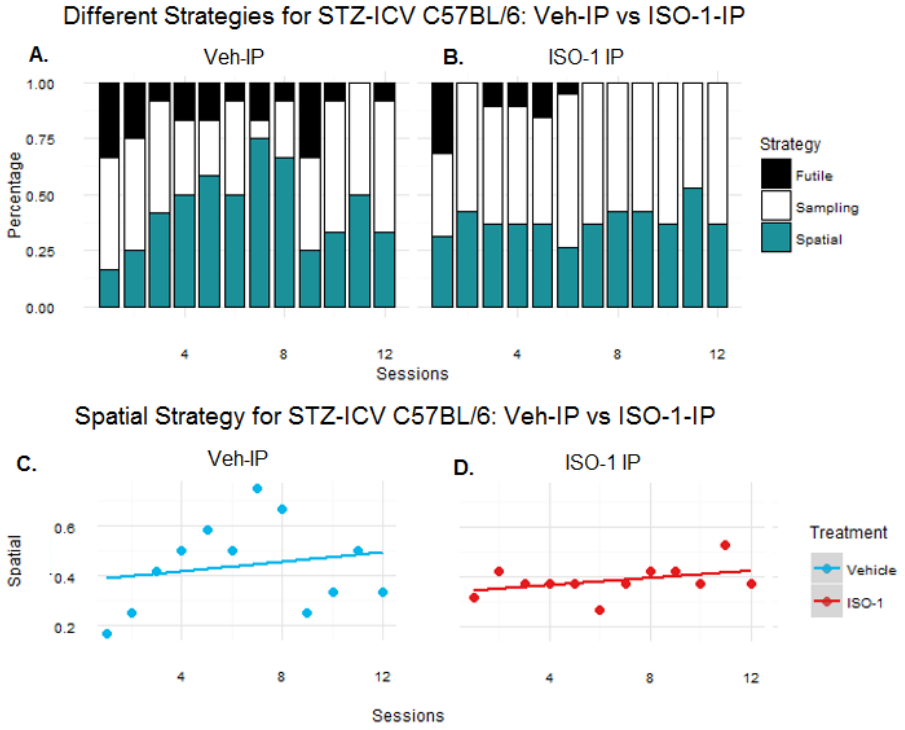


***Figure 5.16. Differences in preferred strategies by STZ-ICV***

***A. and B.*** *Percentage Stack bar chart representing different strategies used by animals to escape the clockmaze over 12 trials (Black; futile, failure in solving the task within 60 sec, white represents sampling or chaining strategy, where animal finds the open exit by testing every tube and green represent Spatial memory, when animal knows the location of the exit. Data is represents as the mean±SEM; Veh-IP n = 20 and ISO.1-IP n = 20). Either of groups showed improvement in solving the task in terms of using spatial strategy.*

***C. and D.*** *Spatial strategy in C57BL/6 mice Veh-IP and ISO.1-IP in STZ-ICV animal. D. ISO.1-IP treated groups performed slightly better in using spatial strategy (Spearman = 0.36, Y = 0.0072x + 0.3349, R2=0.752. P Value (two tailed probability) = 0.3) in comparison to Veh-IP group (C, STZ-ICV + Vehicle IP, Spearman = 0.201, y = 0.0096x + 0.375, R2=0.0379. P Value (two tailed probability) =0.4), however improvement either of the groups was statistically significant.*

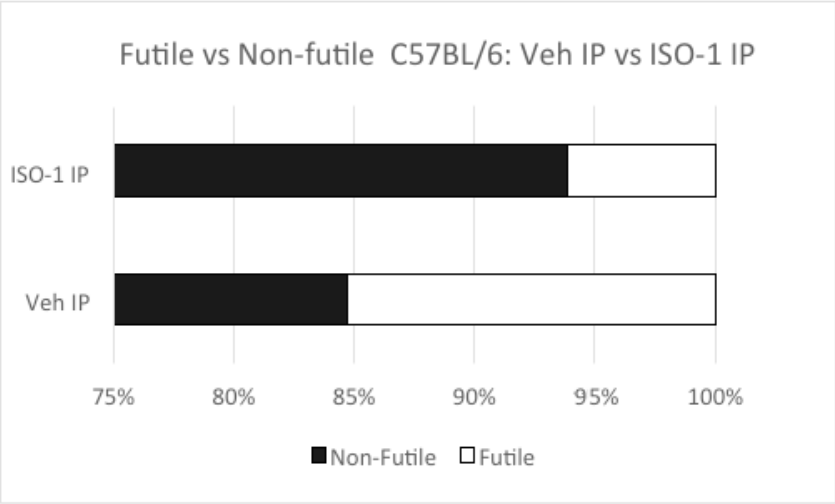


***Figure 5.17. Comparison between futile and non-futile ratio***

*Representation of the non-Futile trials to Futile in the last 4 trials between ISO.1-IP (n=20) and Veh-IP (n=20) in STZ-IC C57BL/6 mice. Although ISO-1 treatment did not significantly improve the choosing of spatial strategy, the general success in solving the task improved in ISO.1-IP group significantly (unpaired student's T test, P value = 0.019171).*

There was no significant main effect of ISO-1 treatment on the average of latencies over 12 trials (**Fig.5.18**). The time needed to escape the maze decreased in both groups (ISO-1 treatment and Veh –IP); however, there was no significant difference between groups.

Both experimental groups exhibited similar rate of errors over 12 tirals (**Fig. 5.19.**), indicating that inhibition of the MIF molecule, did not affect spatial learning in the STZ-ICV animal in the first 12 trials.

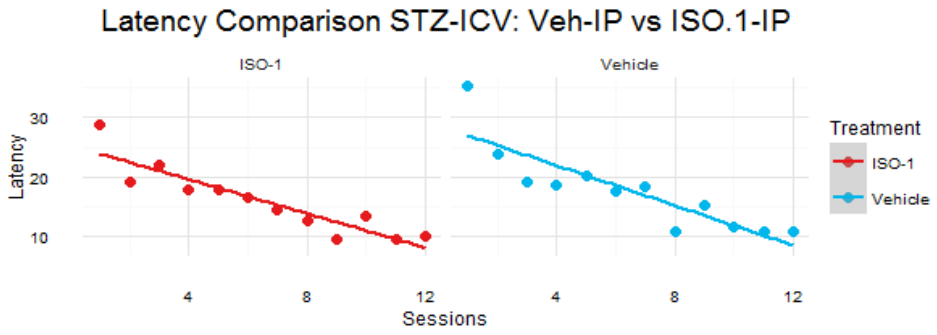


***Figure 5.18. Latency over 12 trials: Veh-IP vs. ISO.1-IP***

*Data represents the average of latencies of each group in 12 successive trials.*

*Improvement was not significant between the first and the last trial in either ISO-1 treatment (**Right,** n=20, Spearman = 0.36, Y = 1.4307x + 25.339, R2=0.8305, P Value (two tailed probability) = 0.1189), or in Veh-IP (**Left,** n=20, Spearman -0.201, y = -1.6789x + 28.695, R2= 0.7515, P Value (two tailed probability) =0.397*

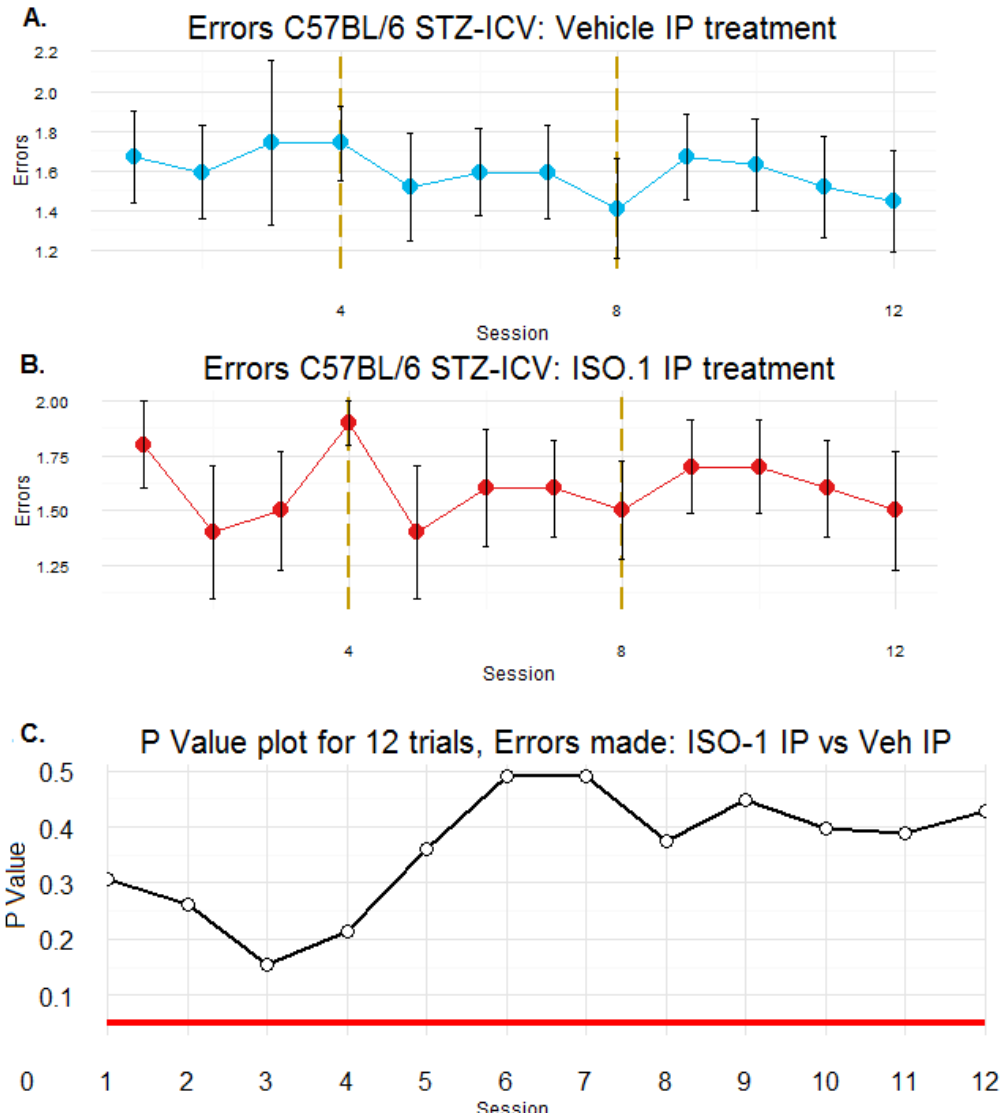


***Figure 5.19. Clockmaze; comparison of the errors made in each group over 12 trials***

***A. and B.*** *Average number of errors made by each group over 12 trials (Errors defined average number of times animals tries a closed exit to solve the task (sampling behavior).* ***A.*** *The amount of errors made by Veh IP animals (n=20) were generally more in comparison to ISO-1 treated group B, n=20). However no significant differences were observed between two experimental groups (****C.*** *plotted P value (two tailed Student T-test), comparing the differences in errors made between Veh-IP and ISO.1-IP)*

### *5.2.4. Probe test for spatial recall:*

Probe trials are designed to measure long-term memory as an act of recalling. We check the percent time spent in the target area, when the tunnel exit is physically blocked. The test is performed after the 12 acquisition trials, with the purpose to check for the long-term memory. Veh-ICV injected animals spend most of the time in the target area and relatively less time searching for other potential exits, differing in this test from STZ-ICV that insist on using a sampling strategy in trying to find the exit (data not shown).

### *5.2.5. Reverse learning trials:*

After the probe trial, reverse trails are performed in which the exit is moved to the opposite location in comparison to the original place. These trials can help confirm the observed spatial impairments. The expectation is for the animal to remember where the platform initially used to be in the first trials if the memory is functional, as a result of which it will take longer for this animal to find the new exit as a result of confusion and a biased search strategy. In the following trials the latency should decrease as a consequence of having a functional memory and learning the new location for the exit.
Veh-ICV and untreated groups tend to spend more time at the old target during the initial reversal trials, but over time they learn the new exit (**Fig.5 20**). The STZ-ICV injected group relies mostly on searching strategy (chaining), and the latency is improved slightly in this group over time. Information from the last day of the reverse trials provides evidence that the inhibition of the MIF was able to improve the cognition in terms of spatial learning, as the mice exhibit capability in using environmental cues to find their ways towards the exit. There is a near significant main effect of ISO-1 treatment on cumulative proximity scores in final reverse trials for spatial memory. As highlighted by the red box in Figure **5.20.B**, the ISO-1 IP group starts showing improvement in learning the task, indicating their capabilities in the spatial navigation, which is as mentioned before, hippocampal dependent.

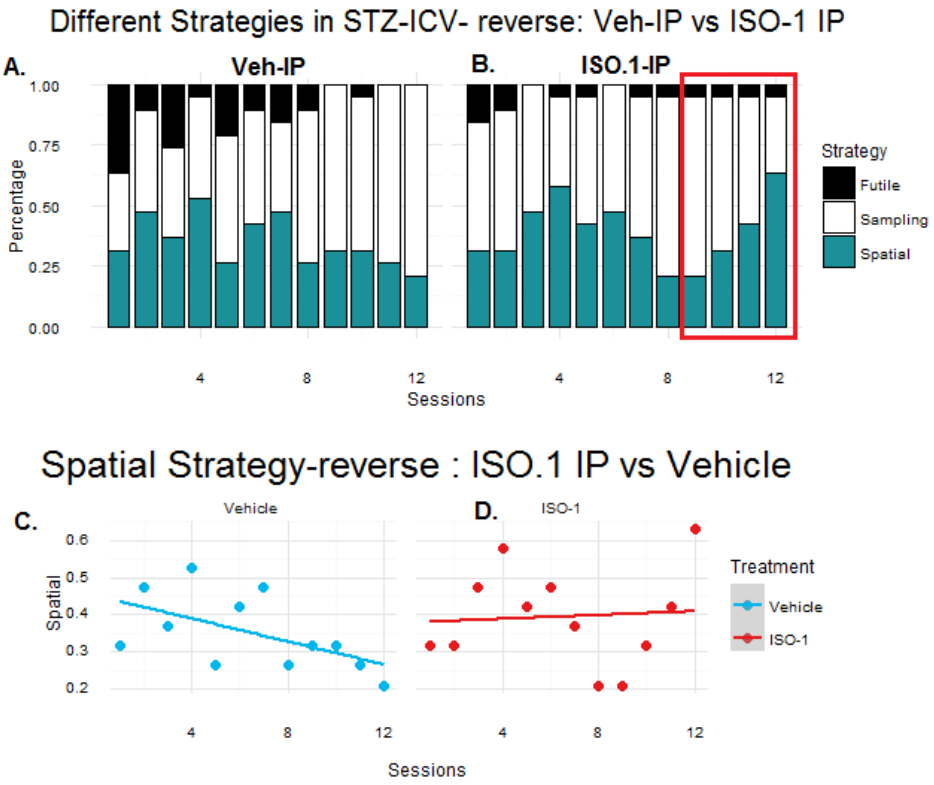


***Figure 5.20. Strategy use during reverse learning trials in the clockmaze task***

***A. and B.*** *Bar chart representing different strategies used by animals to escape the clockmaze in reverse trials (Trial 12 to 24, Data is represents as the mean±SEM; Veh-IP n = 20 and ISO.1-IP n = 20).* ***A*** *and* ***C*** *Animal in the Veh-IP treatment tended to worsen over time in terms of choosing the spatial strategy to solve the task, whereas as ISO-1 treatment group showed a trend in using spatial memory specially at the last four trials-* ***B,*** *(area framed in red) and* ***D.***

An analysis of the latency, the time needed to escape the maze, during the reverse learning trials show that the performance of the animals is not different between treatment groups (**Fig.5.21.**)

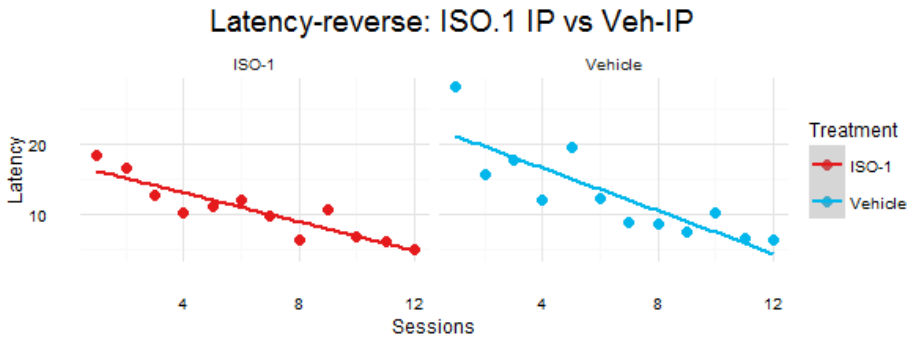


***Figure 5.21. Latency differences in reverse trials for STZ-ICV injected animals, in different treatments***

*Data represents the average of latencies of each group in reverse trials (12 to 24). Latency was significantly decreased in ISO-1 treated group (**Left,** n=20, IP Spearman =0.902, y = -1.0365x + 17.248, R2= 0.8161, P Value (two tailed probability) = 0.00001) and Veh-IP group (**Right,** n=20, Spearman =- 0.895 Y = -1.5275x + 22.772 R2= 0.7093, P Value (two tailed probability) = 0.00001)*

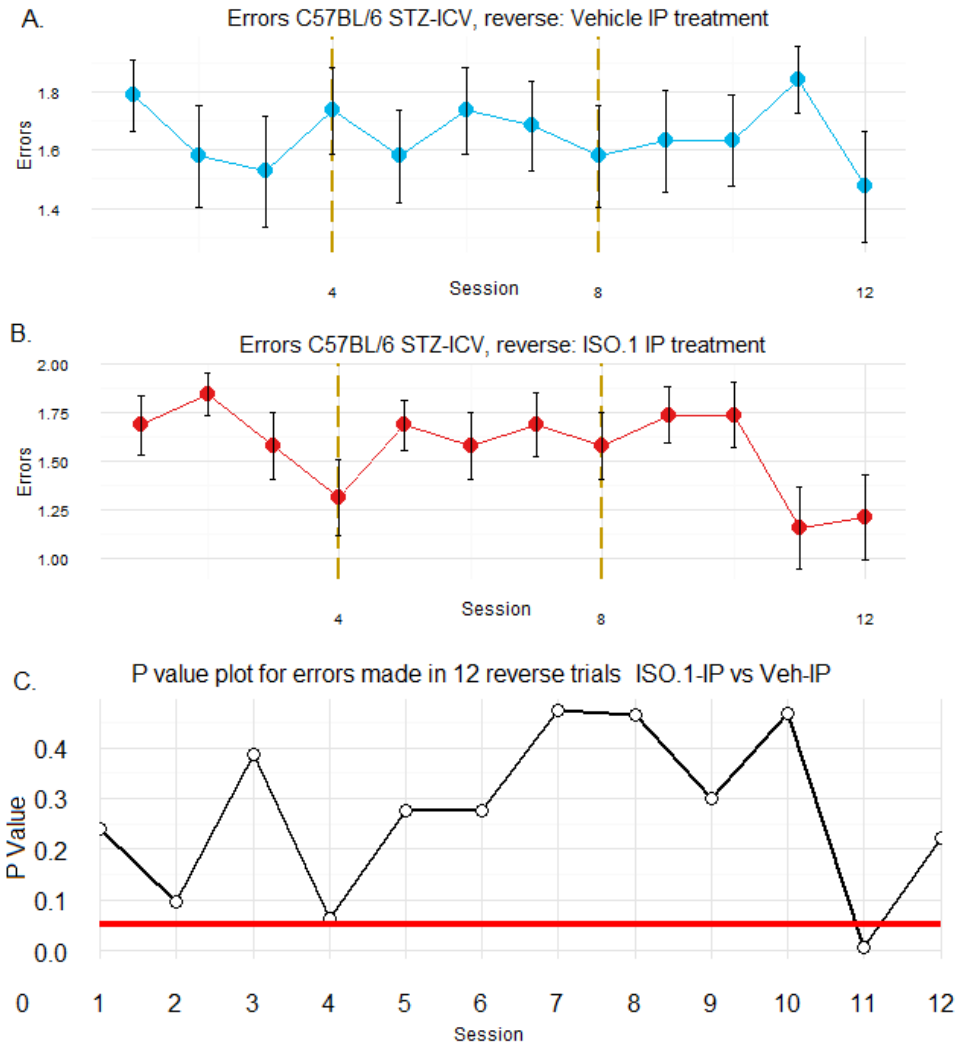


***Figure 5.22. Reverse Trials: comparison of the errors made between two groups.***

***A. and B.*** *Average number of errors made by each group over 12 trials (Errors defined average number of times animals tries a closed exit to solve the task (sampling behavior).* ***A.*** *Veh IP group (n=20) had more errors in comparison to the treatment group (**B**, n=20), the observation however was not supported by statistical analysis (**C.** plotted P value (two tailed Student T-test), comparing the differences in errors made between Veh-IP and ISO.1-IP)*

### 5.2.6. Effects of neuroinflammation on hippocampal cytokine gene expression

In the same time frame in which spatial memory is disrupted, pro-inflammtory cytokines are elevated in hippocampus of the STZ-ICV mice. Vehicle-ICV animals, however, have no upregulation in expression of those mediators. The mRNA was prepared from hippocampus of different experimental groups of mice and reverse-transcribed into cDNA for the purpose of investigating the presence of several inflammatory cytokines to determine any lasting changes in immune milieu of the hippocampus. As a first step, we looked for upregulation in GFAP and Iba1, which are well known markers for astrocytic and microglia cells. We observed an obvious and significant increase in both of these markers, meaning that ICV injection of the STZ molecule, leads to the activation of the glial population of the hippocampus (**Fig.5.23.**). These cells are considered responsible for the ongoing and constant expression of inflammatory mediators such as cytokines.

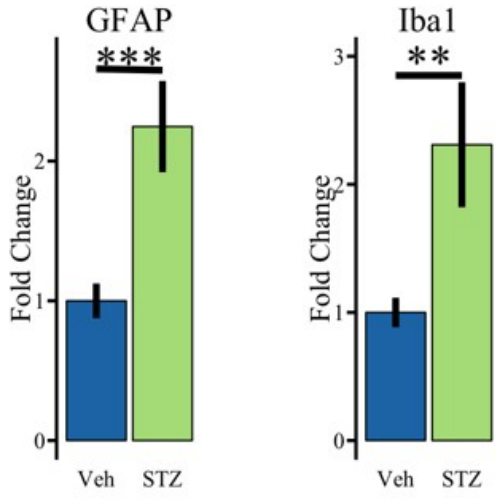


***Figure 5.23. Upregulation of glial markers in transcriptional levels in hippocampus of STZ-ICV C57BL/6 mice***

*mRNA was isolated from hippocampus and reverse transcribed, followed by performing a quantitative PCR, using Bactin as housekeeping gene. The bars represent the mean ± SEM for 10 mice per group (10 biological replicate, each of which having two technical replicates- **P<0.02 and ***P<0.01 (Mann-Whitney Test).**Left:** Increase in Glial fibrillary acidic protein (GFAP) marker in STZ-ICV animal, as an indicator of astrogliosis, as well as upregulation oflba-1 (**Right**) the microglial marker.*

The study was followed by testing the upregulation of different inflammatory cytokines, such as interferon-α, interferon-β, IL-6 and IL-12 (**Fig.5.24.)**. Except for IFN-β, there has been a clear upregulation in the inflammatory cytokines in the hippocampus.

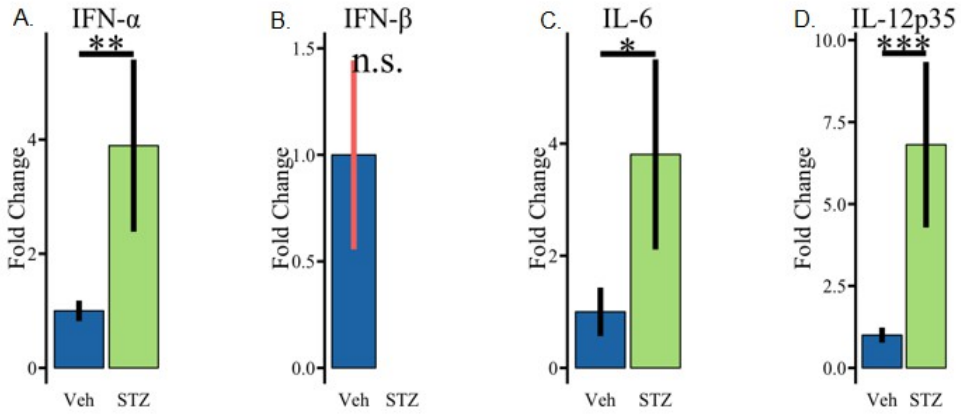


***Figure 5.24. cytokine response of hippocampus in STZ-ICV model***

*qPCR analysis, using Beta-actin as housekeeping gene. Graphs represent the mean ± SEM for 10 mice per group (10 biological replicate, each of which having two technical replicates- *P<0.05, **P<0.02 and ***P<0.01 Mann-Whitney Test).*

*STZ-ICV lead to the significant upregulation of many Inflammatory mediators such as TNF-alpha* ***(A.),*** *IL-6* ***(C.)*** *and IL-12p35* ***(D.)*** *in hippocampus of C57BL/6 animals. The mRNA expression levels for IFN-beta remained unaffected* ***(B.)****.*

### *5.2.7. MIF inhibition by ISO-1 molecule does not affect activation of the glial cells:*

Based on qPCR results, inhibition of MIF did not affect the activation of microglia or astrocyte cells, which was reflected in the similar expression levels of GFAP and Iba-1. Similar observations were made in the case of different inflammatory cytokines. Except for some levels of down-regulation on IL-6, no obvious down-regulation has been observed in other cytokines in the mRNA levels (**Fig.5.25**).

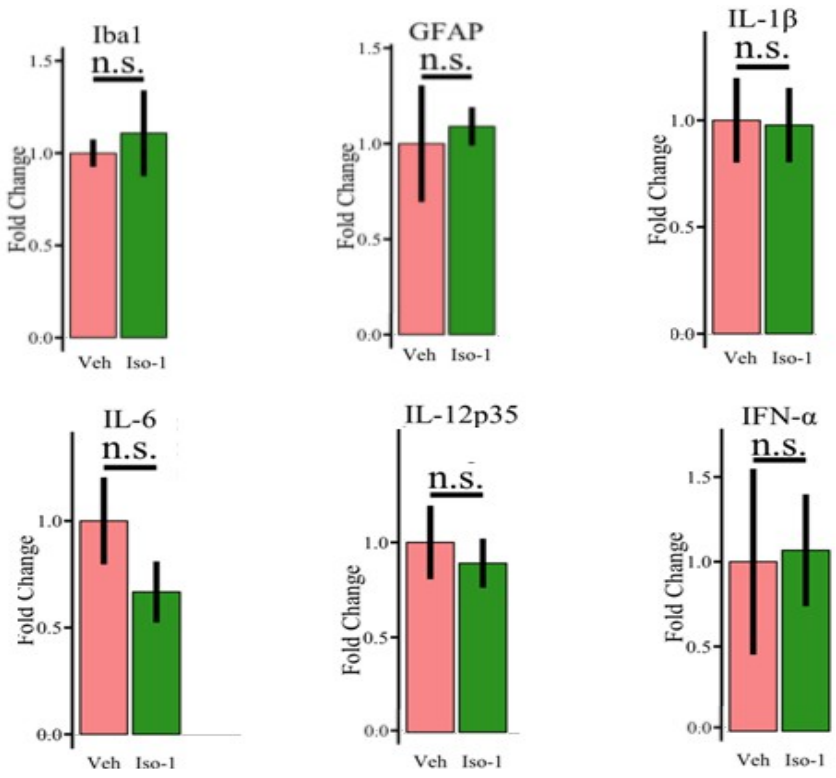


***Figure 5.25. cytokine expression in hippocampus of STZ-ICV C57BL/6, under different treatments: Veh-IP vs ISO.1-IP***

*Reverse transcribed hippocampal mRNA was used to perform a quantitative PCR, using Bactin as housekeeping gene. The bars represent the mean ± SEM for 10 mice per group (10 biological replicate, each of which having two technical replicates). ISO-1 treatment did not affect the mRNA level glial markers (GFAP and Iba-1) or cytokines, however a trend was seen in the case of IL-6 and IL-12p35.*

## 5.3. Effects of STZ-ICV related neuroinflammation on context memory

Considering the potential important role of the MIF inhibition for preventing this inflammatory-related pathology, we decided to test another hippocampal related paradigm checking for contextual fear memory in male animals (MIF-KO, C57BL/6).

In this section the experiments were designed to:

- Investigate the regulation of inflammatory markers in the hippocampus of the MIF-KO STZ injected mice in comparison to MIF-KO Veh injected mice and WT control mice.
- Train animals for contextual fear conditioning paradigm to associate a conditioned stimulus (CS) with an unconditioned stimulus (US), looking for deficits in learning and memory.
- Monitor the weight change in MIF-KO mice in comparison to WT mice.
- In order to investigate the answers to these questions, MIF-KO animals were injected intracerebroventricularly by STZ and 4 weeks later were trained for fear conditioning paradigm. The mice from previous experimental setup were also tested in the fear-conditioning task as a source of comparison.

The cued fear conditioning was conducted over the period of four days:

1. Day 1: the animals are habituated to the experiment chamber for 10 min.
2. Day 2: animals learn to associate an unconditioned stimulus (foot shock) with conditioned stimulus (auditory tone) over 20 min.
3. Day 3: Context memory: animals are returned to the same chamber in which they were shocked and the software scores the amount of freezing.
4. Day 4: Tone memory: the original chamber is replaced and animal is exposed to the new chamber followed by presentation the conditioned stimulus (auditory tone).

In the fear-conditioning paradigm the mice learned to associate the tone with a foot shock (**Fig.5.26.**).

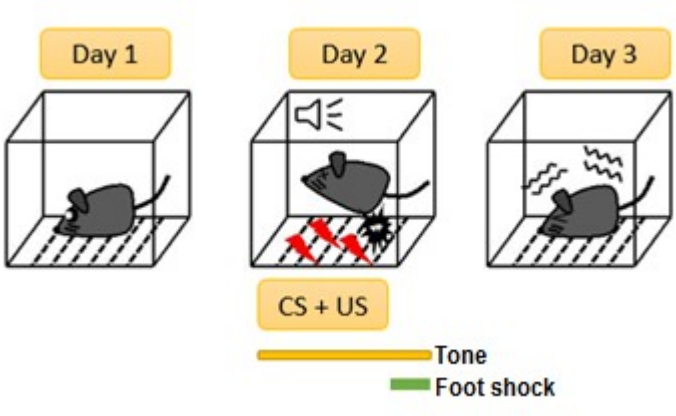


***Figure 5.26. Fear conditioning: concept***

*The rodent is habituated to a chamber, at this point there is nothing novel about this particular context. On the second day a tone in displayed and during that time the foot shock (unconditioned stimulus) is represented to the animal, which is just aversive and not painful. The animal is exposed to the tone for five times. A day later animal is returned to the chamber. Under normal healthy brain conditions the profound fear should be present, which is measured with freezing.*

The amygdala, hippocampus and sensory neocortical are the areas of the brain that play the critical role in learning process in the fear conditioning task. While the lesions in the hippocampal area cause problems exclusively with contextual memory (Philips and LeDoux 1992), any lesions in amygdala will affect both tone and contextual memory (Stephen Maren 1999).

### *5.3.1. Chronic inflammation caused by STZ-ICV in hippocampus disrupts contextual fear conditioning in C57BL/6 mice:*

In the behavioral studies we focused on the role of chronic hippocampal neuroinflammation during contextual fear conditioning. WT and MIF-KO mice underwent the fear conditioning protocol and the percent freezing during each phase of the task was measured. In a pilot study, STZ-ICV animals were compared to Veh-ICV treated mice (**Fig. 5. 27.**). The results presented here showed a clear cognitive difference between STZ-ICV injected and untreated WT animals. Vehicle injected C57BL/6 animals froze significantly more than STZ-ICV animals. The tone memory response (amygdala dependent) was similar in both groups.

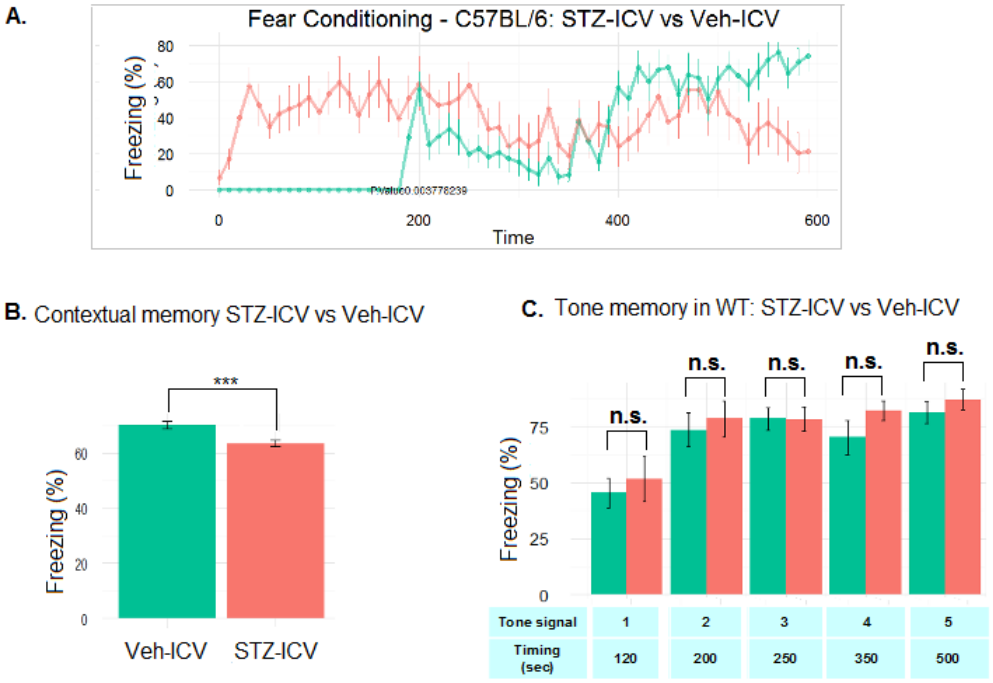


***Figure 5.27. Fear conditioning STZ-ICV vs Veh-ICV***

***A.*** *During the training session, STZ-ICV (n=20) and Veh-ICV (n=20)- mice received 5 light shocks pairing with a tone signal, data is represented as* mean ± SEM.

***B.*** *During the context memory task, the Veh-ICV group froze significantly more in comparison to STZ-ICV animals, analysis of variance (one way ANOVA) followed by unpaired t-test was used to calculate the statistical significance (***P<0.001, F=29.691)*

***C.*** *Graphs represent the reaction of each treatment group in the tone-memory task. Bars represent the average percentage of freezing in each group in exposure to the tone cue* ± SEM. *indicates no difference between experimental groups (all p>0.05)*

Knowing that STZ-induced inflammation in the hippocampus disrupts the formation of the context memory, we intended to investigate the effects of MIF inhibition on the context memory. For this purpose, two groups of STZ-ICV animal were treated daily with either ISO-1 IP or Veh-IP as a source of control. **Figure 5. 28.A.** illustrates the behavior of these animals during the fear conditioning itself. There are no significant differences to be observed at this step of the experiments.

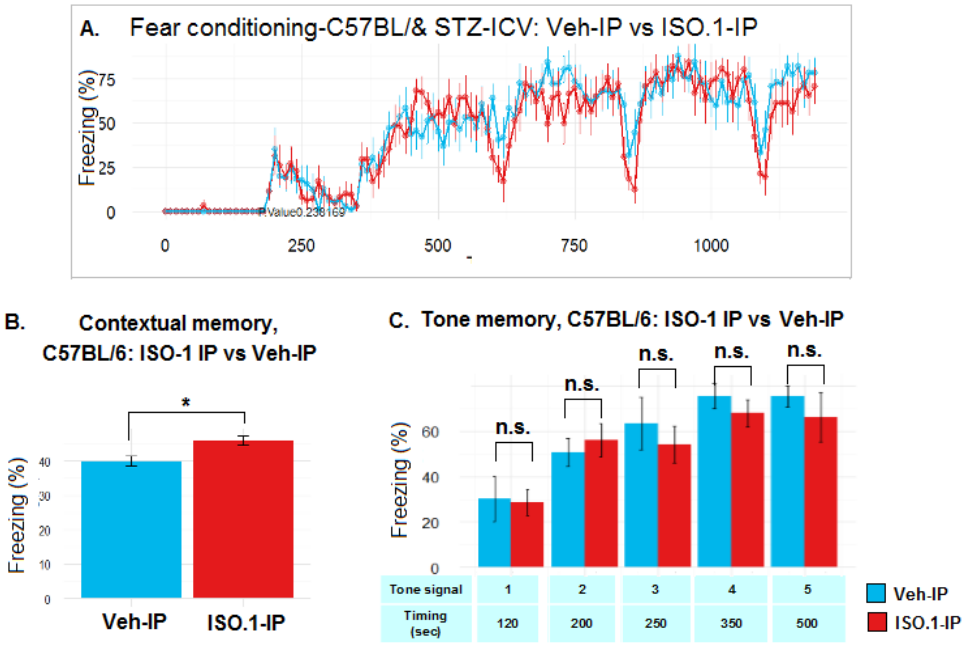


***Figure 5.28. Effects of MIF inhibition on fear conditioning in C57BL/6***

***A.*** *The graph illustrates the average ± SEM of freezing in ISO.1-IP (red, n=20) and Veh-IP (blue, n=20) during the training session(a tone plus shock pairing, 0.5-mA, repeated 5 times). Both groups showed similar reaction during the Fear conditioning session.*

***B.*** *Contextual fear conditioning in STZ-ICV C57BL/6 mice, Veh vs ISO-1 treatment: Mice were tested for this task 24h after fear conditioning session. ISO-1 treated animals exhibit significantly higher freezing in comparison to Veh-IP, Values are mean ± SEM, *P<0.05 (One- way ANOVA: df=1, F=4.751, Student T test P.value = 0.033)*

*C. Tone memory task, designed to test the amygdala dependent auditory fear memory, statistical analysis revealed no significant differences between two groups.*

As tested in the fear-conditioning chamber, we observed improvements in the contextual memory in C57BL/6 mice after ISO-1 treatment (**Fig.5.28.B.**). Inhibition of MIF using ISO-1, significantly increased the amount of freezing in the animals, which indicates the improvement in contextual memory, which is considered to be hippocampus dependent ((Philips and LeDoux 1992). The animals exhibit no differences in response to tone memory.

To make sure that the observation made is indeed driven by MIF deficiency, another strain of mice were added to the project. In this step we injected MIF-KO male mice with either STZ or vehicle intracerebroventricularly. Six weeks after injection the animals were tested in the same fear-conditioning chamber under the same protocol performed for C57BL/6 mice. The reaction of the MIF-KO mice in the different treatment groups is summarized in figure **5.29.**

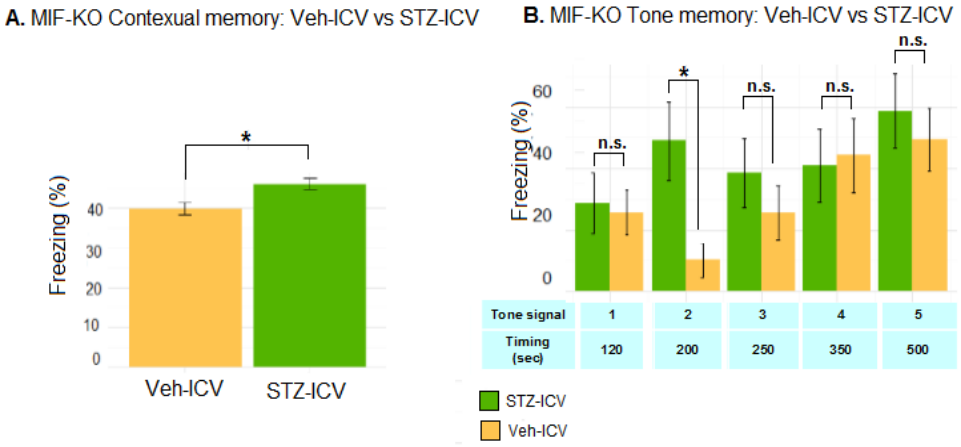


***Figure 5.29. Effects of MIF deficiency in MIF-KO mice on fear conditioning, contextual memory and tone memory in STZ-ICV mice.*** All results are presented as a mean ± SEM.

***A.*** *comparison of hippocampus dependent contextual memory between umanipulated MIF KO represented in yellow (n=10), and STZ-ICV group (green, n=10). Contextual memory was improved in STZ-ICV animals (One-way ANOVA, unpaired students T testF=12.8, P=0.01).*

***B.*** *Tone-memory, after being fear conditioned with 5 tone-shock pairings, the degree of freezing in response to tone was scored,one way ANOVA revealed no significant differences between two groups for this task.*

Interestingly, we observed that the MIF-KO mice at the baseline acted differently in comparison to C57BL/6 mice (**Fig.5.30.**). And the STZ-ICV increased the alertness and cognitive performance in these animals in the case of both context and tone memory.

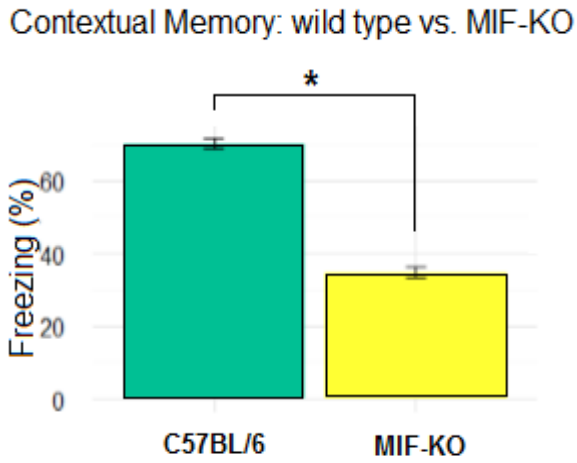


***Figure 5.30. Contextual fear conditioning in un-manipulated C57BL/6 and MIF-KO mice***

*(n = 20 in each group) - Bar graphs show mean ± s.e.m.*P< 0.05.*
*The contextual memory was measured by total freezing time as the animal was placed back inside the same chamber where the fear conditioning took place after 24 hours.*
*Green bars represent the un-manipulated wild type in comparison to the un-manipulated MIF-KO (yellow), Significant difference was observed between two group.*

After making the observation about the cognition in MIF-KO animals, we were interested in monitoring the behavior of these animals in the extinction of the fear. As mentioned before, exposure to the tone, which is associated with foot-shock during fear conditioning, results in freezing behavior. The degree of this freezing response depends on the level of the anxiety and functional brain structures especially hippocampus and amygdala when it comes to testing the contextual memory. This test consists of two trials in two successive days, where the animal is repeatedly exposed to the conditioned stimulus, without any punishment following. Under normal circumstances, this results in a decline in response to fear. We observed a steady decline in the fear response of both STZ-ICV and Veh-ICV animals almost to the floor level at the end of the second day (**Fig.5.31.**). Further studies are necessary for explaining the exact role on MIF in this neural circuitry.

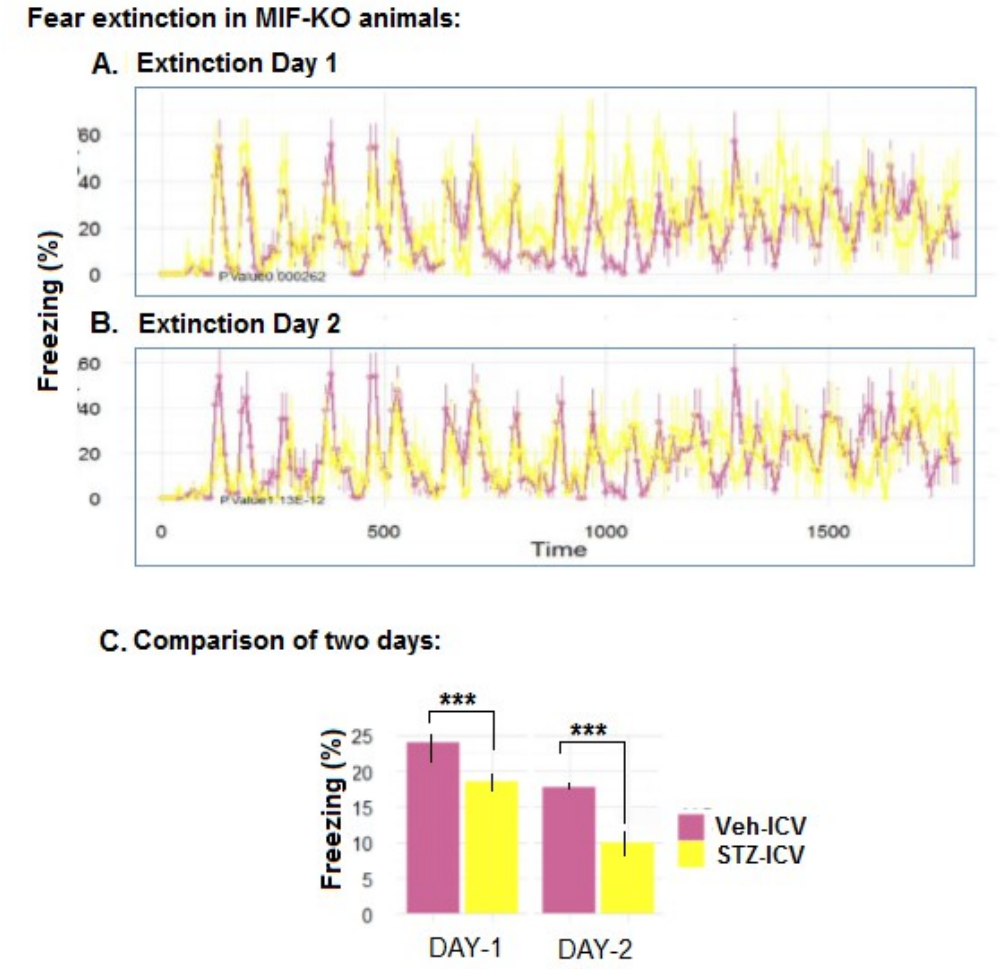


***Figure 5.31. Fear extinction in MIF-KO animals***

***A. and B.*** *graphs represent comparison of Veh-ICV MIF-KO and STZ-ICV MIF-KO in terms of percentage of freezing on the Day one (**A.**) and Day two (**B.**) of the extinction learning protocol. The reaction to the auditory cue over time (measured in seconds) was recorded continuously on the camera, and is represented as average freezing for each group in 10 sec bins in purple for Veh-ICV (n=10) and yellow for STZ-ICV (n=10).*

***C.*** *Bar graphs presentation of the average freezing response during the last three tones of each Trial for the same experiment as described above. The freezing level decreased in both groups in from the first to the second extinction training session. STZ-ICV group froze significantly less than Veh-ICV group.( one way ANOVA, Day 1 F= 24.39, ***P<0.001. Day 2 F=22.838 ***P<0.001)*

### *5.3.2. Inhibition of MIF using ISO-1 and MIF deficiency (in MIF-KO) rescue the weight loss after STZ-ICV surgery:*

Throughout the duration of the study, the weight of the animals were documented on a weekly basis to monitor the changes as a result of inflammation as well as making sure that it does not drop dramatically below the pre-surgery weight. The food intake was also documented on a daily basis. **Figure 5.32**. represents the graph for weigh measurement for different groups of animals throughout the study.

After surgery the body weight markedly decreased in STZ-ICV *C57BL/6* animals, which was inhibited by ISO-1 treatment. Interestingly the MIF-KO mice were protected from weight loss.

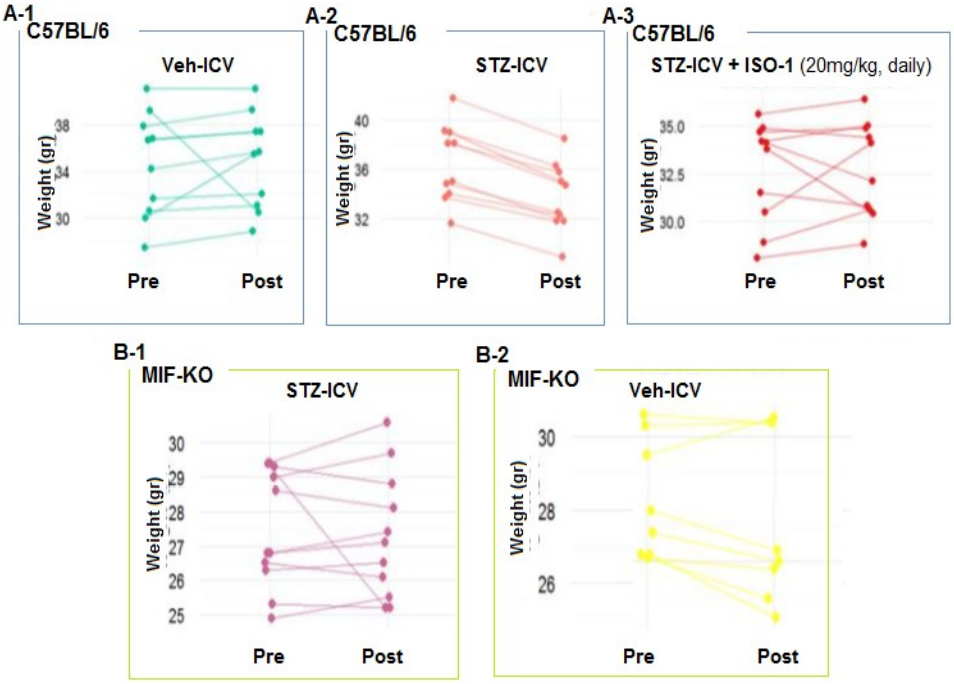


***Figure 5.32. Spaghetti plot of weight in different groups pre and post surgery,*** *each line represent the weight for one individual mouse before and 6 weeks after surgery.*

***C57BL/6:***

***A-1*** *the horizontal line representing the steady weight before and after the surgery in vehicle injected mice, in comparison to* ***A-2*** *where a steep drop was observed after the surgery,which was inhibited by ISO-1 treatment (****A-3:*** *daily dose of 20mg/kg, IP).*

***MIF-KO:***

***B-1*** *represents the weight of MIF-KO animal before and 6 weeks after the surgery, the horizontal lines represent the steady weight in these animals in contrast to STZ-ICV in wild type* ***(A-2). B-2*** *represents the weight documentation in the control group.*

# 7. Discussion

Sustained inflammation is recognized to have a critical role in development and progression of neurodegenerative diseases.

The innate immune response in the brain is mainly regulated by microglia and astrocytes (Hernangómez et al. 2014), these cells are the main contributors to neuroinflammation. Under normal conditions, they are transiently activated by a variety of triggers, including pathogens, providing inflammatory mechanisms to defeat the pathogen.

However chronic activation of microglia and astrocytes can instigate inflammatory events leading to neurotoxicity through various molecular signaling pathways, as a result of a toxic neuronal microenvironment. The release of various cytokines constitutes the hallmark of the inflammatory response. Indeed, cytokines have gained increased recognition as critical factors in neuroinflammation, and consequently in neuro-degeneration, both as biomarkers (diagnosis), as well as therapeutic targets. For instance, the levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 are considered as indicators of inflammation (Koleva-Georgieva et al. 2011). Recent evidence indicates that MIF, as an early cytokine, may play a critical role in regulating the expression and release of other inflammation mediators (Bucala 1994). This cytokine is detected in different areas of the brain like hypothalamus, hippocampus, and perivascular epithelial cells (Silverman et al. 2005, Bacher et al. 1998). Elevation in MIF's expression overtime is associated with chronic inflammation (Alexander et al. 2012). In our project, we hypothesized that inhibition of MIF as an early released cytokine upstream of other mediators of inflammation can down regulate the inflammation. Our *in vitro* data indicates a baseline level of MIF released by glial cells under normal, unstimulated conditions, as was reported before (Bacher et al. 1998). The presence of this protein under normal conditions can be explained by the fact that MIF functions also as a hormone, or neuroendocrine mediator, in addition to the role that it plays as a proinflammatory cytokine (Mitchell et al. 2002). MIF is known to be an early phase mediator released as soon as 30 min after stimulation (FingerleRowson et al. 2002) and plays a critical role in regulating expression and release of other inflammatory cytokines.

One major question that remains to be answered is the role of MIF in neurons. We made the interesting observation that unlike glia cells, the presence or lack of MIF was uncoupled from control of cytokine production in

MIF-KO and wild type neurons. This result provides basis for future investigations on which downstream-mediators of STZ are inducing the cytokine expression from neurons.

Our *in vitro* data support the hypothesis that MIF acts as an early phase cytokine released both from microglia and astrocytes resulting in upregulation and release of other inflammatory mediators. Inhibition of MIF leads to attenuation of this response. Although we observed attenuation of the cytokine storm in response to MIF inhibition *in vitro,* further investigation (using other inhibitors for MIF, as well as using different molecules to stimulate the cells such as LPS, Aß, etc...) is required to prove causality of inflammatory changes. Following *in vitro* studies, we aimed to investigate the effects of acute inflammatory response and MIF inhibition in the brain. Neuroinflammation was achieved by ICV injection of the STZ molecule into the hippocampus. Unlike other Alzheimer's models, the glial activation and cytokine involvement in cognitive deficits can be extensively researched in STZ-ICV model. It has been demonstrated that acute inflammatory response subsequent to STZ treatment is sufficient to mount a microglial and astrocytic response along with histological changes. We observed dramatic histological alterations in the STZ-ICV group in terms of upregulation in the glial markers such as GFAP and Iba1 in 6 weeks after the surgery.

The *in vivo* qPCR results for inflammatory mediators after ISO-1 treatments appear to be inconclusive. On one hand we observed in preliminary in vitro studies that MIF inhibition can effectively down regulate glial cytokine production. A down regulatory trend in production of cytokines in C57BL/6 STZ-ICV animals, however it was not significant. On the other hand however, we detected absolutely no upregulation in any of inflammatory markers in MIF-KO strain after STZ-ICV, showing the importance of MIF in initiating the inflammatory response and enhancing the expression levels for other cytokines.

There is a possibility that one should check the time point later than 6 weeks after beginning the ISO-1 IP (20mg/kg) treatment to investigate whether MIF inhibition can effectively control the local inflammation in hippocampus. Also considering the fact that mice start to exhibit improvements in spatial learning in later trials of clockmaze followed by a significant improvement in contextual memory in fear conditioning task while they are still receiving the treatment, could point out to the fact that the chosen time frame was still early to investigate the effects of MIF inhibition in the brain. In a different scenario, the

detectable level of cytokines after ISO-1 treatment can be explained by the presence of D-DT or MIF-2, which might be upregulated in a negative feedback loop and as a result of MIF inhibition.

However, due to the presence of both MIF and D-DT mRNA at baseline levels both *in vivo* and *in vitro*, we were unable to detect any changes in mRNA expression of either of the proteins. In order to complete inhibition of MIF and D-DT activity, further experiments are necessary to test the effects of a co-treatment against D-DT along with using MIF inhibitor.

It is known that excessive expression of the pro-inflammatory cytokines in response to central or peripheral stimulation can cause behavioral pathology and cognitive deficits. For example, it has been shown that ICV administration of IL-1β affects learning and memory especially in contextual memory (Mencl et al. 2000). Also, IL-6 elevation and cognitive dysfunction are positively correlated (Maggio et al. 2006; Weaver et al. 2002). Considering the evidence on the role of MIF in regulating pro-inflammatory cytokines, we hypothesized the attenuation of the inflammatory mediators as a result of MIF (genetically knock-out or chemical inhibition of the molecule) will improve the hippocampal based behavioral dysfunction observed in STZ-ICV group. Cognitive deficits were evident in both learning and memory of STZ-ICV group in comparison to vehicle-injected animals. At the same time point in which cognitive impairment were detectable, pro-inflammatory cytokines were upregulated and astrocytic reactivity was increased (upregulation in GFAP), pointing out to the tight relationship between neuroinflammation in hippocampus and changes in structural plasticity which may underlie cognitive dysfunction in neurodegenerative disorders.

In terms of deploying the spatial memory, we observed no differences between untreated and vehicle-injected animals, indicating the surgery itself has no destructive effects on hippocampus and subsequently on learning and memory in these animals. STZ-ICV injection in mice, however, leads to noticeable impairments shown by failure in either learning to solve the task spatially or being entirely unsuccessful. Success or failure in performance of the clockmaze task can be influenced by many factors such sex, species, age, strain, treatment and motor skills. In our experiment, however, animals were chosen from the same litter and sex. Our screening results suggest that sensory and motor skills are no factors that might account for differences observed in memory performance. Even at the training stage of the clockmaze task, there was a significant difference between the performances

of vehicle and STZ-ICV group. This can be explained by the lack of motivation in the STZ-ICV animals, due to the constant presence of inflammatory mediators in CNS, which may cause sickness behavior and difficulties in habituating to a new stressful environment. This study showed the MIF inhibition improved reference memory performance in the final trials in STZ-ICV animal group, as compared to agematched STZ-ICV groups, but had no significant effects on cognitive flexibility. However, some evidence suggests limited beneficial role of MIF inhibition in cognition, which was observed in the late trials, Thus further work is necessary in this model to determine the exact anti-inflammatory therapeutic potential in inhibition of MIF. Our chosen application method (daily IP) may not be the most appropriate option for delivering the drug to the brain of the animals. It has been previously shown that ISO-1 is capable of crossing the blood brain barrier, yet it doesn't mean that all the IP injected substance, finds its way into the CNS. Insufficient dose to elicit the protective effects may be an explanation why the expected effect was not observed. Optionally, one might suggest that intravenous injections or intracerebroventricular treatment with ISO-1 represent better routes of injection. Both routes of administration are not considered easy choices. Intravenous (IV) applications are considered difficult in murine models and ICV interventions are accompanied by many side effects apart from being challenging and tiring for the health status of the animal itself. Increasing daily treatment sessions maybe another option in order to increase the concentration of ISO-1 in the CNS and as a result of that increased inhibition of MIF molecule. Alternatively, we can continue with ISO-1 IP daily regimen and assess the re-learning of the task in these mice after a longer period of time, to control whether there is any sign in recovery in spatial memory. Optionally one may try other inhibitors for the MIF molecule.

Upregulation of the cytokine was used as an indicator for presence of chronic neuroinflammation in the brain, however we detected no expression for different cytokines in the hippocampus of the MIF-KO animals. The fact that STZ injection in these animals did not elevate those cytokines in contrast to WT animals, strongly suggests that MIF plays a key role in this inflammatory response.

In tone memory task, the mice learn to associate the one (CS) and the foot-shock (US), the task is mainly controlled by amygdala. Lack of cognitive impairments in the case of tone memory task in fear conditioning indicates to the fact that the observed hippocampal dependent cognitive deficits are not

as a result of systemic changes, but occur following local changes (either histological or inflammatory) in hippocampus area. However due to the small size of the mouse brain, it was not possible to obtain separate mRNA samples from amygdala to check the levels for expression of different cytokines. Systemic neuroinflammation models can be used in order to test whether MIF inhibition can affect amygdala associated behaviors or not. In contrast to tone memory different treatment groups of mice exhibit significant differences in amount of freezing time in hippocamapal dependent contextual memory. These results showed a deficit in contextual memory, possibly related to neuroinflammation or neurodegeneration caused by STZ in the hippocampal area, which was even more confirmed by having the similar response in the tone memory, which is amygdala dependent. We also observed a significant protective effect followed by ISO-1 IP injection, which was reflected in increased total time of freezing in these mice in comparison to Veh-IP injected STZ-ICV mice. ISO-1 binds to tautomerase active site in the MIF molecule, resulting in inhibition of the cytokine activity of MIF. These findings are congruent with existing literature, which shows that inhibition of MIF leads to down-regulation of the cytokines (Al-Abed et al. 2005). It is therefore reasonable to assume that deficits in hippocampal based cognition can be targeted through attenuation of cytokine production. For this purpose MIF seems be a proper target. Our data provides evidence that due to lack of MIF in the MIF-KO mouse strain, these animals were protected against upregulation of many cytokines after STZ stimulation.

There is a general difference in the overall behavior of the MIF-KO mice and WT animals, which can be interpreted as sickness behavior resulting from the lack of MIF in these MIF-KO mice. It has been previously shown that the MIF-KO mice exhibit depressive like behavior (Bay-Richter et al. 2015). This might as well indicate the role of MIF in inflammatory processes that are present in psychotic disorders, also raising question whether there shared mechanism between sickness like or depressive behavior and dementia exist. It has been suggested that brain inflammation around hippocampal NMDA receptors (NMDA-R1) can lead to neurodegeneration in the glutamatergic neurons expressing these receptors, which is associated with behavioral deficits (Rosi et al. 2004). Our results support this hypothesis by showing that the contextual memory can be rescued by down regulating the inflammation, however one of the limitations of this study is the lack of data on the expression of the NMDA receptors in response to STZ and also after

inhibition of MIF.

In summary, these data delivered further insight into the possible role of MIF as an early cytokine in neuroinflammatory-dependent cognitive deficits in neurodegenerative disorders by showing that MIF inhibition might provide a basis for new therapeutic approaches. However further experiments are necessary to improve the understanding of MIF's role in the pathway of glial activation during the neuroinflammation. One other possible mechanism through which STZ may induce cognitive impairments is via excessive accumulation of NO, which leads to activating the activator protein-1 pathway (AP-1, Angel and Karin 1991), sustained activation of AP-1 induces upregulation of iNOS (Mendes. AF, et al. 2003). at the later time points after the surgery the NO production can be induces by presence of certain inflammatory components such as TNFα (Eberhardt et al. 1996).

In parallel to cognition studies, the weight of the animals was also documented regularly. Interestingly, our results indicate that both inhibition and lack of MIF molecule, rescues the weight loss which is typical after STZ-ICV interventions. The weight drop in the first week by all animals can be explained by the fact that animals may avoid the food as a result of the pain in the head area as a result of surgery. After recovery from the surgical shock however, the STZ-ICV C57BL/6 mice continue losing weight. This anorectic effect may occur as a consequence of changes in the metabolic rate as a result of activation of the inflammatory pathways in the hippocampus. Conditions associated with chronic inflammation, such as sepsis and chronic viral infections like HIV) in CNS are shown to be linked to insulin resistance development (Gregor, Hotamisligil 2011). Increase in the levels of inflammatory cytokines such as IL-6 and TNF a can influence the weight of the animals (Mohamed-Ali et al. 1997). Both IL-6 and TNFa are capable of inhibiting insulin signaling directly. Several serine kinases become activated as a result of upregulation of these cytokines, which affect the phosphorylation of IRS-1, a critical member for insulin signaling pathway. Thus their upregulation will result in losing sensitivity to the insulin or insulin resistance (Gual et al. 2005). It has also been shown that taste aversion can be induced in the rats by injection of IL-1 (Tazi et al. 1988), which might lead to changes in the appetite and thus suppressing the food intake.

Our experimental evidence suggests that lack of MIF molecule as well as inhibiting its cytokine activity by ISO-1 molecule protects the mice from severe weight loss.

The purpose of the current study was to investigate the effect of MIF inhibition on neuroinflammation induced by ICV administration of STZ on cytokine production as well as cognition. Our Initial investigation results have shown that neuroinflammation is playing a pivotal role in causing cognitive deficits as well as improvement in cognition as a results of MIF inhibition. MIF inhibition can affect the inflammatory milieu and cognition, however the effect does not seem to be immediate, and can be first detected after 6 weeks of treatment in the animal (considering the delayed improvement of mice in clockmaze and overall better performance in second context-memory task while on ISO-1 regimen) Neuroinflammation driven cognitive impairment happens as a result of complex interactions between different arms of immune and nervous system. It should be further investigated whether the observed improvements are due to cytokine down regulation or as a results of MIF effects on synaptic plasticity and neurogenesis processes.

Using anti-inflammatory strategies such as MIF inhibition is a potential option to improve cognition in patients suffering from neurodegenerative diseases with a prevalent neuroinflammation. Continued research on MIF inhibition as therapeutic target is necessary to provide further insights in the role of this early cytokine in both neuroinflammation and cognition.

# 8. REFERENCES

Acevedo, Giselles; Padala, Nischal K.; Ni, Li; Jonakait, G. M. (2013): Astrocytes inhibit microglial surface expression of dendritic cell-related co-stimulatory molecules through a contact-mediated process. In *Journal of neurochemistry* 125 (4), pp. 575–587. DOI: 10.1111/jnc.12221.

Adamo, M.; Raizada, M. K.; LeRoith, D. (1989): Insulin and insulin-like growth factor receptors in the nervous system. In *Molecular neurobiology* 3 (1-2), pp. 71–100.

Akiyama, H.; Barger, S.; Barnum, S.; Bradt, B.; Bauer, J.; Cole, G. M. et al. (2000): Inflammation and Alzheimer's disease. In *Neurobiology of aging* 21 (3), pp. 383–421.

Al-Abed, Yousef; Dabideen, Darrin; Aljabari, Bayan; Valster, Aline; Messmer, Davorka; Ochani, Mahendar et al. (2005): ISO-1 binding to the tautomerase active site of MIF inhibits its pro-inflammatory activity and increases survival in severe sepsis. In *The Journal of biological chemistry* 280 (44), pp. 36541–36544. DOI: 10.1074/jbc.C500243200.

Albert, Marilyn S.; Dekosky, Steven T.; Dickson, Dennis; Dubois, Bruno; Feldman, Howard H.; Fox, Nick C. et al. (2011): The diagnosis of mild cognitive impairment due to Alzheimer’s disease: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. In *Alzheimer's & dementia : the journal of the Alzheimer's Association* 7 (3), pp. 270–279. DOI: 10.1016/j.jalz.2011.03.008.

Alexander, Jessica K.; Cox, Gina M.; Tian, Jin-Bin; Zha, Alicia M.; Wei, Ping; Kigerl, Kristina A. et al. (2012): Macrophage Migration Inhibitory Factor (MIF) is Essential for Inflammatory and Neuropathic Pain and Enhances Pain in Response to Stress. In *Experimental Neurology* 236 (2), pp. 351–362. DOI: 10.1016/j.expneurol.2012.04.018.

Allan, S. M.; Harrison, D. C.; Read, S.; Collins, B.; Parsons, A. A.; Philpott, K.; Rothwell, N. J. (2001): Selective increases in cytokine expression in the rat brain in response to striatal injection of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate and interleukin-1. In *Brain research. Molecular brain research* 93 (2), pp. 180–189.

Allan, S. M.; Rothwell, N. J. (2001): Cytokines and acute neurodegeneration. In *Nature reviews. Neuroscience* 2 (10), pp. 734–744. DOI: 10.1038/35094583.

Almolda, Beatriz; Labra, Carmen de; Barrera, Iliana; Gruart, Agnes; Delgado-Garcia, Jose M.; Villacampa, Nadia et al. (2015): Alterations in microglial phenotype and hippocampal neuronal function in transgenic mice with astrocyte-targeted production of interleukin-10. In *Brain, Behavior, and Immunity* 45, pp. 80–97. DOI: 10.1016/j.bbi.2014.10.015.

Andrews, Shea; Das, Debjani; Anstey, Kaarin J.; Easteal, Simon (2015): Interactive effect of APOE genotype and blood pressure on cognitive decline: the PATH through life study. In *Journal of Alzheimer's disease : JAD* 44 (4),

pp. 1087–1098. DOI: 10.3233/JAD-140630.

Antony, Joseph M.; Izad, Maryam; Bar-Or, Amit; Warren, Kenneth G.; Vodjgani, Mohammed; Mallet, Francois; Power, C. (2006): Quantitative analysis of human endogenous retrovirus-W env in neuroinflammatory diseases. In *AIDS research and human retroviruses* 22 (12), pp. 1253–1259. DOI: 10.1089/aid.2006.22.1253.

Anwar, Mamdouh M.; Meki, Abdel-Raheim M. A. (2003): Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. In *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* 135 (4), pp. 539–547.

Araque, Alfonso; Navarrete, Marta (2010): Glial cells in neuronal network function. In *Philosophical Transactions of the Royal Society B: Biological Sciences* 365 (1551), pp. 2375–2381. DOI: 10.1098/rstb.2009.0313.

Atsumi, Toshiya; Cho, You-Ree; Leng, Lin; McDonald, Courtney; Yu, Tim; Danton, Cheryl et al. (2007): The proinflammatory cytokine macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation. In *Journal of immunology (Baltimore, Md. : 1950)* 179 (8), pp. 5399–5406.

Baas, Johanna M.; Nugent, Monique; Lissek, Shmuel; Pine, Daniel S.; Grillon, Christian (2004): Fear conditioning in virtual reality contexts: a new tool for the study of anxiety. In *Biological psychiatry* 55 (11), pp. 1056–1060. DOI: 10.1016/j.biopsych.2004.02.024.

Bacher, M.; Meinhardt, A.; Lan, H. Y.; Dhabhar, F. S.; Mu, W.; Metz, C. N. et al. (1998): MIF expression in the rat brain: implications for neuronal function. In *Molecular Medicine* 4 (4), pp. 217–230.

Bacher, Michael; Dodel, Richard; Aljabari, Bayan; Keyvani, Kathy; Marambaud, Philippe; Kayed, Rakez et al. (2008): CNI-1493 inhibits Abeta production, plaque formation, and cognitive deterioration in an animal model of Alzheimer's disease. In *The Journal of experimental medicine* 205 (7), pp. 1593–1599. DOI: 10.1084/jem.20060467.

Banks, W. A.; Kastin, A. J. (1998): Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin. In *Peptides* 19 (5), pp. 883–889.

Banks, William A.; Owen, Joshua B.; Erickson, Michelle A. (2012): Insulin in the brain: there and back again. In *Pharmacology & therapeutics* 136 (1), pp. 82–93. DOI: 10.1016/j.pharmthera.2012.07.006.

Barrientos, Ruth M.; Sprunger, David B.; Campeau, Serge; Watkins, Linda R.; Rudy, Jerry W.; Maier, Steven F. (2004): BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. In *Journal of neuroimmunology* 155 (1-2), pp. 119–126. DOI: 10.1016/j.jneuroim.2004.06.009.

Bass, David I.; Nizam, Zainab G.; Partain, Kristin N.; Wang, Arick; Manns, Joseph R. (2014): Amygdala-mediated enhancement of memory for specific events depends on the hippocampus. In *Neurobiology of learning and memory* 107, pp. 37–41. DOI: 10.1016/j.nlm.2013.10.020.

Baumann, N.; Pham-Dinh, D. (2001): Biology of oligodendrocyte and myelin in the mammalian central nervous system. In *Physiological reviews* 81 (2), pp. 871–927.

Bay-Richter, Cecilie; Janelidze, Shorena; Sauro, Analise; Bucala, Richard; Lipton, Jack; Deierborg, Tomas; Brundin, Lena (2015): Behavioural and neurobiological consequences of macrophage migration inhibitory factor gene deletion in mice. In *Journal of Neuroinflammation* 12, p. 163. DOI: 10.1186/s12974-015-0387-4.

Bearer, Elaine L. (2012): HSV, axonal transport and Alzheimer's disease: in vitro and in vivo evidence for causal relationships. In *Future virology* 7 (9), pp. 885–899. DOI: 10.2217/fvl.12.81.

Beattie, E. C.; Carroll, R. C.; Yu, X.; Morishita, W.; Yasuda, H.; Zastrow, M. von; Malenka, R. C. (2000): Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. In *Nature neuroscience* 3 (12), pp. 1291–1300. DOI: 10.1038/81823.

Bekris, Lynn M.; Lutz, Franziska; Yu, Chang-En (2012): Functional analysis of APOE locus genetic variation implicates regional enhancers in the regulation of both TOMM40 and APOE. In *Journal of human genetics* 57 (1), pp. 18–25. DOI: 10.1038/jhg.2011.123.

Bendrat, K.; Al-Abed, Y.; Callaway, D. J.; Peng, T.; Calandra, T.; Metz, C. N.; Bucala, R. (1997): Biochemical and mutational investigations of the enzymatic activity of macrophage migration inhibitory factor. In *Biochemistry* 36 (49), pp. 15356–15362. DOI: 10.1021/bi971153a.

Benitez, Bruno A.; Jin, Sheng Chih; Guerreiro, Rita; Graham, Rob; Lord, Jenny; Harold, Denise et al. (2014): Missense variant in TREML2 protects against Alzheimer's disease. In *Neurobiology of aging* 35 (6), 1510.e19-26. DOI: 10.1016/j.neurobiolaging.2013.12.010.

Benzing, T.; Brandes, R.; Sellin, L.; Schermer, B.; Lecker, S.; Walz, G.; Kim, E. (1999): Upregulation of RGS7 may contribute to tumor necrosis factor-induced changes in central nervous function. In *Nature medicine* 5 (8), pp. 913–918. DOI: 10.1038/11354.

Bernhagen, J.; Calandra, T.; Bucala, R. (1998): Regulation of the immune response by macrophage migration inhibitory factor: biological and structural features. In *Journal of molecular medicine (Berlin, Germany)* 76 (3-4), pp. 151–161.

Bernhagen, Jurgen; Krohn, Regina; Lue, Hongqi; Gregory, Julia L.; Zernecke, Alma; Koenen, Rory R. et al. (2007): MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. In *Nature medicine* 13 (5), pp. 587–596. DOI: 10.1038/nm1567.

Beyaert, R.; Vanhaesebroeck, B.; Declercq, W.; van Lint, J.; Vandenabele, P.; Agostinis, P. et al. (1995): Casein kinase-1 phosphorylates the p75 tumor necrosis factor receptor and negatively regulates tumor necrosis factor signaling for apoptosis. In *The Journal of biological chemistry* 270 (40), pp. 23293–23299.

Bezzina, Charlotte; Verret, Laure; Juan, Cecile; Remaud, Jessica; Halley,

Helene; Rampon, Claire; Dahan, Lionel (2015): Early onset of hypersynchronous network activity and expression of a marker of chronic seizures in the Tg2576 mouse model of Alzheimer's disease. In *PloS one* 10 (3), e0119910. DOI: 10.1371/journal.pone.0119910.

Bhaskar, Kiran; Konerth, Megan; Kokiko-Cochran, Olga N.; Cardona, Astrid; Ransohoff, Richard M.; Lamb, Bruce T. (2010): Regulation of tau pathology by the microglial fractalkine receptor. In *Neuron* 68 (1), pp. 19–31. DOI: 10.1016/j.neuron.2010.08.023.

Bingham, Emma M.; Hopkins, David; Smith, Diarmuid; Pernet, Andrew; Hallett, William; Reed, Laurence et al. (2002): The role of insulin in human brain glucose metabolism: an 18fluoro-deoxyglucose positron emission tomography study. In *Diabetes* 51 (12), pp. 3384–3390.

Bird, Chris M.; Burgess, Neil (2008): The hippocampus and memory: insights from spatial processing. In *Nat Rev Neurosci* 9 (3), pp. 182–194. DOI: 10.1038/nrn2335.

Blanchard, D. C.; Hori, K.; Rodgers, R. J.; Hendrie, C. A.; Blanchard, R. J. (1989): Attenuation of defensive threat and attack in wild rats (Rattus rattus) by benzodiazepines. In *Psychopharmacology* 97 (3), pp. 392–401.

Blázquez, Enrique; Velázquez, Esther; Hurtado-Carneiro, Verónica; Ruiz-Albusac, Juan Miguel (2014): Insulin in the Brain: Its Pathophysiological Implications for States Related with Central Insulin Resistance, Type 2 Diabetes and Alzheimer's Disease. In *Frontiers in endocrinology* 5, p. 161. DOI: 10.3389/fendo.2014.00161.

Block, Michelle L.; Zecca, Luigi; Hong, Jau-Shyong (2007): Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. In *Nat Rev Neurosci* 8 (1), pp. 57–69. DOI: 10.1038/nrn2038.

Bochud, Pierre-Yves; Calandra, Thierry (2003): Pathogenesis of sepsis: new concepts and implications for future treatment. In *BMJ : British Medical Journal* 326 (7383), pp. 262–266.

Bock, F. de; Dornand, J.; Rondouin, G. (1996): Release of TNF alpha in the rat hippocampus following epileptic seizures and excitotoxic neuronal damage. In *Neuroreport* 7 (6), pp. 1125–1129.

Bond, C. E.; Patel, P.; Crouch, L.; Tetlow, N.; Day, T.; Abu-Hayyeh, S. et al. (2006): Astroglia up-regulate transcription and secretion of 'readthrough' acetylcholinesterase following oxidative stress. In *The European journal of neuroscience* 24 (2), pp. 381–386. DOI: 10.1111/j.1460-9568.2006.04898.x.

Bouton, Mark E.; Moody, Erik W. (2004): Memory processes in classical conditioning. In *Neuroscience and biobehavioral reviews* 28 (7), pp. 663–674. DOI: 10.1016/j.neubiorev.2004.09.001.

Bouton, Mark E.; Westbrook, R. Frederick; Corcoran, Kevin A.; Maren, Stephen (2006): Contextual and temporal modulation of extinction: behavioral and biological mechanisms. In *Biological psychiatry* 60 (4), pp. 352–360. DOI: 10.1016/j.biopsych.2005.12.015.

Bozza, M.; Satoskar, A. R.; Lin, G.; Lu, B.; Humbles, A. A.; Gerard, C.; David,

J. R. (1999): Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. In *The Journal of experimental medicine* 189 (2), pp. 341–346.

Brabers, N A C H; Nottet, H S L M (2006): Role of the pro-inflammatory cytokines TNF-alpha and IL-1beta in HIV-associated dementia. In *European journal of clinical investigation* 36 (7), pp. 447–458. DOI: 10.1111/j.1365-2362.2006.01657.x.

Brackenbury, William J.; Davis, Tigwa H.; Chen, Chunling; Slat, Emily A.; Detrow, Matthew J.; Dickendesher, Travis L. et al. (2008): Voltage-gated Na+ channel beta1 subunit-mediated neurite outgrowth requires Fyn kinase and contributes to postnatal CNS development in vivo. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28 (12), pp. 3246–3256. DOI: 10.1523/JNEUROSCI.5446-07.2008.

Broe, G. A.; Grayson, D. A.; Creasey, H. M.; Waite, L. M.; Casey, B. J.; Bennett, H. P. et al. (2000): Anti-inflammatory drugs protect against Alzheimer disease at low doses. In *Archives of neurology* 57 (11), pp. 1586–1591.

Bucala, R. (1994): Identification of MIF as a new pituitary hormone and macrophage cytokine and its role in endotoxic shock. In *Immunology letters* 43 (1-2), pp. 23–26.

Budarf, M.; McDonald, T.; Sellinger, B.; Kozak, C.; Graham, C.; Wistow, G. (1997): Localization of the human gene for macrophage migration inhibitory factor (MIF) to chromosome 22q11.2. In *Genomics* 39 (2), pp. 235–236. DOI: 10.1006/geno.1996.4505.

Buko, Vyacheslav; Belonovskaya, Elena; Naruta, Elena; Lukivskaya, Oxana; Kanyuka, Olena; Zhuk, Olga et al. (2015): Pituitary tumor transforming gene as a novel regulatory factor of liver fibrosis. In *Life sciences* 132, pp. 34–40. DOI: 10.1016/j.lfs.2015.04.010.

Burger-Kentischer, Anke; Goebel, Heike; Seiler, Rudiger; Fraedrich, Gustav; Schaefer, Hans E.; Dimmeler, Stefanie et al. (2002): Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. In *Circulation* 105 (13), pp. 1561–1566.

Burton, Michael D.; Johnson, Rodney W. (2012): Interleukin-6 trans-signaling in the senescent mouse brain is involved in infection-related deficits in contextual fear conditioning. In *Brain, Behavior, and Immunity* 26 (5), pp. 732–738. DOI: 10.1016/j.bbi.2011.10.008.

Butters, Meryl A.; Young, Jeffrey B.; Lopez, Oscar; Aizenstein, Howard J.; Mulsant, Benoit H.; Reynolds, Charles F. 3rd et al. (2008): Pathways linking late-life depression to persistent cognitive impairment and dementia. In *Dialogues in clinical neuroscience* 10 (3), pp. 345–357.

Cabal-Hierro, Lucia; Lazo, Pedro S. (2012): Signal transduction by tumor necrosis factor receptors. In *Cellular signalling* 24 (6), pp. 1297–1305. DOI: 10.1016/j.cellsig.2012.02.006.

Cabral, Henrique O.; Fouquet, Celine; Rondi-Reig, Laure; Pennartz, Cyriel M. A.; Battaglia, Francesco P. (2014): Single-trial properties of place cells in control and CA1 NMDA receptor subunit 1-KO mice. In *The Journal of*

*neuroscience : the official journal of the Society for Neuroscience* 34 (48), pp. 15861–15869. DOI: 10.1523/JNEUROSCI.5320-13.2014.

Cai, Wen-Hui; Blundell, Jacqueline; Han, Jie; Greene, Robert W.; Powell, Craig M. (2006): Postreactivation glucocorticoids impair recall of established fear memory. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26 (37), pp. 9560–9566. DOI: 10.1523/JNEUROSCI.2397-06.2006.

Calandra, T.; Spiegel, L. A.; Metz, C. N.; Bucala, R. (1998): Macrophage migration inhibitory factor is a critical mediator of the activation of immune cells by exotoxins of Gram-positive bacteria. In *Proceedings of the National Academy of Sciences of the United States of America* 95 (19), pp. 11383–11388.

Candeias, Emanuel; Duarte, Ana I.; Carvalho, Cristina; Correia, Sonia C.; Cardoso, Susana; Santos, Renato X. et al. (2012): The impairment of insulin signaling in Alzheimer's disease. In *IUBMB life* 64 (12), pp. 951–957. DOI: 10.1002/iub.1098.

Capri, Miriam; Yani, Stella Lukas; Chattat, Rabih; Fortuna, Daniela; Bucci, Laura; Lanzarini, Catia et al. (2014): Pre-Operative, High-IL-6 Blood Level is a Risk Factor of Post-Operative Delirium Onset in Old Patients. In *Frontiers in endocrinology* 5, p. 173. DOI: 10.3389/fendo.2014.00173.

Caracciolo, Barbara; Xu, Weili; Collins, Stephen; Fratiglioni, Laura (2014): Cognitive decline, dietary factors and gut-brain interactions. In *Mechanisms of ageing and development* 136-137, pp. 59–69. DOI: 10.1016/j.mad.2013.11.011.

Caraci, Filippo; Molinaro, Gemma; Battaglia, Giuseppe; Giuffrida, Maria Laura; Riozzi, Barbara; Traficante, Anna et al. (2011): Targeting group II metabotropic glutamate (mGlu) receptors for the treatment of psychosis associated with Alzheimer's disease: selective activation of mGlu2 receptors amplifies beta-amyloid toxicity in cultured neurons, whereas dual activation of mGlu2 and mGlu3 receptors is neuroprotective. In *Molecular pharmacology* 79 (3), pp. 618–626. DOI: 10.1124/mol.110.067488.

Caramanos, Z.; Shapiro, M. L. (1994): Spatial memory and N-methyl-D-aspartate receptor antagonists APV and MK-801: memory impairments depend on familiarity with the environment, drug dose, and training duration. In *Behavioral neuroscience* 108 (1), pp. 30–43.

Cardona, Astrid E.; Pioro, Erik P.; Sasse, Margaret E.; Kostenko, Volodymyr; Cardona, Sandra M.; Dijkstra, Ineke M. et al. (2006): Control of microglial neurotoxicity by the fractalkine receptor. In *Nature neuroscience* 9 (7), pp. 917–924. DOI: 10.1038/nn1715.

Cartier, Laetitia; Hartley, Oliver; Dubois-Dauphin, Michel; Krause, Karl-Heinz (2005): Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. In *Brain research. Brain research reviews* 48 (1), pp. 16–42. DOI: 10.1016/j.brainresrev.2004.07.021.

Chan, Gail; White, Charles C.; Winn, Phoebe A.; Cimpean, Maria; Replogle, Joseph M.; Glick, Laura R. et al. (2015): CD33 modulates TREM2:

convergence of Alzheimer loci. In *Nature neuroscience* 18 (11), pp. 1556–1558. DOI: 10.1038/nn.4126.

Chan, W. Y.; Kohsaka, S.; Rezaie, P. (2007): The origin and cell lineage of microglia: new concepts. In *Brain research reviews* 53 (2), pp. 344–354. DOI: 10.1016/j.brainresrev.2006.11.002.

Chen, Yanxing; Liang, Zhihou; Blanchard, Julie; Dai, Chun-Ling; Sun, Shenggang; Lee, Moon H. et al. (2012a): A non-transgenic mouse model (icv-STZ mouse) of Alzheimer's disease: Similarities to and differences from the transgenic model (3xTg-AD mouse). In *Molecular neurobiology* 47 (2), pp. 711–725. DOI: 10.1007/s12035-012-8375-5.

Chen, Yanxing; Tian, Zhu; Liang, Zhihou; Sun, Shenggang; Dai, Chun-Ling; Lee, Moon H. et al. (2012b): Brain gene expression of a sporadic (icv-STZ Mouse) and a familial mouse model (3xTg-AD mouse) of Alzheimer's disease. In *PloS one* 7 (12), e51432. DOI: 10.1371/journal.pone.0051432.

Chiara, Giovanna de; Marcocci, Maria Elena; Civitelli, Livia; Argnani, Rafaela; Piacentini, Roberto; Ripoli, Cristian et al. (2010): APP processing induced by herpes simplex virus type 1 (HSV-1) yields several APP fragments in human and rat neuronal cells. In *PloS one* 5 (11), e13989. DOI: 10.1371/journal.pone.0013989.

Cho, Seo-Hyun; Sun, Binggui; Zhou, Yungui; Kauppinen, Tiina M.; Halabisky, Brian; Wes, Paul et al. (2011): CX3CR1 protein signaling modulates microglial activation and protects against plaque-independent cognitive deficits in a mouse model of Alzheimer disease. In *The Journal of biological chemistry* 286 (37), pp. 32713–32722. DOI: 10.1074/jbc.M111.254268.

Chua, A. O.; Wilkinson, V. L.; Presky, D. H.; Gubler, U. (1995): Cloning and characterization of a mouse IL-12 receptor-beta component. In *Journal of immunology (Baltimore, Md. : 1950)* 155 (9), pp. 4286–4294.

Chuang, Chia-Chang; Chuang, Yin-Ching; Chang, Wen-Teng; Chen, Chi-Chung; Hor, Lien-I; Huang, A-Ming et al. (2010): Macrophage migration inhibitory factor regulates interleukin-6 production by facilitating nuclear factor-kappa B activation during Vibrio vulnificus infection. In *BMC immunology* 11, p. 50. DOI: 10.1186/1471-2172-11-50.

Cnop, Miriam; Welsh, Nils; Jonas, Jean-Christophe; Jorns, Anne; Lenzen, Sigurd; Eizirik, Decio L. (2005): Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. In *Diabetes* 54 Suppl 2, S97-107.

Conboy, L.; Varea, E.; Castro, J. E.; Sakouhi-Ouertatani, H.; Calandra, T.; Lashuel, H. A.; Sandi, C. (2011): Macrophage migration inhibitory factor is critically involved in basal and fluoxetine-stimulated adult hippocampal cell proliferation and in anxiety, depression, and memory-related behaviors. In *Molecular psychiatry* 16 (5), pp. 533–547. DOI: 10.1038/mp.2010.15.

Cook, David G.; Leverenz, James B.; McMillan, Pamela J.; Kulstad, J. Jacob; Ericksen, Sasha; Roth, Richard A. et al. (2003): Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. In *The American journal of pathology* 162

(1), pp. 313–319.

Craft, S.; Teri, L.; Edland, S. D.; Kukull, W. A.; Schellenberg, G.; McCormick, W. C. et al. (1998): Accelerated decline in apolipoprotein E-epsilon4 homozygotes with Alzheimer's disease. In *Neurology* 51 (1), pp. 149–153.

Daryadel, Arezoo; Grifone, Remo Filippo; Simon, Hans-Uwe; Yousefi, Shida (2006): Apoptotic neutrophils release macrophage migration inhibitory factor upon stimulation with tumor necrosis factor-alpha. In *The Journal of biological chemistry* 281 (37), pp. 27653–27661. DOI: 10.1074/jbc.M604051200.

Daun, J. M.; Cannon, J. G. (2000): Macrophage migration inhibitory factor antagonizes hydrocortisone-induced increases in cytosolic IkappaBalpha. In *American journal of physiology. Regulatory, integrative and comparative physiology* 279 (3), R1043-9.

Daynes, R. A.; Araneo, B. A.; Ershler, W. B.; Maloney, C.; Li, G. Z.; Ryu, S. Y. (1993): Altered regulation of IL-6 production with normal aging. Possible linkage to the age-associated decline in dehydroepiandrosterone and its sulfated derivative. In *Journal of immunology (Baltimore, Md. : 1950)* 150 (12), pp. 5219–5230.

Debiec, J.; Ledoux, J. E. (2004): Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. In *Neuroscience* 129 (2), pp. 267–272. DOI: 10.1016/j.neuroscience.2004.08.018.

Del Puerto, Ana; Wandosell, Francisco; Garrido, Juan Jose (2013): Neuronal and glial purinergic receptors functions in neuron development and brain disease. In *Frontiers in cellular neuroscience* 7, p. 197. DOI: 10.3389/fncel.2013.00197.

Dembic, Z.; Loetscher, H.; Gubler, U.; Pan, Y. C.; Lahm, H. W.; Gentz, R. et al. (1990): Two human TNF receptors have similar extracellular, but distinct intracellular, domain sequences. In *Cytokine* 2 (4), pp. 231–237.

Derecki, Noel C.; Katzmarski, Natalie; Kipnis, Jonathan; Meyer-Luehmann, Melanie (2014): Microglia as a critical player in both developmental and late-life CNS pathologies. In *Acta neuropathologica* 128 (3), pp. 333–345. DOI: 10.1007/s00401-014-1321-z.

DeWitt, D. A.; Perry, G.; Cohen, M.; Doller, C.; Silver, J. (1998): Astrocytes regulate microglial phagocytosis of senile plaque cores of Alzheimer's disease. In *Experimental Neurology* 149 (2), pp. 329–340. DOI: 10.1006/exnr.1997.6738.

Di Bona, Danilo; Candore, Giuseppina; Franceschi, Claudio; Licastro, Federico; Colonna-Romano, Giuseppina; Camma, Calogero et al. (2009): Systematic review by meta-analyses on the possible role of TNF-alpha polymorphisms in association with Alzheimer's disease. In *Brain research reviews* 61 (2), pp. 60–68. DOI: 10.1016/j.brainresrev.2009.05.001.

Di Santo, E.; Adami, M.; Bertorelli, R.; Ghezzi, P. (1997): Systemic interleukin 10 administration inhibits brain tumor necrosis factor production in mice. In *European journal of pharmacology* 336 (2-3), pp. 197–202.

Dinarello, C. A. (1998): Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. In *Annals of the New York Academy of Sciences* 856, pp. 1–11.

Dinarello, C. A. (2000): The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. In *The New England journal of medicine* 343 (10), pp. 732–734. DOI: 10.1056/NEJM200009073431011.

Dios, Angeles; Mitchell, Robert A.; Aljabari, Bayan; Lubetsky, Jodi; O'Connor, KerryAnn; Liao, Hong et al. (2002): Inhibition of MIF bioactivity by rational design of pharmacological inhibitors of MIF tautomerase activity. In *Journal of medicinal chemistry* 45 (12), pp. 2410–2416.

Donn, Rachelle P.; Plant, Darren; Jury, Francine; Richards, Helen L.; Worthington, Jane; Ray, David W.; Griffiths, Christopher E. M. (2004): Macrophage migration inhibitory factor gene polymorphism is associated with psoriasis. In *The Journal of investigative dermatology* 123 (3), pp. 484–487. DOI: 10.1111/j.0022-202X.2004.23314.x.

Drake, Caroline; Boutin, Hervé; Jones, Matthew S.; Denes, Adam; McColl, Barry W.; Selvarajah, Johann R. et al. (2011): Brain inflammation is induced by co-morbidities and risk factors for stroke. In *Brain, Behavior, and Immunity* 25 (6-4), pp. 1113–1122. DOI: 10.1016/j.bbi.2011.02.008.

Duron, E.; Hanon, Olivier (2008): Vascular risk factors, cognitve decline, and dementia. In *Vascular Health and Risk Management* 4 (2), pp. 363–381.

Edwards, Kate M.; Tomfohr, Lianne M.; Mills, Paul J.; Bosch, Jos A.; Ancoli-Israel, Sonia; Loredo, Jose S.; Dimsdale, Joel (2011): Macrophage migratory inhibitory factor (MIF) may be a key factor in inflammation in obstructive sleep apnea. In *Sleep* 34 (2), pp. 161–163.

Eikelenboom, P.; Veerhuis, R.; van Exel, E.; Hoozemans, J. J. M.; Rozemuller, A. J. M.; van Gool, W. A. (2011): The early involvement of the innate immunity in the pathogenesis of late-onset Alzheimer's disease: neuropathological, epidemiological and genetic evidence. In *Current Alzheimer research* 8 (2), pp. 142–150.

Ekstrom, Arne D.; Kahana, Michael J.; Caplan, Jeremy B.; Fields, Tony A.; Isham, Eve A.; Newman, Ehren L.; Fried, Itzhak (2003): Cellular networks underlying human spatial navigation. In *Nature* 425 (6954), pp. 184–188. DOI: 10.1038/nature01964.

Elahy, Mina; Jackaman, Connie; Mamo, John Cl; Lam, Virginie; Dhaliwal, Satvinder S.; Giles, Corey et al. (2015): Blood–brain barrier dysfunction developed during normal aging is associated with inflammation and loss of tight junctions but not with leukocyte recruitment. In *Immunity & Ageing : I & A* 12, p. 2. DOI: 10.1186/s12979-015-0029-9.

Eleazu, Chinedum Ogbonnaya; Eleazu, Kate Chinedum; Chukwuma, Sonia; Essien, Udeme Nelson (2013): Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. In *Journal of diabetes and metabolic disorders* 12 (1), p. 60. DOI: 10.1186/2251-6581-12-60.

El-Turk, Farah; Cascella, Michele; Ouertatani-Sakouhi, Hajer; Narayanan,

Raghavendran Lakshmi; Leng, Lin; Bucala, Richard et al. (2008): The conformational flexibility of the carboxy terminal residues 105-114 is a key modulator of the catalytic activity and stability of macrophage migration inhibitory factor. In *Biochemistry* 47 (40), pp. 10740–10756. DOI: 10.1021/bi800603x.

Erta, Maria; Quintana, Albert; Hidalgo, Juan (2012): Interleukin-6, a major cytokine in the central nervous system. In *International journal of biological sciences* 8 (9), pp. 1254–1266. DOI: 10.7150/ijbs.4679.

Etminan, Mahyar; Gill, Sudeep; Samii, Ali (2003): Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. In *BMJ (Clinical research ed.)* 327 (7407), p. 128. DOI: 10.1136/bmj.327.7407.128.

Farina, Cinthia; Aloisi, Francesca; Meinl, Edgar (2007): Astrocytes are active players in cerebral innate immunity. In *Trends in immunology* 28 (3), pp. 138–145. DOI: 10.1016/j.it.2007.01.005.

Fellin, Tommaso; Carmignoto, Giorgio (2004): Neurone-to-astrocyte signalling in the brain represents a distinct multifunctional unit. In *The Journal of physiology* 559 (Pt 1), pp. 3–15. DOI: 10.1113/jphysiol.2004.063214.

Fields, R. Douglas; Stevens-Graham, Beth (2002): New insights into neuron-glia communication. In *Science (New York, N.Y.)* 298 (5593), pp. 556–562. DOI: 10.1126/science.298.5593.556.

Fingerle-Rowson, Gunter; Koch, Peter; Bikoff, Rachel; Lin, Xinchun; Metz, Christine N.; Dhabhar, Firdaus S. et al. (2002): Regulation of Macrophage Migration Inhibitory Factor Expression by Glucocorticoids in Vivo. In *The American journal of pathology* 162 (1), pp. 47–56.

Fisher, Mark; Vasilevko, Vitaly; Passos, Giselle F.; Ventura, Christopher; Quiring, Daniel; Cribbs, David H. (2011): Therapeutic modulation of cerebral microhemorrhage in a mouse model of cerebral amyloid angiopathy. In *Stroke; a journal of cerebral circulation* 42 (11), pp. 3300–3303. DOI: 10.1161/STROKEAHA.111.626655.

Flieger, Oliver; Engling, Andre; Bucala, Richard; Lue, Hongqi; Nickel, Walter; Bernhagen, Jurgen (2003): Regulated secretion of macrophage migration inhibitory factor is mediated by a non-classical pathway involving an ABC transporter. In *FEBS letters* 551 (1-3), pp. 78–86.

Frank, Matthew G.; Barrientos, Ruth M.; Biedenkapp, Joseph C.; Rudy, Jerry W.; Watkins, Linda R.; Maier, Steven F. (2006): mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. In *Neurobiology of aging* 27 (5), pp. 717–722. DOI: 10.1016/j.neurobiolaging.2005.03.013.

Frank-Cannon, Tamy C.; Alto, Laura T.; McAlpine, Fiona E.; Tansey, Malu G. (2009): Does neuroinflammation fan the flame in neurodegenerative diseases? In *Molecular neurodegeneration* 4, p. 47. DOI: 10.1186/1750-1326-4-47.

Franke, Heike; Verkhratsky, Alexei; Burnstock, Geoffrey; Illes, Peter (2012): Pathophysiology of astroglial purinergic signalling. In *Purinergic signalling* 8 (3), pp. 629–657. DOI: 10.1007/s11302-012-9300-0.

Friedman, Daniel; Honig, Lawrence S.; Scarmeas, Nikolaos (2011): Seizures and Epilepsy in Alzheimer's Disease. In *CNS neuroscience & therapeutics* 18 (4), pp. 285–294. DOI: 10.1111/j.1755-5949.2011.00251.x.

Friedman, W. J. (2001): Cytokines regulate expression of the type 1 interleukin-1 receptor in rat hippocampal neurons and glia. In *Experimental Neurology* 168 (1), pp. 23–31. DOI: 10.1006/exnr.2000.7595.

Gabay, C.; Kushner, I. (1999): Acute-phase proteins and other systemic responses to inflammation. In *The New England journal of medicine* 340 (6), pp. 448–454. DOI: 10.1056/NEJM199902113400607.

Garden, Gwenn A.; Moller, Thomas (2006): Microglia biology in health and disease. In *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology* 1 (2), pp. 127–137. DOI: 10.1007/s11481-006-9015-5.

Gaul, C.; Jordan, B.; Wustmann, T.; Preuss, U. W. (2007): Kluver-Bucy syndrome in humans. In *Der Nervenarzt* 78 (7), pp. 821–823. DOI: 10.1007/s00115-007-2271-7.

Gehrmann, J.; Banati, R. B. (1995): Microglial turnover in the injured CNS: activated microglia undergo delayed DNA fragmentation following peripheral nerve injury. In *Journal of neuropathology and experimental neurology* 54 (5), pp. 680–688.

Geisterfer, M.; Richards, C. D.; Gauldie, J. (1995): Cytokines oncostatin M and interleukin 1 regulate the expression of the IL-6 receptor (gp80, gp130). In *Cytokine* 7 (6), pp. 503–509. DOI: 10.1006/cyto.1995.0068.

Gemberling, G. A.; Domjan, M. (1982): Selective associations in one-day-old rats: taste-toxicosis and texture-shock aversion learning. In *Journal of comparative and physiological psychology* 96 (1), pp. 105–113.

Gemma, Carmelina; Fister, Matthew; Hudson, Charles; Bickford, Paula C. (2005): Improvement of memory for context by inhibition of caspase-1 in aged rats. In *The European journal of neuroscience* 22 (7), pp. 1751–1756. DOI: 10.1111/j.1460-9568.2005.04334.x.

Gemma, Carmelina; Mesches, Michael H.; Sepesi, Boris; Choo, Kevin; Holmes, Douglas B.; Bickford, Paula C. (2002): Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22 (14), pp. 6114–6120.

Glass, Christopher K.; Saijo, Kaoru; Winner, Beate; Marchetto, Maria Carolina; Gage, Fred H. (2010): Mechanisms underlying inflammation in neurodegeneration. In *Cell* 140 (6), pp. 918–934. DOI: 10.1016/j.cell.2010.02.016.

Godbout, J. P.; Chen, J.; Abraham, J.; Richwine, A. F.; Berg, B. M.; Kelley, K. W.; Johnson, R. W. (2005): Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. In *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 19 (10), pp. 1329–1331. DOI:

10.1096/fj.05-3776fje.

Godbout, J. P.; Johnson, R. W. (2004): Interleukin-6 in the aging brain. In *Journal of neuroimmunology* 147 (1-2), pp. 141–144.

Goldeck, David; Larbi, Anis; Pellicanó, Mariavaleria; Alam, Iftikhar; Zerr, Inga; Schmidt, Christian et al. (2013): Enhanced Chemokine Receptor Expression on Leukocytes of Patients with Alzheimer's Disease. In *PloS one* 8 (6), e66664. DOI: 10.1371/journal.pone.0066664.

Gonzalez-Scarano, F.; Baltuch, G. (1999): Microglia as mediators of inflammatory and degenerative diseases. In *Annual review of neuroscience* 22, pp. 219–240. DOI: 10.1146/annurev.neuro.22.1.219.

Goosens, Ki A.; Hobin, Jennifer A.; Maren, Stephen (2003): Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? In *Neuron* 40 (5), pp. 1013–1022.

Gore, Yael; Starlets, Diana; Maharshak, Nitsan; Becker-Herman, Shirly; Kaneyuki, Utako; Leng, Lin et al. (2008): Macrophage migration inhibitory factor induces B cell survival by activation of a CD74-CD44 receptor complex. In *The Journal of biological chemistry* 283 (5), pp. 2784–2792. DOI: 10.1074/jbc.M703265200.

Graff-Radford, Jonathan; Lesnick, Timothy G.; Boeve, Bradley F.; Przybelski, Scott A.; Jones, David T.; Senjem, Matthew L. et al. (2016): Predicting Survival in Dementia With Lewy Bodies With Hippocampal Volumetry. In *Movement disorders : official journal of the Movement Disorder Society. DOI:* 10.1002/mds.26666.

Grand, Jacob Hg; Caspar, Sienna; MacDonald, Stuart Ws (2011): Clinical features and multidisciplinary approaches to dementia care. In *Journal of Multidisciplinary Healthcare* 4, pp. 125–147. DOI: 10.2147/JMDH.S17773.

Gregor, Margaret F.; Hotamisligil, Gokhan S. (2011): Inflammatory mechanisms in obesity. In *Annual review of immunology* 29, pp. 415–445. DOI: 10.1146/annurev-immunol-031210-101322.

Gregory, Julia L.; Morand, Eric F.; McKeown, Sonja J.; Ralph, Jennifer A.; Hall, Pamela; Yang, Yuan H. et al. (2006): Macrophage migration inhibitory factor induces macrophage recruitment via CC chemokine ligand 2. In *Journal of immunology (Baltimore, Md. : 1950)* 177 (11), pp. 8072–8079.

Griciuc, Ana; Serrano-Pozo, Alberto; Parrado, Antonio R.; Lesinski, Andrea N.; Asselin, Caroline N.; Mullin, Kristina et al. (2013a): Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. In *Neuron* 78 (4), pp. 631–643. DOI: 10.1016/j.neuron.2013.04.014.

Griciuc, Ana; Serrano-Pozo, Alberto; Parrado, Antonio R.; Lesinski, Andrea N.; Asselin, Caroline N.; Mullin, Kristina et al. (2013b): Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. In *Neuron* 78 (4), pp. 631–643. DOI: 10.1016/j.neuron.2013.04.014.

Grieb, Pawel (2016): Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's Disease: in Search of a Relevant Mechanism. In *Molecular neurobiology* 53 (3), pp. 1741–1752. DOI: 10.1007/s12035-015-

9132-3.

Gual, Philippe; Le Marchand-Brustel, Yannick; Tanti, Jean-Francois (2005): Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. In *Biochimie* 87 (1), pp. 99–109. DOI: 10.1016/j.biochi.2004.10.019.

Guerreiro, Rita J.; Gustafson, Deborah R.; Hardy, John (2010): THE GENETIC ARCHITECTURE OF ALZHEIMER'S DISEASE: BEYOND APP, PSENs AND APOE. In *Neurobiology of aging* 33 (3), pp. 437–456. DOI: 10.1016/j.neurobiolaging.2010.03.025.

Guma, Monica; Ronacher, Lisa; Liu-Bryan, Ru; Takai, Shinji; Karin, Michael; Corr, Maripat (2009): Caspase-1 Independent IL-1β Activation in Neutrophil Dependent Inflammation. In *Arthritis and rheumatism* 60 (12), pp. 3642–3650. DOI: 10.1002/art.24959.

Havrankova, J.; Schmechel, D.; Roth, J.; Brownstein, M. (1978): Identification of insulin in rat brain. In *Proceedings of the National Academy of Sciences of the United States of America* 75 (11), pp. 5737–5741.

Heidenreich, K. A.; Toledo, S. P. (1989): Insulin receptors mediate growth effects in cultured fetal neurons. II. Activation of a protein kinase that phosphorylates ribosomal protein S6. In *Endocrinology* 125 (3), pp. 1458–1463. DOI: 10.1210/endo-125-3-1458.

Heneka, Michael T.; Kummer, Markus P.; Latz, Eicke (2014): Innate immune activation in neurodegenerative disease. In *Nat Rev Immunol* 14 (7), pp. 463–477.

Heneka, Michael T.Frederic Brosseron, Marius Krauthausen, Markus Kummer, Body Fluid Cytokine Levels in Mild Cognitive Impairment and Alzheimer's Disease: a Comparative Overview, doi: 10.1007/s12035-014-8657-1 Mol Neurobiol. 2014; 50(2): 534–544.Published online 2014 Feb 2

Heras-Sandoval, David; Pedraza-Chaverri, Jose; Perez-Rojas, Jazmin M. (2016): Role of docosahexaenoic acid in the modulation of glial cells in Alzheimer's disease. In *Journal of Neuroinflammation* 13 (1), p. 61. DOI: 10.1186/s12974-016-0525-7.

Hermes, Gretchen; Ajioka, James W.; Kelly, Krystyna A.; Mui, Ernest; Roberts, Fiona; Kasza, Kristen et al. (2008): Neurological and behavioral abnormalities, ventricular dilatation, altered cellular functions, inflammation, and neuronal injury in brains of mice due to common, persistent, parasitic infection. In *Journal of Neuroinflammation* 5, p. 48. DOI: 10.1186/1742-2094-5-48.

Hernangómez, Miriam; Carrillo-Salinas, Francisco J.; Mecha, Miriam; Correa, Fernando; Mestre, Leyre; Loría, Frida et al. (2014): Brain Innate Immunity in the Regulation of Neuroinflammation: Therapeutic Strategies by Modulating CD200-CD200R Interaction Involve the Cannabinoid System. In *Current Pharmaceutical Design* 20 (29), pp. 4707–4722. DOI: 10.2174/13816128206661401302029 11.

Heyen, JR; Ye, S.; Finck, B. N.; Johnson, R. W. (2000): Interleukin (IL)-10 inhibits IL-6 production in microglia by preventing activation of NF-kappaB. In

*Brain research. Molecular brain research* 77 (1), pp. 138–147.

Hirrlinger, Johannes; Hulsmann, Swen; Kirchhoff, Frank (2004): Astroglial processes show spontaneous motility at active synaptic terminals in situ. In *The European journal of neuroscience* 20 (8), pp. 2235–2239. DOI: 10.1111/j.1460-9568.2004.03689.x.

Hobin, Jennifer A.; Goosens, Ki A.; Maren, Stephen (2003): Context-dependent neuronal activity in the lateral amygdala represents fear memories after extinction. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23 (23), pp. 8410–8416.

Hoek, R. M.; Ruuls, S. R.; Murphy, C. A.; Wright, G. J.; Goddard, R.; Zurawski, S. M. et al. (2000): Down-regulation of the macrophage lineage through interaction with OX2 (CD200). In *Science (New York, N.Y.)* 290 (5497), pp. 1768–1771.

Holmes, Fiona E.; Arnott, Nighat; Vanderplank, Penny; Kerr, Niall C. H.; Longbrake, Erin E.; Popovich, Philip G. et al. (2008): Intra-neural administration of fractalkine attenuates neuropathic pain-related behaviour. In *Journal of neurochemistry* 106 (2), pp. 640–649. DOI: 10.1111/j.1471-4159.2008.05419.x.

Hoshino, Tadashi; Sato, Yoshitake; Toyonaga, Yoshikiyo; Hanaki, Hideaki; Sunakawa, Keisuke (2013): Nationwide survey of the development of drug resistance in the pediatric field in 2007 and 2010: drug sensitivity of Haemophilus influenzae in Japan (second report). In *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* 19 (3), pp. 495–503. DOI: 10.1007/s10156-013-0591-z.

Hoyer, S.; Henneberg, N.; Knapp, S.; Lannert, H.; Martin, E. (1996): Brain glucose metabolism is controlled by amplification and desensitization of the neuronal insulin receptor. In *Annals of the New York Academy of Sciences* 777, pp. 374–379.

Hu, Xiangyou; Zhou, Xiangdong; He, Wanxia; Yang, Jun; Xiong, Wenchen; Wong, Philip et al. (2010): BACE1 deficiency causes altered neuronal activity and neurodegeneration. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30 (26), pp. 8819–8829. DOI: 10.1523/JNEUROSCI.1334-10.2010.

Hughes, Paula Marie; Botham, Michelle Sandra; Frentzel, Stefan; Mir, Anis; Perry, Victor Hugh (2002): Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, during acute and chronic inflammation in the rodent CNS. In *Glia* 37 (4), pp. 314–327.

Humpel, Christian; Hochstrasser, Tanja (2011): Cerebrospinal fluid and blood biomarkers in Alzheimer's disease. In *World journal of psychiatry* 1 (1), pp. 8–18. DOI: 10.5498/wjp.v1.i1.8.

Hundhausen, Christian; Misztela, Dominika; Berkhout, Theo A.; Broadway, Neil; Saftig, Paul; Reiss, Karina et al. (2003): The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX3CL1 (fractalkine) and regulates CX3CL1-mediated cell-cell adhesion. In *Blood* 102 (4), pp. 1186–1195. DOI: 10.1182/blood-2002-12-3775.

Hurley, Laura L.; Tizabi, Yousef (2013): Neuroinflammation, neurodegeneration, and depression. In *Neurotoxicity research* 23 (2), pp. 131–144. DOI: 10.1007/s12640-012-9348-1.

Inacio, Ana R.; Ruscher, Karsten; Leng, Lin; Bucala, Richard; Deierborg, Tomas (2011): Macrophage migration inhibitory factor promotes cell death and aggravates neurologic deficits after experimental stroke. In *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 31 (4), pp. 1093–1106. DOI: 10.1038/jcbfm.2010.194.

Ischiropoulos, H.; al-Mehdi, A. B. (1995): Peroxynitrite-mediated oxidative protein modifications. In *FEBS letters* 364 (3), pp. 279–282.

Jana, Malabendu; Anderson, Jamar A.; Saha, Ramendra N.; Liu, Xiaojuan; Pahan, Kalipada (2005): Regulation of inducible nitric oxide synthase in proinflammatory cytokine-stimulated human primary astrocytes. In *Free radical biology & medicine* 38 (5), pp. 655–664. DOI: 10.1016/j.freeradbiomed.2004.11.021.

Jebelli, Joseph; Hooper, Claudie; Pocock, Jennifer M. (2014): Microglial p53 activation is detrimental to neuronal synapses during activation-induced inflammation: Implications for neurodegeneration. In *Neuroscience letters* 583, pp. 92–97. DOI: 10.1016/j.neulet.2014.08.049.

Jia, Hong-bin; Jin, Yi; Ji, Qing; Hu, Yi-feng; Zhou, Zhi-qiang; Xu, Jian-guo; Yang, Jian-jun (2009): Effects of recombinant human erythropoietin on neuropathic pain and cerebral expressions of cytokines and nuclear factor-kappa B. In *Canadian journal of anaesthesia = Journal canadien d'anesthesie* 56 (8), pp. 597–603. DOI: 10.1007/s12630-009-9111-0.

Jimbo, Kotaro; Park, Jin Soo; Yokosuka, Kimiaki; Sato, Kimiaki; Nagata, Kensei (2005): Positive feedback loop of interleukin-1beta upregulating production of inflammatory mediators in human intervertebral disc cells in vitro. In *Journal of neurosurgery. Spine* 2 (5), pp. 589–595. DOI: 10.3171/spi.2005.2.5.0589.

Jin, Hong-Jian; Xiang, Li-Xin; Shao, Jian-Zhong (2007a): Molecular cloning and identification of macrophage migration inhibitory factor (MIF) in teleost fish. In *Developmental and comparative immunology* 31 (11), pp. 1131–1144. DOI: 10.1016/j.dci.2007.02.004.

Jin, Hong-Jian; Xiang, Li-Xin; Shao, Jian-Zhong (2007b): Molecular cloning and identification of macrophage migration inhibitory factor (MIF) in teleost fish. In *Developmental and comparative immunology* 31 (11), pp. 1131–1144. DOI: 10.1016/j.dci.2007.02.004.

Jonsson, Thorlakur; Stefansson, Kari (2013): TREM2 and neurodegenerative disease. In *The New England journal of medicine* 369 (16), pp. 1568–1569. DOI: 10.1056/NEJMc1306509.

Jorgensen, William L.; Trofimov, Alexander; Du, Xin; Hare, Alissa A.; Leng, Lin; Bucala, Richard (2011): Benzisothiazolones as modulators of macrophage migration inhibitory factor. In *Bioorganic & medicinal chemistry letters* 21 (15), pp. 4545–4549. DOI: 10.1016/j.bmcl.2011.05.127.

Jung, S.; Aliberti, J.; Graemmel, P.; Sunshine, M. J.; Kreutzberg, G. W.; Sher, A.; Littman, D. R. (2000): Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. In *Molecular and cellular biology* 20 (11), pp. 4106–4114.

Juttler, Eric; Tarabin, Victoria; Schwaninger, Markus (2002): Interleukin-6 (IL-6): a possible neuromodulator induced by neuronal activity. In *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 8 (3), pp. 268–275.

Kaiyala, K. J.; Prigeon, R. L.; Kahn, S. E.; Woods, S. C.; Schwartz, M. W. (2000): Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. In *Diabetes* 49 (9), pp. 1525–1533.

Kamat, Pradip Kumar (2015a): Streptozotocin induced Alzheimer's disease like changes and the underlying neural degeneration and regeneration mechanism. In *Neural regeneration research* 10 (7), pp. 1050–1052. DOI: 10.4103/1673-5374.160076.

Kamat, Pradip Kumar (2015b): Streptozotocin induced Alzheimer's disease like changes and the underlying neural degeneration and regeneration mechanism. In *Neural regeneration research* 10 (7), pp. 1050–1052. DOI: 10.4103/1673-5374.160076.

Kaushal, Alka; Wani, Willayat Yousuf; Anand, R.; Gill, Kiran Dip (2013): Spontaneous and induced nontransgenic animal models of AD: modeling AD using combinatorial approach. In *American journal of Alzheimer's disease and other dementias* 28 (4), pp. 318–326. DOI: 10.1177/1533317513488914.

Kawabori, Masahito; Kacimi, Rachid; Kauppinen, Tiina; Calosing, Cyrus; Kim, Jong Youl; Hsieh, Christine L. et al. (2015): Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 35 (8), pp. 3384–3396. DOI: 10.1523/JNEUROSCI.2620-14.2015.

Kerschbaumer Randolf J, Manfred Rieger, Neutralization of Macrophage Migration Inhibitory Factor (MIF) by Fully Human Antibodies Correlates with Their Specificity for the β-Sheet Structure of MIF, J Biol Chem. 2012 Mar 2; 287(10): 7446–7455. Published online 2012 Jan 11. doi: 10.1074/jbc.M111.329664

Kerti, Lucia; Witte, A. Veronica; Winkler, Angela; Grittner, Ulrike; Rujescu, Dan; Floel, Agnes, Higher glucose levels associated with lower memory and reduced hippocampal microstructure, 10.1212/01.wnl.0000435561.00234.ee , 2013

Kennedy, D. W.; Abkowitz, J. L. (1997): Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. In *Blood* 90 (3), pp. 986–993.

Kim, Bhumsoo; Figueroa-Romero, Claudia; Pacut, Crystal; Backus, Carey; Feldman, Eva L. (2015): Insulin Resistance Prevents AMPK-induced Tau Dephosphorylation through Akt-mediated Increase in AMPKSer-485

Phosphorylation. In *The Journal of biological chemistry* 290 (31), pp. 19146–19157. DOI: 10.1074/jbc.M115.636852.

Kıray, Hülya; Lindsay, Susan L.; Hosseinzadeh, Sara; Barnett, Susan C.: The multifaceted role of astrocytes in regulating myelination. In *Experimental Neurology. DOI:* 10.1016/j.expneurol.2016.03.009.

Kirk-Sanchez, Neva J.; McGough, Ellen L. (2013): Physical exercise and cognitive performance in the elderly: current perspectives. In *Clinical Interventions in Aging* 9, pp. 51–62. DOI: 10.2147/CIA.S39506.

Kleemann, R.; Hausser, A.; Geiger, G.; Mischke, R.; Burger-Kentischer, A.; Flieger, O. et al. (2000): Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. In *Nature* 408 (6809), pp. 211–216. DOI: 10.1038/35041591.

Knoblach, S. M.; Faden, A. I. (1998): Interleukin-10 improves outcome and alters proinflammatory cytokine expression after experimental traumatic brain injury. In *Experimental Neurology* 153 (1), pp. 143–151. DOI: 10.1006/exnr.1998.6877.

Koda, Masao; Nishio, Yutaka; Hashimoto, Masayuki; Kamada, Takahito; Koshizuka, Shuhei; Yoshinaga, Katsunori et al. (2004): Up-regulation of macrophage migration-inhibitory factor expression after compression-induced spinal cord injury in rats. In *Acta neuropathologica* 108 (1), pp. 31–36. DOI: 10.1007/s00401-004-0853-z.

Koleva-Georgieva, Desislava N.; Sivkova, Nelly P.; Terzieva, Dora (2011): Serum inflammatory cytokines IL-1beta, IL-6, TNF-alpha and VEGF have influence on the development of diabetic retinopathy. In *Folia medica* 53 (2), pp. 44–50.

Kolodziejczyk, Karolina; Saab, Aiman S.; Nave, Klaus-Armin; Attwell, David (2010): Why do oligodendrocyte lineage cells express glutamate receptors? In *F1000 Biology Reports* 2, p. 57. DOI: 10.3410/B2-57.

Konat, Gregory W.; Kielian, Tammy; Marriott, Ian (2006): The role of Toll-like receptors in CNS response to microbial challenge. In *Journal of neurochemistry* 99 (1), pp. 1–12. DOI: 10.1111/j.1471-4159.2006.04076.x.

Koning, Nathalie; Bo, Lars; Hoek, Robert M.; Huitinga, Inge (2007): Downregulation of macrophage inhibitory molecules in multiple sclerosis lesions. In *Annals of neurology* 62 (5), pp. 504–514. DOI: 10.1002/ana.21220.

Konsman, Jan Pieter; Parnet, Patricia; Dantzer, Robert (2002): Cytokine-induced sickness behaviour: mechanisms and implications. In *Trends in neurosciences* 25 (3), pp. 154–159.

Koo, Ja Wook; Duman, Ronald S. (2008): IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. In *Proceedings of the National Academy of Sciences of the United States of America* 105 (2), pp. 751–756. DOI: 10.1073/pnas.0708092105.

Koscso, Balazs; Csoka, Balazs; Selmeczy, Zsolt; Himer, Leonora; Pacher, Pal; Virag, Laszlo; Hasko, Gyorgy (2012): Adenosine augments IL-10 production by microglial cells through an A2B adenosine receptor-mediated

process. In *Journal of immunology (Baltimore, Md. : 1950)* 188 (1), pp. 445–453. DOI: 10.4049/jimmunol.1101224.

Kozlowska, Kasia; Walker, Peter; McLean, Loyola; Carrive, Pascal (2015): Fear and the Defense Cascade: Clinical Implications and Management. In *Harvard Review of Psychiatry* 23 (4), pp. 263–287. DOI: 10.1097/HRP.0000000000000065.

Kraft-Terry, Stephanie D.; Stothert, Andrew R.; Buch, Shilpa; Gendelman, Howard E. (2010): HIV-1 neuroimmunity in the era of antiretroviral therapy. In *Neurobiology of disease* 37 (3), pp. 542–548. DOI: 10.1016/j.nbd.2009.12.015.

Kraska, Audrey; Santin, Mathieu D.; Dorieux, Olene; Joseph-Mathurin, Nelly; Bourrin, Emmanuel; Petit, Fanny et al. (2012): In vivo cross-sectional characterization of cerebral alterations induced by intracerebroventricular administration of streptozotocin. In *PloS one* 7 (9), e46196. DOI: 10.1371/journal.pone.0046196.

Kroncke, K. D.; Fehsel, K.; Sommer, A.; Rodriguez, M. L.; Kolb-Bachofen, V. (1995): Nitric oxide generation during cellular metabolization of the diabetogenic N-methyl-N-nitroso-urea streptozotozin contributes to islet cell DNA damage. In *Biological chemistry Hoppe-Seyler* 376 (3), pp. 179–185.

Kunze, Albrecht; Congreso, Marga Rubenecia; Hartmann, Christian; Wallraff-Beck, Anke; Hüttmann, Kerstin; Bedner, Peter et al. (2008): Connexin expression by radial glia-like cells is required for neurogenesis in the adult dentate gyrus. In *Proceedings of the National Academy of Sciences of the United States of America* 106 (27), pp. 11336–11341. DOI: 10.1073/pnas.0813160106.

Lanahan, A.; Williams, J. B.; Sanders, L. K.; Nathans, D. (1992): Growth factor-induced delayed early response genes. In *Molecular and cellular biology* 12 (9), pp. 3919–3929.

Lawson, L. J.; Perry, V. H.; Dri, P.; Gordon, S. (1990): Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. In *Neuroscience* 39 (1), pp. 151–170.

Ledoux, Kathleen (2015): Understanding compassion fatigue: understanding compassion. In *Journal of advanced nursing* 71 (9), pp. 2041–2050. DOI: 10.1111/jan.12686.

Lee, Sungho; Varvel, Nicholas H.; Konerth, Megan E.; Xu, Guixiang; Cardona, Astrid E.; Ransohoff, Richard M.; Lamb, Bruce T. (2010): CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. In *The American journal of pathology* 177 (5), pp. 2549–2562. DOI: 10.2353/ajpath.2010.100265.

Leech, M.; Metz, C.; Hall, P.; Hutchinson, P.; Gianis, K.; Smith, M. et al. (1999): Macrophage migration inhibitory factor in rheumatoid arthritis: evidence of proinflammatory function and regulation by glucocorticoids. In *Arthritis and rheumatism* 42 (8), pp. 1601–1608. DOI: 10.1002/1529-0131(199908)42:8<1601::AID-ANR6>3.0.CO;2-B.

Leng, Lin; Chen, Liang; Fan, Juan; Greven, Dorothee; Arjona, Alvaro; Du, Xin

et al. (2011): A small-molecule macrophage migration inhibitory factor antagonist protects against glomerulonephritis in lupus-prone NZB/NZW F1 and MRL/lpr mice. In *Journal of immunology (Baltimore, Md. : 1950)* 186 (1), pp. 527–538. DOI: 10.4049/jimmunol.1001767.

Lenzen, S. (2008): The mechanisms of alloxan- and streptozotocin-induced diabetes. In *Diabetologia* 51 (2), pp. 216–226. DOI: 10.1007/s00125-007-0886-7.

Leonard, B. E.; Myint, A. (2006): Inflammation and depression: is there a causal connection with dementia? In *Neurotoxicity research* 10 (2), pp. 149–160.

Lester-Coll, Nataniel; Rivera, Enrique J.; Soscia, Stephanie J.; Doiron, Kathryn; Wands, Jack R.; de la Monte, Suzanne M (2006): Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. In *Journal of Alzheimer's disease : JAD* 9 (1), pp. 13–33.

Leung, Kelvin K.; Malone, Ian M.; Ourselin, Sebastien; Gunter, Jeffrey L.; Bernstein, Matt A.; Thompson, Paul M. et al. (2015): Effects of changing from non-accelerated to accelerated MRI for follow-up in brain atrophy measurement. In *NeuroImage* 107, pp. 46–53. DOI: 10.1016/j.neuroimage.2014.11.049.

Liu, Chia-Chen; Kanekiyo, Takahisa; Xu, Huaxi; Bu, Guojun (2013): Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. In *Nature reviews. Neurology* 9 (2), pp. 106–118. DOI: 10.1038/nrneurol.2012.263.

Li Zhou, Monica Miranda-Saksena and Nitin K Saksena, Viruses and neurodegeneration, DOI: 10.1186/1743-422X-10-172, virology Journal201310:172, 2013

Llamas-Covarrubias, Ma; Valle, Y.; Bucala, R.; Navarro-Hernández, Re; Palafox-Sánchez, Ca; Padilla-Gutiérrez, JR et al. (2013): Macrophage migration inhibitory factor (MIF): genetic evidence for participation in early onset and early stage rheumatoid arthritis. In *Cytokine* 61 (3), pp. 759–765. DOI: 10.1016/j.cyto.2012.12.032.

Loane, David J.; Byrnes, Kimberly R. (2010): Role of microglia in neurotrauma. In *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* 7 (4), pp. 366–377. DOI: 10.1016/j.nurt.2010.07.002.

Lokensgard, James R.; Mutnal, Manohar B.; Prasad, Sujata; Sheng, Wen; Hu, Shuxian (2016): Glial cell activation, recruitment, and survival of B-lineage cells following MCMV brain infection. In *Journal of Neuroinflammation* 13 (1), p. 114. DOI: 10.1186/s12974-016-0582-y.

Lolis, Elias; Bucala, Richard (2003): Macrophage migration inhibitory factor. In *Expert opinion on therapeutic targets* 7 (2), pp. 153–164. DOI: 10.1517/14728222.7.2.153.

Longhi, L.; Ortolano, F.; Zanier, E. R.; Perego, C.; Stocchetti, N.; Simoni, M.

G. de (2008): Effect of traumatic brain injury on cognitive function in mice lacking p55 and p75 tumor necrosis factor receptors. In *Acta neurochirurgica. Supplement* 102, pp. 409–413.

Longhi, Luca; Perego, Carlo; Ortolano, Fabrizio; Aresi, Silvia; Fumagalli, Stefano; Zanier, Elisa R. et al. (2013): Tumor necrosis factor in traumatic brain injury: effects of genetic deletion of p55 or p75 receptor. In *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 33 (8), pp. 1182–1189. DOI: 10.1038/jcbfm.2013.65.

Lue, H.; Thiele, M.; Franz, J.; Dahl, E.; Speckgens, S.; Leng, L. et al. (2007): Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. In *Oncogene* 26 (35), pp. 5046–5059. DOI: 10.1038/sj.onc.1210318.

Lue, Hongqi; Dewor, Manfred; Leng, Lin; Bucala, Richard; Bernhagen, Jurgen (2011): Activation of the JNK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on CXCR4 and CD74. In *Cellular signalling* 23 (1), pp. 135–144. DOI: 10.1016/j.cellsig.2010.08.013.

Lue, Hongqi; Kapurniotu, Aphrodite; Fingerle-Rowson, Gunter; Roger, Thierry; Leng, Lin; Thiele, Michael et al. (2006): Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. In *Cellular signalling* 18 (5), pp. 688–703. DOI: 10.1016/j.cellsig.2005.06.013.

Lull, Melinda E.; Block, Michelle L. (2010): Microglial Activation & Chronic Neurodegeneration. In *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* 7 (4), pp. 354–365. DOI: 10.1016/j.nurt.2010.05.014.

Madeira, Mila Fernandes Moreira; Queiroz-Junior, Celso Martins; Costa, Graciela Mitre; Santos, Patricia Campi; Silveira, Elcia Maria; Garlet, Gustavo Pompermaier et al. (2012): MIF induces osteoclast differentiation and contributes to progression of periodontal disease in mice. In *Microbes and infection / Institut Pasteur* 14 (2), pp. 198–206. DOI: 10.1016/j.micinf.2011.09.005.

Maggio, Marcello; Guralnik, Jack M.; Longo, Dan L.; Ferrucci, Luigi (2006): Interleukin-6 in aging and chronic disease: a magnificent pathway. In *The journals of gerontology. Series A, Biological sciences and medical sciences* 61 (6), pp. 575–584.

Mainardi, Marco; Fusco, Salvatore; Grassi, Claudio (2015): Modulation of Hippocampal Neural Plasticity by Glucose-Related Signaling. In *Neural Plasticity* 2015, p. 657928. DOI: 10.1155/2015/657928.

Maiti, Panchanan; Piacentini, Roberto; Ripoli, Cristian; Grassi, Claudio; Bitan, Gal (2011): Surprising toxicity and assembly behaviour of amyloid beta-protein oxidized to sulfone. In *The Biochemical journal* 433 (2), pp. 323–332. DOI: 10.1042/BJ20101391.

Maldonado, Roberto A.; Andrian, Ulrich H. von (2010): How tolerogenic

dendritic cells induce regulatory T cells. In *Advances in immunology* 108, pp. 111–165. DOI: 10.1016/B978-0-12-380995-7.00004-5.

Malik, Manasi; Simpson, James F.; Parikh, Ishita; Wilfred, Bernard R.; Fardo, David W.; Nelson, Peter T.; Estus, Steven (2013): CD33 Alzheimer's risk-altering polymorphism, CD33 expression, and exon 2 splicing. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33 (33), pp. 13320–13325. DOI: 10.1523/JNEUROSCI.1224-13.2013.

Marambaud, Philippe; Dreses-Werringloer, Ute; Vingtdeux, Valerie (2009): Calcium signaling in neurodegeneration. In *Molecular neurodegeneration* 4, p. 20. DOI: 10.1186/1750-1326-4-20.

Marchetti, Lara; Klein, Matthias; Schlett, Katalin; Pfizenmaier, Klaus; Eisel, Ulrich L. M. (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. In *The Journal of biological chemistry* 279 (31), pp. 32869–32881. DOI: 10.1074/jbc.M311766200.

Marshall, J. D.; Chehimi, J.; Gri, G.; Kostman, J. R.; Montaner, L. J.; Trinchieri, G. (1999): The interleukin-12-mediated pathway of immune events is dysfunctional in human immunodeficiency virus-infected individuals. In *Blood* 94 (3), pp. 1003–1011.

Masutani, M.; Suzuki, H.; Kamada, N.; Watanabe, M.; Ueda, O.; Nozaki, T. et al. (1999): Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes. In *Proceedings of the National Academy of Sciences of the United States of America* 96 (5), pp. 2301–2304.

Matough, Fatmah A.; Budin, Siti B.; Hamid, Zariyantey A.; Alwahaibi, Nasar; Mohamed, Jamaludin (2011): The Role of Oxidative Stress and Antioxidants in Diabetic Complications. In *Sultan Qaboos University Medical Journal* 12 (1), pp. 5–18.

Matus-Amat, Patricia; Higgins, Emily A.; Sprunger, David; Wright-Hardesty, Karli; Rudy, Jerry W. (2007): The role of dorsal hippocampus and basolateral amygdala NMDA receptors in the acquisition and retrieval of context and contextual fear memories. In *Behavioral neuroscience* 121 (4), pp. 721–731. DOI: 10.1037/0735-7044.121.4.721.

McAlpine, Fiona E.; Tansey, Malu G. (2008): Neuroinflammation and tumor necrosis factor signaling in the pathophysiology of Alzheimer's disease. In *Journal of inflammation research* 1, pp. 29–39.

McGaugh, James L.; Roozendaal, Benno (2002): Role of adrenal stress hormones in forming lasting memories in the brain. In *Current opinion in neurobiology* 12 (2), pp. 205–210.

McGeer, Patrick L.; McGeer, Edith G. (2007): NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies. In *Neurobiology of aging* 28 (5), pp. 639–647. DOI: 10.1016/j.neurobiolaging.2006.03.013.

Melia, K. R.; Falls, W. A.; Davis, M. (1992): Involvement of pertussis toxin sensitive G-proteins in conditioned fear-potentiated startle: possible

involvement of the amygdala. In *Brain research* 584 (1-2), pp. 141–148.

Mencl, W. E.; Pugh, K. R.; Shaywitz, S. E.; Shaywitz, B. A.; Fulbright, R. K.; Constable, R. T. et al. (2000): Network analysis of brain activations in working memory: behavior and age relationships. In *Microscopy research and technique* 51 (1), pp. 64–74. DOI: 10.1002/1097-0029(20001001)51:1<64::AID-JEMT7>3.0.CO;2-D.

Merk, Melanie; Mitchell, Robert A.; Endres, Stefan; Bucala, Richard (2012a): D-dopachrome tautomerase (D-DT or MIF-2): doubling the MIF cytokine family. In *Cytokine* 59 (1), pp. 10–17. DOI: 10.1016/j.cyto.2012.03.014.

Merk, Melanie; Mitchell, Robert A.; Endres, Stefan; Bucala, Richard (2012b): D-dopachrome tautomerase (D-DT or MIF-2): doubling the MIF cytokine family. In *Cytokine* 59 (1), pp. 10–17. DOI: 10.1016/j.cyto.2012.03.014.

Meyer-Siegler, Katherine L.; Leifheit, Erica C.; Vera, Pedro L. (2004): Inhibition of macrophage migration inhibitory factor decreases proliferation and cytokine expression in bladder cancer cells. In *BMC cancer* 4, p. 34. DOI: 10.1186/1471-2407-4-34.

Migliore, Lucia; Coppede, Fabio (2002): Genetic and environmental factors in cancer and neurodegenerative diseases. In *Mutation research* 512 (2-3), pp. 135–153.

Mildner, Alexander; Schmidt, Hauke; Nitsche, Mirko; Merkler, Doron; Hanisch, Uwe-Karsten; Mack, Matthias et al. (2007): Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. In *Nature neuroscience* 10 (12), pp. 1544–1553. DOI: 10.1038/nn2015.

Minas, Konstantinos; Liversidge, Janet (2006): Is the CD200/CD200 receptor interaction more than just a myeloid cell inhibitory signal? In *Critical reviews in immunology* 26 (3), pp. 213–230.

Mischke, R.; Kleemann, R.; Brunner, H.; Bernhagen, J. (1998): Cross-linking and mutational analysis of the oligomerization state of the cytokine macrophage migration inhibitory factor (MIF). In *FEBS letters* 427 (1), pp. 85–90.

Mitchell, Robert A.; Liao, Hong; Chesney, Jason; Fingerle-Rowson, Gunter; Baugh, John; David, John; Bucala, Richard (2002): Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. In *Proceedings of the National Academy of Sciences of the United States of America* 99 (1), pp. 345–350. DOI: 10.1073/pnas.012511599.

Mohamed-Ali, V.; Pinkney, J. H.; Panahloo, A.; Goodrick, S.; Coppack, S. W.; Yudkin, J. S. (1997): Relationships between plasma leptin and insulin concentrations, but not insulin resistance, in non-insulin-dependent (type 2) diabetes mellitus. In *Diabetic medicine : a journal of the British Diabetic Association* 14 (5), pp. 376–380. DOI: 10.1002/(SICI)1096-9136(199705)14:5<376::AID-DIA370>3.0.CO;2-9.

Morales-Corraliza, Jose; Wong, Harrison; Mazzella, Matthew J.; Che, Shaoli; Lee, Sang Han; Petkova, Eva et al. (2016): Brain-Wide Insulin Resistance, Tau Phosphorylation Changes, and Hippocampal Neprilysin and Amyloid-beta

Alterations in a Monkey Model of Type 1 Diabetes. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 36 (15), pp. 4248–4258. DOI: 10.1523/JNEUROSCI.4640-14.2016.

Moran, Chris; Beare, Richard; Phan, Thanh G.; Bruce, David G.; Callisaya, Michele L.; Srikanth, Velandai (2015): Type 2 diabetes mellitus and biomarkers of neurodegeneration. In *Neurology* 85 (13), pp. 1123–1130. DOI: 10.1212/WNL.0000000000001982.

Moustafa, Ahmed A.; Gilbertson, Mark W.; Orr, Scott P.; Herzallah, Mohammad M.; Servatius, Richard J.; Myers, Catherine E. (2012): A model of amygdala-hippocampal-prefrontal interaction in fear conditioning and extinction in animals. In *Brain and cognition* 81 (1), 10.1016/j.bandc.2012.10.005. DOI: 10.1016/j.bandc.2012.10.005.

Mouton, Peter R.; Long, Jeffrey M.; Lei, De-Liang; Howard, Victor; Jucker, Mathias; Calhoun, Michael E.; Ingram, Donald K. (2002): Age and gender effects on microglia and astrocyte numbers in brains of mice. In *Brain research* 956 (1), pp. 30–35.

Muller, Alexandre P.; Zimmer, Eduardo Rigon; Kalinine, Eduardo; Haas, Clarissa B.; Oses, Jean Pierre; Martimbianco de Assis, Adriano et al. (2012): Physical exercise exacerbates memory deficits induced by intracerebroventricular STZ but improves insulin regulation of H(2)O(2) production in mice synaptosomes. In *Journal of Alzheimer's disease : JAD* 30 (4), pp. 889–898. DOI: 10.3233/JAD-2012-112066.

Munoz, Lenka; Ralay Ranaivo, Hantamalala; Roy, Saktimayee M.; Hu, Wenhui; Craft, Jeffrey M.; McNamara, Laurie K. et al. (2007): A novel p38 alpha MAPK inhibitor suppresses brain proinflammatory cytokine up-regulation and attenuates synaptic dysfunction and behavioral deficits in an Alzheimer's disease mouse model. In *Journal of Neuroinflammation* 4, p. 21. DOI: 10.1186/1742-2094-4-21.

Munoz, Lenka; Ramsay, Emma E.; Manetsch, Melanie; Ge, Qi; Peifer, Christian; Laufer, Stefan; Ammit, Alaina J. (2010): Novel p38 MAPK inhibitor ML3403 has potent anti-inflammatory activity in airway smooth muscle. In *European journal of pharmacology* 635 (1-3), pp. 212–218. DOI: 10.1016/j.ejphar.2010.02.037.

Muzzio, Isabel A.; Kentros, Clifford; Kandel, Eric (2009): What is remembered? Role of attention on the encoding and retrieval of hippocampal representations. In *The Journal of physiology* 587 (Pt 12), pp. 2837–2854. DOI: 10.1113/jphysiol.2009.172445.

Nazem, Amir; Sankowski, Roman; Bacher, Michael; Al-Abed, Yousef (2015): Rodent models of neuroinflammation for Alzheimer’s disease. In *Journal of Neuroinflammation* 12, p. 74. DOI: 10.1186/s12974-015-0291-y.

Neuroinflammation Working Group; Akiyama, Haruhiko; Barger, Steven; Barnum, Scott; Bradt, Bonnie; Bauer, Joachim et al. (2000): Inflammation and Alzheimer’s disease. In *Neurobiology of aging* 21 (3), pp. 383–421.

Nilsson, Jakob; Kinloch-de-Loes, Sabine; Granath, Anna; Sonnerborg, Anders; Goh, Li-Ean; Andersson, Jan (2007): Early immune activation in gut-

associated and peripheral lymphoid tissue during acute HIV infection. In *AIDS (London, England)* 21 (5), pp. 565–574. DOI: 10.1097/QAD.0b013e3280117204.

Nishibori, M.; Nakaya, N.; Tahara, A.; Kawabata, M.; Mori, S.; Saeki, K. (1996): Presence of macrophage migration inhibitory factor (MIF) in ependyma, astrocytes and neurons in the bovine brain. In *Neuroscience letters* 213 (3), pp. 193–196.

Nishihira, Jun; Sakaue, Shinji (2012): Overview of Macrophage Migration Inhibitory Factor (MIF) as a Potential Biomarker Relevant to Adiposity. In *Journal of Traditional and Complementary Medicine* 2 (3), pp. 186–191.

Nishino, T.; Bernhagen, J.; Shiiki, H.; Calandra, T.; Dohi, K.; Bucala, R. (1995): Localization of macrophage migration inhibitory factor (MIF) to secretory granules within the corticotrophic and thyrotrophic cells of the pituitary gland. In *Molecular medicine (Cambridge, Mass.)* 1 (7), pp. 781–788.

Nishiyama, A.; Chang, A.; Trapp, B. D. (1999): NG2+ glial cells: a novel glial cell population in the adult brain. In *Journal of neuropathology and experimental neurology* 58 (11), pp. 1113–1124.

Okamoto, Hiroshi; Kimura, Mitsutoshi; Watanabe, Noriyuki; Ogihara, Masahiko (2009): Tumor necrosis factor (TNF) receptor-2-mediated DNA synthesis and proliferation in primary cultures of adult rat hepatocytes: The involvement of endogenous transforming growth factor-alpha. In *European journal of pharmacology* 604 (1-3), pp. 12–19. DOI: 10.1016/j.ejphar.2008.12.004.

Olivera, Gabriela C.; Ren, Xiaoyuan; Vodnala, Suman K.; Lu, Jun; Coppo, Lucia; Leepiyasakulchai, Chaniya et al. (2016): Nitric Oxide Protects against Infection-Induced Neuroinflammation by Preserving the Stability of the Blood-Brain Barrier. In *PLoS pathogens* 12 (2), e1005442. DOI: 10.1371/journal.ppat.1005442.

Parkhurst, Christopher N.; Yang, Guang; Ninan, Ipe; Savas, Jeffrey N.; Yates, John R. 3rd; Lafaille, Juan J. et al. (2013): Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. In *Cell* 155 (7), pp. 1596–1609. DOI: 10.1016/j.cell.2013.11.030.

Pearson-Leary, Jiah; Osborne, Danielle Maria; McNay, Ewan C. (2015): Role of Glia in Stress-Induced Enhancement and Impairment of Memory. In *Frontiers in integrative neuroscience* 9, p. 63. DOI: 10.3389/fnint.2015.00063.

Peng, Qisheng; Malhotra, Shikha; Torchia, James A.; Kerr, William G.; Coggeshall, K. Mark; Humphrey, Mary Beth (2010): TREM2- and DAP12-dependent activation of PI3K requires DAP10 and is inhibited by SHIP1. In *Science signaling* 3 (122), ra38. DOI: 10.1126/scisignal.2000500.

Peters, R. (2006): Ageing and the brain. In *Postgraduate medical journal* 82 (964), pp. 84–88. DOI: 10.1136/pgmj.2005.036665.

Phelps, Elizabeth A.; Delgado, Mauricio R.; Nearing, Katherine I.; LeDoux, Joseph E. (2004): Extinction learning in humans: role of the amygdala and vmPFC. In *Neuron* 43 (6), pp. 897–905. DOI: 10.1016/j.neuron.2004.08.042.

Phillips, M. I.; Palovcik, R. A. (1989): Dose-response testing of peptides by hippocampal brain slice recording. In *Methods in enzymology* 168, pp. 129–144.

Piacentini, Roberto; Civitelli, Livia; Ripoli, Cristian; Marcocci, Maria Elena; Chiara, Giovanna de; Garaci, Enrico et al. (2011): HSV-1 promotes Ca2+ -mediated APP phosphorylation and Abeta accumulation in rat cortical neurons. In *Neurobiology of aging* 32 (12), 2323.e13-26. DOI: 10.1016/j.neurobiolaging.2010.06.009.

Pieper, A. A.; Brat, D. J.; Krug, D. K.; Watkins, C. C.; Gupta, A.; Blackshaw, S. et al. (1999): Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. In *Proceedings of the National Academy of Sciences of the United States of America* 96 (6), pp. 3059–3064.

Planel, Emmanuel; Tatebayashi, Yoshitaka; Miyasaka, Tomohiro; Liu, Li; Wang, Lili; Herman, Mathieu et al. (2007): Insulin dysfunction induces in vivo tau hyperphosphorylation through distinct mechanisms. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 27 (50), pp. 13635–13648. DOI: 10.1523/JNEUROSCI.3949-07.2007.

Porte, Daniel, JR; Baskin, Denis G.; Schwartz, Michael W. (2005): Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. In *Diabetes* 54 (5), pp. 1264–1276.

Prather, M. D.; Lavenex, P.; Mauldin-Jourdain, M. L.; Mason, W. A.; Capitanio, J. P.; Mendoza, S. P.; Amaral, D. G. (2001): Increased social fear and decreased fear of objects in monkeys with neonatal amygdala lesions. In *Neuroscience* 106 (4), pp. 653–658.

Presky, D. H.; Yang, H.; Minetti, L. J.; Chua, A. O.; Nabavi, N.; Wu, C. Y. et al. (1996): A functional interleukin 12 receptor complex is composed of two beta-type cytokine receptor subunits. In *Proceedings of the National Academy of Sciences of the United States of America* 93 (24), pp. 14002–14007.

Qinna, Nidal A.; Badwan, Adnan A. (2015): Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. In *Drug design, development and therapy* 9, pp. 2515–2525. DOI: 10.2147/DDDT.S79885.

Quintana, Albert; Erta, Maria; Ferrer, Beatriz; Comes, Gemma; Giralt, Mercedes; Hidalgo, Juan (2013): Astrocyte-specific deficiency of interleukin-6 and its receptor reveal specific roles in survival, body weight and behavior. In *Brain, Behavior, and Immunity* 27 (1), pp. 162–173. DOI: 10.1016/j.bbi.2012.10.011.

Raivich, Gennadij (2005): Like cops on the beat: the active role of resting microglia. In *Trends in neurosciences* 28 (11), pp. 571–573. DOI: 10.1016/j.tins.2005.09.001.

Ramos-Rodriguez, Juan Jose; Infante-Garcia, Carmen; Galindo-Gonzalez, Lucia; Garcia-Molina, Yaiza; Lechuga-Sancho, Alfonso; Garcia-Alloza, Monica (2016): Increased Spontaneous Central Bleeding and Cognition Impairment in APP/PS1 Mice with Poorly Controlled Diabetes Mellitus. In *Molecular*

*neurobiology* 53 (4), pp. 2685–2697. DOI: 10.1007/s12035-015-9311-2.

Rauch, Scott L.; Shin, Lisa M.; Phelps, Elizabeth A. (2006): Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research--past, present, and future. In *Biological psychiatry* 60 (4), pp. 376–382. DOI: 10.1016/j.biopsych.2006.06.004.

Rivera, Enrique J.; Goldin, Alison; Fulmer, Noah; Tavares, Rose; Wands, Jack R.; de la Monte, Suzanne M (2005): Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. In *Journal of Alzheimer's disease : JAD* 8 (3), pp. 247–268.

Rivest, Serge (2009): Regulation of innate immune responses in the brain. In *Nat Rev Immunol* 9 (6), pp. 429–439. DOI: 10.1038/nri2565.

Roger, Thierry; Froidevaux, Celine; Martin, Christian; Calandra, Thierry (2003): Macrophage migration inhibitory factor (MIF) regulates host responses to endotoxin through modulation of Toll-like receptor 4 (TLR4). In *Journal of endotoxin research* 9 (2), pp. 119–123. DOI: 10.1179/096805103125001513.

Rosengren, E.; Bucala, R.; Aman, P.; Jacobsson, L.; Odh, G.; Metz, C. N.; Rorsman, H. (1996): The immunoregulatory mediator macrophage migration inhibitory factor (MIF) catalyzes a tautomerization reaction. In *Molecular medicine (Cambridge, Mass.)* 2 (1), pp. 143–149.

Rosi, Susanna; Ramirez-Amaya, Victor; Hauss-Wegrzyniak, Beatrice; Wenk, Gary L. (2004): Chronic brain inflammation leads to a decline in hippocampal NMDA-R1 receptors. In *Journal of Neuroinflammation* 1 (1), p. 12. DOI: 10.1186/1742-2094-1-12.

Ross, Fiona M.; Allan, Stuart M.; Rothwell, Nancy J.; Verkhratsky, Alexej (2003): A dual role for interleukin-1 in LTP in mouse hippocampal slices. In *Journal of neuroimmunology* 144 (1-2), pp. 61–67.

Rossi, A. G.; Haslett, C.; Hirani, N.; Greening, A. P.; Rahman, I.; Metz, C. N. et al. (1998): Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. In *The Journal of clinical investigation* 101 (12), pp. 2869–2874. DOI: 10.1172/JCI1524.

Rousselet, Estelle; Traver, Sabine; Monnet, Yann; Perrin, Aline; Mandjee, Nathalie; Hild, Audrey et al. (2012): Tumor necrosis factor-like weak inducer of apoptosis induces astrocyte proliferation through the activation of transforming-growth factor-alpha/epidermal growth factor receptor signaling pathway. In *Molecular pharmacology* 82 (5), pp. 948–957. DOI: 10.1124/mol.112.079608.

Rovensky, J.; Svejcar, J.; Pekarek, J.; Zitnan, D.; Hajzok, O.; Cebecauer, L. (1975): Correlation between the results of the migration inhibitory factor production test with DNA and the severity of the disease in the systemic lupus erythematosus patients. In *Zeitschrift fur Immunitatsforschung, experimentelle und klinische Immunologie* 150 (1), pp. 24–30.

Rumbaugh, Jeffrey A.; Bachani, Muznabanu; Li, Wenxue; Butler, Tracy R.; Smith, Katherine J.; Bianchet, Mario A. et al. (2013): HIV immune complexes

prevent excitotoxicity by interaction with NMDA receptors. In *Neurobiology of disease* 49, pp. 169–176. DOI: 10.1016/j.nbd.2012.08.013.

Ryan, Lee; Lin, Chun-Yu; Ketcham, Katie; Nadel, Lynn (2010): The role of medial temporal lobe in retrieving spatial and nonspatial relations from episodic and semantic memory. In *Hippocampus* 20 (1), pp. 11–18. DOI: 10.1002/hipo.20607.

Sacchetti, B.; Lorenzini, C. A.; Baldi, E.; Tassoni, G.; Bucherelli, C. (1999): Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 19 (21), pp. 9570–9578.

Saijo, Kaoru; Crotti, Andrea; Glass, Christopher K. (2010): Nuclear receptors, inflammation, and neurodegenerative diseases. In *Advances in immunology* 106, pp. 21–59. DOI: 10.1016/S0065-2776(10)06002-5.

Salkovic-Petrisic, M.; Hoyer, S. (2007): Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. In *Journal of neural transmission. Supplementum* (72), pp. 217–233.

Salkovic-Petrisic, Melita; Osmanovic, Jelena; Grunblatt, Edna; Riederer, Peter; Hoyer, Siegfried (2009): Modeling sporadic Alzheimer's disease: the insulin resistant brain state generates multiple long-term morphobiological abnormalities including hyperphosphorylated tau protein and amyloid-beta. In *Journal of Alzheimer's disease : JAD* 18 (4), pp. 729–750. DOI: 10.3233/JAD-2009-1184.

Sanders, Matthew J.; Fanselow, Michael S. (2003): Pre-training prevents context fear conditioning deficits produced by hippocampal NMDA receptor blockade. In *Neurobiology of learning and memory* 80 (2), pp. 123–129.

Sandhir, Rajat; Gupta, Smriti (2015): Molecular and biochemical trajectories from diabetes to Alzheimer's disease: A critical appraisal. In *World Journal of Diabetes* 6 (12), pp. 1223–1242. DOI: 10.4239/wjd.v6.i12.1223.

Sashinami, Hiroshi; Sakuraba, Hirotake; Ishiguro, Yoh; Munakata, Akihiro; Nishihira, Jun; Nakane, Akio (2006): The role of macrophage migration inhibitory factor in lethal Listeria monocytogenes infection in mice. In *Microbial pathogenesis* 41 (2-3), pp. 111–118. DOI: 10.1016/j.micpath.2006.06.001.

Schechter, R.; Beju, D.; Gaffney, T.; Schaefer, F.; Whetsell, L. (1996): Preproinsulin I and II mRNAs and insulin electron microscopic immunoreaction are present within the rat fetal nervous system. In *Brain research* 736 (1-2), pp. 16–27.

Scheller, Jurgen; Chalaris, Athena; Schmidt-Arras, Dirk; Rose-John, Stefan (2011): The pro- and anti-inflammatory properties of the cytokine interleukin-6. In *Biochimica et biophysica acta* 1813 (5), pp. 878–888. DOI: 10.1016/j.bbamcr.2011.01.034.

Schmidt-Supprian, M.; Murphy, C.; While, B.; Lawler, M.; Kapurniotu, A.; Voelter, W. et al. (2000): Activated protein C inhibits tumor necrosis factor and macrophage migration inhibitory factor production in monocytes. In *European*

*cytokine network* 11 (3), pp. 407–413.

Scott, Julia A.; Braskie, Meredith N.; Tosun, Duygu; Thompson, Paul M.; Weiner, Michael; DeCarli, Charles; Carmichael, Owen T. (2015): Cerebral Amyloid and Hypertension are Independently Associated with White Matter Lesions in Elderly. In *Frontiers in aging neuroscience* 7, p. 221. DOI: 10.3389/fnagi.2015.00221.

Senter, Peter D.; Al-Abed, Yousef; Metz, Christine N.; Benigni, Fabio; Mitchell, Robert A.; Chesney, J. et al. (2002): Inhibition of macrophage migration inhibitory factor (MIF) tautomerase and biological activities by acetaminophen metabolites. In *Proceedings of the National Academy of Sciences of the United States of America* 99 (1), pp. 144–149. DOI: 10.1073/pnas.011569399.

Shaftel, Solomon S.; Griffin, W. Sue T.; O'Banion, M. Kerry (2008): The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. In *Journal of Neuroinflammation* 5, p. 7. DOI: 10.1186/1742-2094-5-7.

Shah, Kaushik; Desilva, Shanal; Abbruscato, Thomas (2012): The role of glucose transporters in brain disease: diabetes and Alzheimer's Disease. In *International journal of molecular sciences* 13 (10), pp. 12629–12655. DOI: 10.3390/ijms131012629.

Shi, Lei; Argenta, Anne Elizabeth; Winseck, Adam Kerrissey; Brunso-Bechtold, Judy Karen (2004): Stereological quantification of GAD-67-immunoreactive neurons and boutons in the hippocampus of middle-aged and old Fischer 344 x Brown Norway rats. In *The Journal of comparative neurology* 478 (3), pp. 282–291. DOI: 10.1002/cne.20303.

Simons, David; Grieb, Gerrit; Hristov, Mihail; Pallua, Norbert; Weber, Christian; Bernhagen, Jurgen; Steffens, Guy (2011): Hypoxia-induced endothelial secretion of macrophage migration inhibitory factor and role in endothelial progenitor cell recruitment. In *Journal of cellular and molecular medicine* 15 (3), pp. 668–678. DOI: 10.1111/j.1582-4934.2010.01041.x.

Simons, Mikael; Nave, Klaus-Armin (2016): Oligodendrocytes: Myelination and Axonal Support. In *Cold Spring Harbor perspectives in biology* 8 (1), a020479. DOI: 10.1101/cshperspect.a020479.

Smith, David M.; Bulkin, David A. (2014): The form and function of hippocampal context representations. In *Neuroscience and biobehavioral reviews* 40, pp. 52–61. DOI: 10.1016/j.neubiorev.2014.01.005.

Sofroniew, Michael V.; Vinters, Harry V. (2010): Astrocytes: biology and pathology. In *Acta neuropathologica* 119 (1), pp. 7–35. DOI: 10.1007/s00401-009-0619-8.

Sohal, R. S.; Weindruch, R. (1996): Oxidative stress, caloric restriction, and aging. In *Science (New York, N.Y.)* 273 (5271), pp. 59–63.

Sokolowski, Jennifer D.; Chabanon-Hicks, Chloe N.; Han, Claudia Z.; Heffron, Daniel S.; Mandell, James W. (2014): Fractalkine is a "find-me" signal released by neurons undergoing ethanol-induced apoptosis. In *Frontiers in cellular neuroscience* 8, p. 360. DOI: 10.3389/fncel.2014.00360.

Steele, R. J.; Morris, R. G. (1999): Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. In *Hippocampus* 9 (2), pp. 118–136. DOI: 10.1002/(SICI)1098-1063(1999)9:2<118::AID-HIPO4>3.0.CO;2-8.

Stenvinkel, Peter; Ketteler, Markus; Johnson, Richard J.; Lindholm, Bengt; Pecoits-Filho, Roberto; Riella, Miguel et al. (2005): IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. In *Kidney international* 67 (4), pp. 1216–1233. DOI: 10.1111/j.1523-1755.2005.00200.x.

Stranahan, Alexis M.; Arumugam, Thiruma V.; Cutler, Roy G.; Lee, Kim; Egan, Josephine M.; Mattson, Mark P. (2008a): Diabetes impairs hippocampal function via glucocorticoid–mediated effects on new and mature neurons. In *Nature neuroscience* 11 (3), pp. 309–317. DOI: 10.1038/nn2055.

Stranahan, Alexis M.; Norman, Eric D.; Lee, Kim; Cutler, Roy G.; Telljohann, Richard S.; Egan, Josephine M.; Mattson, Mark P. (2008b): Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. In *Hippocampus* 18 (11), pp. 1085–1088. DOI: 10.1002/hipo.20470.

Streit, W. J.; Sparks, D. L. (1997): Activation of microglia in the brains of humans with heart disease and hypercholesterolemic rabbits. In *Journal of molecular medicine (Berlin, Germany)* 75 (2), pp. 130–138.

Strle, K.; Zhou, J. H.; Shen, W. H.; Broussard, S. R.; Johnson, R. W.; Freund, G. G. et al. (2001): Interleukin-10 in the brain. In *Critical reviews in immunology* 21 (5), pp. 427–449.

Sugimoto, H.; Suzuki, M.; Nakagawa, A.; Tanaka, I.; Nishihira, J. (1996): Crystal structure of macrophage migration inhibitory factor from human lymphocyte at 2.1 A resolution. In *FEBS letters* 389 (2), pp. 145–148.

Sürmeli, Gülşen; Marcu, Daniel Cosmin; McClure, Christina; Garden, Derek Lf; Pastoll, Hugh; Nolan, Matthew F. (2015): Molecularly Defined Circuitry Reveals Input-Output Segregation in Deep Layers of the Medial Entorhinal Cortex. In *Neuron* 88 (5), pp. 1040–1053. DOI: 10.1016/j.neuron.2015.10.041.

Szkudelski, T. (2001): The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. In *Physiological research / Academia Scientiarum Bohemoslovaca* 50 (6), pp. 537–546.

Takeuchi, Osamu; Akira, Shizuo (2010): Pattern recognition receptors and inflammation. In *Cell* 140 (6), pp. 805–820. DOI: 10.1016/j.cell.2010.01.022.

Talbot, Konrad; Wang, Hoau-Yan; Kazi, Hala; Han, Li-Ying; Bakshi, Kalindi P.; Stucky, Andres et al. (2012): Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. In *The Journal of clinical investigation* 122 (4), pp. 1316–1338. DOI: 10.1172/JCI59903.

Tarnowski, Maciej; Grymula, Katarzyna; Liu, Rui; Tarnowska, Joanna; Drukala, Justyna; Ratajczak, Janina et al. (2010): Macrophage migration inhibitory factor is secreted by rhabdomyosarcoma cells, modulates tumor

metastasis by binding to CXCR4 and CXCR7 receptors and inhibits recruitment of cancer-associated fibroblasts. In *Molecular cancer research : MCR* 8 (10), pp. 1328–1343. DOI: 10.1158/1541-7786.MCR-10-0288.

Tazi, A.; Dantzer, R.; Crestani, F.; Le Moal, M. (1988): Interleukin-1 induces conditioned taste aversion in rats: a possible explanation for its pituitary-adrenal stimulating activity. In *Brain research* 473 (2), pp. 369–371.

Tesfaye, Nolawit; Seaquist, Elizabeth R. (2010): Neuroendocrine Responses to Hypoglycemia. In *Annals of the New York Academy of Sciences* 1212, pp. 12–28. DOI: 10.1111/j.1749-6632.2010.05820.x.

Thal, D. R.; Hartig, W.; Schober, R. (1999): Diffuse plaques in the molecular layer show intracellular A beta(8-17)-immunoreactive deposits in subpial astrocytes. In *Clinical neuropathology* 18 (5), pp. 226–231.

Tilg, H.; Shapiro, L.; Vannier, E.; Poutsiaka, D. D.; Trehu, E.; Atkins, M. B. et al. (1994): Induction of circulating antagonists to IL-1 and TNF by IL-2 administration and their effects on IL-2 induced cytokine production in vitro. In *Journal of immunology (Baltimore, Md. : 1950)* 152 (6), pp. 3189–3198.

Toh, Myew-Ling; Aeberli, Daniel; Lacey, Derek; Yang, Yuan; Santos, Leilani L.; Clarkson, Michael et al. (2006): Regulation of IL-1 and TNF receptor expression and function by endogenous macrophage migration inhibitory factor. In *Journal of immunology (Baltimore, Md. : 1950)* 177 (7), pp. 4818–4825.

Tracy, Andrea L.; Phillips, Robert J.; Chi, Michael M.; Powley, Terry L.; Davidson, T. L. (2004): The gastrointestinal tract "tastes" nutrients: evidence from the intestinal taste aversion paradigm. In *American journal of physiology. Regulatory, integrative and comparative physiology* 287 (5), R1086-100. DOI: 10.1152/ajpregu.00047.2004.

Trinchieri, Giorgio (2007): Interleukin-10 production by effector T cells: Th1 cells show self control. In *The Journal of experimental medicine* 204 (2), pp. 239–243. DOI: 10.1084/jem.20070104.

Trollor, Julian N.; Smith, Evelyn; Agars, Emmeline; Kuan, Stacey A.; Baune, Bernhard T.; Campbell, Lesley et al. (2012): The association between systemic inflammation and cognitive performance in the elderly: the Sydney Memory and Ageing Study. In *Age (Dordrecht, Netherlands)* 34 (5), pp. 1295–1308. DOI: 10.1007/s11357-011-9301-x.

Uttara, Bayani; Singh, Ajay V.; Zamboni, Paolo; Mahajan, R. T. (2009): Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. In *Current neuropharmacology* 7 (1), pp. 65–74. DOI: 10.2174/157015909787602823.

van der Heide, Lars P.; Ramakers, Geert M.J.; Smidt, Marten P. (2006): Insulin signaling in the central nervous system: Learning to survive. In *Progress in Neurobiology* 79 (4), pp. 205–221. DOI: 10.1016/j.pneurobio.2006.06.003.

van Exel, Eric; Eikelenboom, Piet; Comijs, Hannie; Frolich, Marijke; Smit, Johannes H.; Stek, Max L. et al. (2009): Vascular factors and markers of inflammation in offspring with a parental history of late-onset Alzheimer

disease. In *Archives of general psychiatry* 66 (11), pp. 1263–1270. DOI: 10.1001/archgenpsychiatry.2009.146.

van Rossum, Denise; Hanisch, Uwe-Karsten (2004): Microglia. In *Metabolic brain disease* 19 (3-4), pp. 393–411.

van Wagoner, N. J.; Oh, J. W.; Repovic, P.; Benveniste, E. N. (1999): Interleukin-6 (IL-6) production by astrocytes: autocrine regulation by IL-6 and the soluble IL-6 receptor. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 19 (13), pp. 5236–5244.

Veerhuis, R.; Janssen, I.; Groot, C. J. de; van Muiswinkel, F. L.; Hack, C. E.; Eikelenboom, P. (1999): Cytokines associated with amyloid plaques in Alzheimer's disease brain stimulate human glial and neuronal cell cultures to secrete early complement proteins, but not C1-inhibitor. In *Experimental Neurology* 160 (1), pp. 289–299. DOI: 10.1006/exnr.1999.7199.

Vlček, Kamil; Laczó, Jan (2014): Neural Correlates of Spatial Navigation Changes in Mild Cognitive Impairment and Alzheimer's Disease. In *Frontiers in Behavioral Neuroscience* 8, p. 89. DOI: 10.3389/fnbeh.2014.00089.

Volterra, Andrea; Meldolesi, Jacopo (2005): Astrocytes, from brain glue to communication elements: the revolution continues. In *Nature reviews. Neuroscience* 6 (8), pp. 626–640. DOI: 10.1038/nrn1722.

Vossel, Keith A.; Beagle, Alexander J.; Rabinovici, Gil D.; Shu, Huidy; Lee, Suzee E.; Naasan, Georges et al. (2013): Seizures and epileptiform activity in the early stages of Alzheimer disease. In *JAMA neurology* 70 (9), pp. 1158–1166. DOI: 10.1001/jamaneurol.2013.136.

Watson GS, Craft S. The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. CNS Drugs. 2003;17(1):27-45.

Wada, Ryuichi; Yagihashi, Soroku (2004): Nitric oxide generation and poly(ADP ribose) polymerase activation precede beta-cell death in rats with a single high-dose injection of streptozotocin. In *Virchows Archiv : an international journal of pathology* 444 (4), pp. 375–382. DOI: 10.1007/s00428-003-0967-z.

Walker, Douglas G.; Dalsing-Hernandez, Jessica E.; Campbell, Nicole A.; Lue, Lih-Fen (2009): Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation. In *Experimental Neurology* 215 (1), pp. 5–19. DOI: 10.1016/j.expneurol.2008.09.003.

Wang, Melissa E.; Wann, Ellen G.; Yuan, Robin K.; Ramos Alvarez, Manuel M.; Stead, Squire M.; Muzzio, Isabel A. (2012a): Long-term stabilization of place cell remapping produced by a fearful experience. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 32 (45), pp. 15802–15814. DOI: 10.1523/JNEUROSCI.0480-12.2012.

Wang, Tongguang; Lee, Myoung-Hwa; Choi, Elliot; Pardo-Villamizar, Carlos A.; Lee, Sung Bin; Yang, In Hong et al. (2012b): Granzyme B-induced

neurotoxicity is mediated via activation of PAR-1 receptor and Kv1.3 channel. In *PloS one* 7 (8), e43950. DOI: 10.1371/journal.pone.0043950.

Wang, Wen-Ying; Tan, Meng-Shan; Yu, Jin-Tai; Tan, Lan (2015): Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. In *Annals of translational medicine* 3 (10), p. 136. DOI: 10.3978/j.issn.2305-5839.2015.03.49.

Watkins, Trent A.; Emery, Ben; Mulinyawe, Sara; Barres, Ben A. (2008): Distinct stages of myelination regulated by gamma-secretase and astrocytes in a rapidly myelinating CNS coculture system. In *Neuron* 60 (4), pp. 555–569. DOI: 10.1016/j.neuron.2008.09.011.

Weaver, J. D.; Huang, M-H; Albert, M.; Harris, T.; Rowe, J. W.; Seeman, T. E. (2002): Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging. In *Neurology* 59 (3), pp. 371–378.

Weinstein, Jonathan R.; Quan, Yi; Hanson, Josiah F.; Colonna, Lucrezia; Iorga, Michael; Honda, Shin-ichiro et al. (2015): IgM-Dependent Phagocytosis in Microglia Is Mediated by Complement Receptor 3, Not Fcalpha/mu Receptor. In *Journal of immunology (Baltimore, Md. : 1950)* 195 (11), pp. 5309–5317. DOI: 10.4049/jimmunol.1401195.

Weiser, W. Y.; Temple, P. A.; Witek-Giannotti, J. S.; Remold, H. G.; Clark, S. C.; David, J. R. (1989): Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. In *Proceedings of the National Academy of Sciences of the United States of America* 86 (19), pp. 7522–7526.

WEISKRANTZ, L. (1956): Behavioral changes associated with ablation of the amygdaloid complex in monkeys. In *Journal of comparative and physiological psychology* 49 (4), pp. 381–391.

Weiss, R. B. (1982): Streptozocin: a review of its pharmacology, efficacy, and toxicity. In *Cancer treatment reports* 66 (3), pp. 427–438.

White, Douglas W.; Suzanne Beard, R.; Barton, Erik S. (2012): Immune modulation during latent herpesvirus infection. In *Immunological reviews* 245 (1), pp. 189–208. DOI: 10.1111/j.1600-065X.2011.01074.x.

Wiltgen, Brian J.; Brown, Robert A. M.; Talton, Lynn E.; Silva, Alcino J. (2004): New circuits for old memories: the role of the neocortex in consolidation. In *Neuron* 44 (1), pp. 101–108. DOI: 10.1016/j.neuron.2004.09.015.

Windhagen, A.; Anderson, D. E.; Carrizosa, A.; Williams, R. E.; Hafler, D. A. (1996): IL-12 induces human T cells secreting IL-10 with IFN-gamma. In *Journal of immunology (Baltimore, Md. : 1950)* 157 (3), pp. 1127–1131.

Winner, Millicent; Meier, Jason; Zierow, Swen; Rendon, Beatriz E.; Crichlow, Gregg V.; Riggs, Randall et al. (2008): A novel, macrophage migration inhibitory factor suicide substrate inhibits motility and growth of lung cancer cells. In *Cancer research* 68 (18), pp. 7253–7257. DOI: 10.1158/0008-5472.CAN-07-6227.

Wold, Loren E.; Ceylan-Isik, Asli F.; Fang, Cindy X.; Yang, Xiaoping; Li, Shi-Yan; Sreejayan, Nair et al. (2006): Metallothionein alleviates cardiac

dysfunction in streptozotocin-induced diabetes: role of Ca2+ cycling proteins, NADPH oxidase, poly(ADP-Ribose) polymerase and myosin heavy chain isozyme. In *Free radical biology & medicine* 40 (8), pp. 1419–1429. DOI: 10.1016/j.freeradbiomed.2005.12.009.

Woods, S. C.; Figlewicz Lattemann, D. P.; Schwartz, M. W.; Porte, D., JR (1990): A re-assessment of the regulation of adiposity and appetite by the brain insulin system. In *International journal of obesity* 14 Suppl 3, 69-73; discussion 74-6.

Xie, Ling; Helmerhorst, Erik; Taddei, Kevin; Plewright, Brian; van Bronswijk, Wilhelm; Martins, Ralph (2002): Alzheimer's beta-amyloid peptides compete for insulin binding to the insulin receptor. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22 (10), RC221.

Yadav, Anjana; Collman, Ronald G. (2009): CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. In *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology* 4 (4), pp. 430–447. DOI: 10.1007/s11481-009-9174-2.

Yuan, Peng; Condello, Carlo; Keene, C. Dirk; Wang, Yaming; Bird, Thomas D.; Paul, Steven M. et al. (2016): TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. In *Neuron* 90 (4), pp. 724–739. DOI: 10.1016/j.neuron.2016.05.003.

Zhang, Jun-Ming; An, Jianxiong (2007): Cytokines, Inflammation and Pain. In *International anesthesiology clinics* 45 (2), pp. 27–37. DOI: 10.1097/AIA.0b013e318034194e.

Zhang, Kun; Xu, Haiyun; Cao, Longlong; Li, Kangsheng; Huang, Qingjun (2013): Interleukin-1beta inhibits the differentiation of hippocampal neural precursor cells into serotonergic neurons. In *Brain research* 1490, pp. 193–201. DOI: 10.1016/j.brainres.2012.10.025.

Zhang, Shi; Wang, Xi-Jin; Tian, Li-Peng; Pan, Jing; Lu, Guo-Qiang; Zhang, Ying-Jie et al. (2011): CD200-CD200R dysfunction exacerbates microglial activation and dopaminergic neurodegeneration in a rat model of Parkinson's disease. In *Journal of Neuroinflammation* 8, p. 154. DOI: 10.1186/1742-2094-8-154.

Zhao, H.; Du, L. N.; Jiang, J. W.; Wu, G. C.; Cao, X. D. (2001a): Modulatory effect of orphanin FQ on the IL-1beta transcripts in hippocampus. In *Sheng li xue bao : [Acta physiologica Sinica]* 53 (3), pp. 209–214.

Zhao, M. L.; Kim, M. O.; Morgello, S.; Lee, S. C. (2001b): Expression of inducible nitric oxide synthase, interleukin-1 and caspase-1 in HIV-1 encephalitis. In *Journal of neuroimmunology* 115 (1-2), pp. 182–191.

Zhao, Ming; Cribbs, David H.; Anderson, Aileen J.; Cummings, Brian J.; Su, Joseph H.; Wasserman, Andrea J.; Cotman, Carl W. (2003): The induction of the TNFalpha death domain signaling pathway in Alzheimer's disease brain. In *Neurochemical research* 28 (2), pp. 307–318.

Zhao, W.; Chen, H.; Xu, H.; Moore, E.; Meiri, N.; Quon, M. J.; Alkon, D. L.

(1999): Brain insulin receptors and spatial memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats. In *The Journal of biological chemistry* 274 (49), pp. 34893–34902.

Zhou, J. H.; Broussard, S. R.; Strle, K.; Freund, G. G.; Johnson, R. W.; Dantzer, R.; Kelley, K. W. (2001): IL-10 inhibits apoptosis of promyeloid cells by activating insulin receptor substrate-2 and phosphatidylinositol 3'-kinase. In *Journal of immunology (Baltimore, Md. : 1950)* 167 (8), pp. 4436–4442.

Zhou, Lu; Cheng, Li; He, Yun; Gu, Yin; Wang, Yejun; Wang, Chenhong (2016): Association of gene polymorphisms of FV, FII, MTHFR, SERPINE1, CTLA4, IL10, and TNFalpha with pre-eclampsia in Chinese women. In *Inflammation research : official journal of the European Histamine Research Society … [et al.]. DOI:* 10.1007/s00011-016-0953-y.

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## Ehrenwörtliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die dem Fachbereich Medizin Marburg zur Promotionsprüfung eingereichte Arbeit mit dem Titel „Effects of Macrophage migration inhibitory factors' inhibition on chronic neuroinflammation" im Institut für mmunologie unter Leitung von Prof. Dr. Stefan Bauer ohne sonstige Hilfe selbst durchgeführt und bei der Abfassung der Arbeit keine anderen als die in der Dissertation aufgeführten Hilfsmittel benutzt habe. Ich habe bisher an keinem in- oder ausländischen Medizinischen Fachbereich ein Gesuch um Zulassung zur Promotion eingereicht, noch die vorliegende oder eine andere Arbeit als Dissertation vorgelegt.

Ich versichere, dass ich sämtliche wörtlichen oder sinngemäßen Übernahmen und Zitate kenntlich gemacht habe.

Mit dem Einsatz von Software zur Erkennung von Plagiaten bin ich einverstanden.

Vorliegende Arbeit wurde (oder wird) in folgenden Publikationsorganen veröffentlicht.

Ort, Datum, Unterschrift

Die Hinweise zur Erkennung von Plagiaten habe ich zur Kenntnis genommen, die Angebote der Philipps-Universität zur Plagiatserkennung (Plagiatssoftware zu beziehen über das Hochschulrechenzentrum) sind mir bekannt.

Ort, Datum, Unterschrift Betreuer“