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## On the effect of peripheral immune stimulation on depressive-like behaviour and central inflammatory gene expression in rat

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## Contents

## Abbreviations

| | |
|---|---|
| BBB | blood brain barrier |
| BDNF | brain derived neurotrophic factor |
| cAMP | cyclic adenosine tri-phosphat |
| cDNA | complementary DNA |
| CNS | central nervous system |
| DAG | diacylglycerol |
| DNA | deoxy-ribonucleic acid |
| ECM | extracellular matrix |
| ECS | electroconvulsive seizures |
| ECT | electroconvulsive therapy |
| FRL | Flinders resistant line |
| FSL | Flinders sensitive line |
| FST | forced swim test |
| 5-HT | 5-hydroxytryptamin (serotonin) |
| 5-HT R | 5-hydroxytryptamine (serotonin) receptor |
| IDO | indoleamine-2,3-dioxygenase |
| IL | interleukin |
| iNOS | inducible nitric oxide synthase |
| i.p. | intraperitoneal |
| IP3 | inositol-3-phoshat |
| LPS | lippolysaccharide |
| MDD | major depressive disorders |
| MHC | major histocompatibility complex |
| MMP | matrix-metalloproteinase |
| mRNA | messenger RNA |
| PAMP | pathogen-associated molecular pattern |
| PNS | peripheral nervous system |
| RNA | ribonucleic acid |
| SEM | standard error of the mean |
| TIMP | tissue inhibitor of matrix-metalloproteinases |
| TLR | toll-like receptor |
| TNF | tumor necrosis factor |

## Introduction

To distinguish between mental and physical health is a common legacy that already existed back in the 16th century, when René Descartes (1596 – 1650) proposed a distinction between the brain and the mind. He believed that the brain, which was part of the body, controlled behaviour only to the extent that it resembled that of animals, whereas the mind, which was independent of the body, provided us with our inimitable human mental capabilities.

Disorders of thought, behaviour or mood have for a very long time not been shown to have any biological explanation. One of the first successful biological treatments of an illness that was then believed to be a psychiatric disorder was the treatment of *paralytic dementia*, also known as *general paralysis of the insane*. The progressive course of the disease went from symptoms of mania and euphoria to seizures, cognitive deterioration and dementia, ultimately leading to paralysis and death, and affected 10-15% of all institutionalized psychiatric patients at the turn of the century. It was found to be caused by the bacterium *Treponema pallidum*, also known as syphilis, and successfully treated by the antibiotic penicillin. The increasing knowledge about underlying biological mechanisms of mental illnesses, such as mood disorders, gives rise to better treatment and will help to relieve us from the burden they impose on our health, happiness and productivity.

Our behaviour is dependent on brain activity, which is determined by the two factors heredity, which forms the basis for our mental functions, and environment, which determines how that basis is formed and used. Not even identical twins, which were provided with the exact same genetic background, think and behave similar, due to differences during embryonic and adult environment. At the same time, similar variations in genes can be linked to similar behaviour (P Suchankova et al., 2009; Petra Suchankova et al., 2011). Thus, developmental changes in the CNS can be caused by external factors, which, therefore, have the ability to alter structure and function of the brain and

subsequently cause changes in our personality (E. Chen, G. E. Miller, Kobor, & Cole, 2011).

Affecting more than 120 million people worldwide, depression is among the leading causes of disability worldwide (Kessler et al., 2007), yet little is known about the neurobiology of mood disorders. The symptoms include a long-lasting depressed mood, feeling of guilt, anxiety, and reoccurring thoughts of death and suicide (Nestler et al., 2002). Even though there are many different pharmacological treatments available, the cause for depression remains unclear and not all patients can be treated successfully.

Accumulating evidence shows that inflammation plays a crucial role in the pathology of psychiatric disorders (Dowlati et al., 2010; Irwin, 2008; Irwin & A. H. Miller, 2007; Pollak & Yirmiya, 2002; Raison, Lucile Capuron, & A. H. Miller, 2006). Patients suffering from depression and suicide attempters show raised levels of pro-inflammatory cytokines in serum. A favoured hypothesis for the role of the immune system in the pathophysiology of depression is that patients suffering from depression and anxiety are afflicted with mental stress that in turn causes the body´s immune system to induce an inflammatory reaction. Alternatively, a low-grade chronic inflammation could lead to dysfunctions in the brain. A third explanation might be that psychiatric functions partly rely on genetic factors that are coupled to inflammation. It is of great importance to investigate these mechanisms.

As it is difficult to study inflammation-related proteins and their role in psychiatric diseases in the human brain, animal studies prove to be of great importance for exploring these questions. Since it is out of the question to simply ask an animal how it feels, it is impossible to model certain symptoms of depression, such as excessive feelings of guilt or recurrent thoughts of death or suicide, in laboratory animals. Therefore, attempts have been made to develop animal models of depression that focus on other symptoms of MDD. One model in which animals can be tested for sickness or depressive-like behaviour is the forced swim test (FST), which was used in this thesis. The effect of LPS

administration on cytokine expression both centrally and peripherally has been documented before (Terrence Deak, Bellamy, & D'Agostino, 2003; M K Hansen et al., 2000), and it has been shown that this stimulation leads to sickness and depressive-like behavior in rodents (DellaGioia & Hannestad, 2010; Frenois et al., 2007) that can be assessed in the FST.

About 20 years ago the brain was still seen as an immune-privileged organ. It was believed that the blood brain barrier (BBB) restricts the entry of any type of immune cell into the central nervous system (CNS) and that antigen from within the CNS could not reach the thymus, since there was no lymphatic drainage of the brain. Furthermore, cells within the brain were not believed to express major histocompatibility complex (MHC) molecules and thus, no antigen presentation to T-cells could take place, even if a leukocyte made it through the BBB into the brain. It has been shown, however, that there are exceptions to these presumptions. Under inflammatory conditions the properties of both the BBB and cells within the CNS change. The endothelial cells that are part of the BBB express receptors that make it possible for leukocytes to migrate into the brain and cells within the brain express MHC molecules. Furthermore, it was observed that lymphatic drainage could occur at specific sites of the CNS, so leukocytes can exit the CNS and migrate into lymphatic tissues.

Components of the immune system that are harmful under pro-inflammatory conditions can be crucial for a down-regulation of the inflammatory response and for stimulation of repair and e.g. remyelination after stroke lesions or brain trauma. Leukocytes are a potent source for neurotrophic factors, which have anti-inflammatory features and can stimulate regional tissue and limit damage. This points towards a contradictive role of inflammation in the brain, which can both be positive, e.g. for the elimination of pathogens, removal of damaged tissue in lesions and promotion of regeneration and repair, and negative, e.g. causing tissue edema and swelling, vascular damage, collateral damage of inflamed tissue and aberrant regeneration and scar formation which limits further regeneration in the lesion, and the maintenance of an equilibrium that promotes the positive aspects of immune actions within the CNS is crucial.

Accumulating evidence shows a strong correlation between the presence of inflammation and symptoms of MDD (Irwin, 2008; Irwin & A. H. Miller, 2007; Konsman, Parnet, & Dantzer, 2002; B. E. Leonard & Ayemu Myint, 2009; M Maes et al., 1997; Michael Maes, 2008; Michael Maes et al., 2009; N. Müller & Ackenheil, 1998; O'Brien, Scott, & Dinan, 2004; Parnet, Kelley, Bluthé, & Dantzer, 2002; Pollak & Yirmiya, 2002; Raison et al., 2006; Simmons & Broderick, 2005; Zhu et al., 2010) and the role inflammatory mediators in this context needs to be clarified in order to unravel the complexity of this disease.

# Background

## Depression

Major depressive disorder (MDD) is the most common mood disorder and the second cause of disability worldwide (Licinio & Wong, 2011). The symptoms that describe MDD are depressed mood, anhedonia (diminished pleasure and interest), weight loss or weight gain, insomnia or hypersomnia, fatigue, feelings of worthlessness or guilt, diminished ability to concentrate or indecisiveness, recurrent thoughts of death and suicide (The Diagnostic and Statistical Manual of Mental Disorders, DSM-IV-TR, American Psychiatric Association).

MDD has been associated with a decrease in the volume of the amygdala, hippocampus and frontal cortex (Ongür, Drevets, & Price, 1998) and antidepressant treatment has been shown to reverse this phenomena (F. Chen, Madsen, Wegener, & Nyengaard, 2009, 2010; Jansson, Wennström, Johanson, & Tingström, 2009; Jayatissa, Bisgaard, Tingström, Papp, & Wiborg, 2006).

The "monoamine hypothesis of depression" suggests an imbalance in monoamine neurotransmitters such as serotonin, dopamine and noradrenaline. Many effective antidepressants have the common effect of acutely enhancing monoamine function (Ressler & Nemeroff, 2000). Serotonin, also called *5-hydroxytryptamine* (5-HT), belongs to the monoamine family of neurotransmitters and is derived from the amino acid tryptophan. Serotonin-containing neurons are clustered within the nine raphe nuclei, each of which projects to a different region of the brain (Pytliak, Vargová, Mechírová, & Felšöci, 2011). Serotonin-selective reuptake inhibitors (SSRIs) are widely used and effective antidepressants. However, their therapeutic effects develop slowly, and the mechanism of action that mediates antidepressant effects is not believed to be due to the immediate elevation of extracellular serotonin but rather due to structural or functional adaptations of the CNS to chronically elevated serotonin levels. Apart from the 5-HT 3 receptor family, which are ligand-gated ion-

channels, all serotonin receptors are G-protein coupled. The 5-HT R1 family, including 5-HT R1B, is inhibitory, and causes a decrease in intracellular cAMP production. 5-HT 1B receptors are present in many parts of the brain, with the highest concentrations in the basal ganglia and striatum, where they inhibit the release of serotonin, and the frontal cortex, where they are believed to inhibit the release of dopamine 5-HT R2 family members are excitatory, and activation of 5-HT R2A e.g. leads to secretion of the neurotransmitter acetylcholine, which can act directly on nicotinic receptors on immune cells and, thus, exert anti-inflammatory effects (Pavlov & Tracey, 2005). Both 5-HT 1B and 2A receptors are believed to be involved in the modulation of anxiety, addiction, learning, memory and, for this thesis most importantly, mood (Pytliak et al., 2011).

Even though many of the current treatments for depression have in common the increase of neurotransmission at central serotonergic or noradrenergic synapses, the *monoamine hypothesis of mood disorders*, which says that depression is a direct consequence of a deficit in these diffuse modulatory systems, is not sufficient to explain e.g. why the antidepressant effect of these drugs takes several weeks to develop. Another hypothesis argues for the role of the hypothalamic-pituitary-adrenal (HPA) axis in depression. The HPA axis is regulated positively by the amygdala and negatively by the hippocampus and regulates the secretion of cortisol from the adrenal gland in response to stress. Pro-inflammatory cytokines also activate the HPA axis and might inhibit the hippocampal negative feedback regulation, thus contributing to the mechanisms proposed in the *HPA axis hyperactivity hypothesis of depression* (Miura et al., 2008). However, accumulating evidence that inflammatory reactions and neural-immune interactions are involved in the pathogenesis of depression and that they may be the cause for serotonergic and adrenocortical dysregulations lead to the *cytokine hypothesis of depression*. The key findings have been elevated levels of pro-inflammatory cytokines, such as IL-1β and TNFα, and increased oxidative and nitrosative stress, which are induced by internal and external factors (Michael Maes et al., 2009)

A consequence of inflammation in the CNS is the loss of neurons and astrocytes (Rajkowska et al., 1999). The reduction of astrocytes might be a result of higher levels of indoleamine-2,3-dioxygenase (IDO), which itself is induced by cytokines (O'Connor et al., 2009), which leads to higher concentrations of quinolinic acid. This cannot be adequately metabolized and is, therefore, toxic to astrocytes. This shows the complexity of effects mediated by an increased production of pro-inflammatory cytokines and other inflammatory mediators such as nitric oxide, whose synthesis is amplified by elevated levels of iNOS.

## The Immune system

Our immune system consists of two components in which different cells and molecules work cooperatively to provide host defense and to restore homeostasis. These two components are innate and adaptive immunity.

### The Innate immune response

The first line of defense against infections is provided by the innate immune response, which consists of cellular and chemical barriers such as skin, mucosal epithelia and antimicrobial molecules, blood proteins like complement and cellular defense mediated through phagocytes (macrophages and neutrophils) and natural killer cells. Its specificity is against molecules shared by groups of related microbes and molecules produced by damaged host cells and its diversity is limited, as it is germline encoded and not able to "learn". It is highly important for the organism, as it is the only immediate protection against pathogens and tissue injury before e.g. the adaptive immune response has time to develop.

### The Adaptive immune response

There are two types of adaptive immune responses, called humoral immunity and cell-mediated immunity that differ in their components and eliminate different types of microbes. Humoral immunity is mediated by antibodies that

are produced by B-lymphocytes and is the principle defense mechanism against extracellular microbes and their toxins. The receptors on the antibodies have a very large diversity as they are produced by somatic recombination of gene segments. Furthermore, the adaptive immune response has a memory function, thus it is able to remember if it had been presented to a specific antigen before, and if so, can augment the reaction. Cell-mediated immunity is mediated by T-lymphocytes and targets intracellular microbes such as viruses and some bacteria that are inaccessible to circulating antibodies.

## Inflammation

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is characterized by e.g. heat, vasodilation and edema. During acute inflammation, effector molecules and cells are recruited to the site of infection or tissue damage in order to eliminate the cause of the inflammatory reaction. Following detection of the harmful stimulus, an inflammatory cascade is initiated via the secretion of inducers and mediators including cytokines, chemokines, histamines etc. The dominant cell type that is recruited during acute inflammation is neutrophils. When activated, they start releasing toxic effector molecules that were stored in granules by endocytosis and thereby fight the pathogen. After elimination of the pathogen, further macrophages are recruited to remove cell debris and restore the tissue at the site of inflammation. For restoration to happen, an important switch in secretion from pro-inflammatory mediators to anti-inflammatory ones must take place. Instead of neutrophils, monocytes are then recruited to the tissue, which facilitate the removal of dead cells and initiate tissue remodeling.

Prolonged inflammation, known as chronic inflammation, leads to a shift in the cells present at the site of inflammation from neutrophils to macrophages and T-lymphocytes. This can happen when the acute inflammatory response wasn´t strong enough to eliminate the pathogen, when the persistent irritant is not large enough to elicit an acute inflammatory response or by autoimmune reactions (R.

K. Kumar & Wales, 2010). The symptoms are much less severe as during acute inflammation but can still cause harm.
Macrophages present the antigen to T-cells, which start secreting cytokines that in turn activate macrophages to produce chemokines, nitric oxide (NO), reactive oxygen species (ROS) and certain metalloproteinases. Thereby, the effector cells stimulate each other and the inflammatory response builds up, ultimately turning into a harmful situation for the host with tissue damage and cell loss. This detrimental cascade of chronic inflammation is suggested to be involved in diseases like cardiovascular diseases and cancer.

Matrix metalloproteinases (MMPs) are proteolytic enzymes that are involved in the maintenance and remodeling of the extracellular matrix in a variety of physiological processes and pathological conditions, such as inflammation and cancer. Among the four identified TIMPs in vertebrates, TIMP-1 is the inducible form, which is sensitive to up-regulation by e.g. IL-1β and IL-6. TIMP-1 binds to an active zinc-binding site on MMPs with its N-terminal domain and thereby mediates inhibition (Gomez, D. Alonso, Yoshiji, & Thorgeirsson, 1997). MMPs and TIMPs play a central role in the structural plasticity of the brain, and a balance between them is crucial for maintaining physiological conditions. An imbalance between MMP and TIMP is implicated in the pathogenesis of CNS disorders involving inflammation (Gardner & Ghorpade, 2003). A differential regulation of TIMP-1 in acute vs. chronic inflammatory conditions has been proposed. Pro-inflammatory cytokines like e.g. IL-1β cause acute activation of astrocytes, which leads to increased secretion of TIMP-1 into the tissue. This elicits a repair response that is typical in early injury. During chronic inflammation, however, a decline of TIMP-1 levels below homeostatic conditions is observed (Gardner & Ghorpade, 2003).

**Cytokines**

Soluble pro- and anti-inflammatory proteins such as cytokines are the key players that orchestrate initiation, maintenance and termination of inflammation. Cytokines form a vast and heterogeneous group of secreted proteins that are produced by many different cell types. They mediate and regulate innate and adaptive immune responses. Cytokines are not stored in the cell that expresses them but their synthesis is initiated upon cell activation. Newly transcribed mRNA of cytokines is often instable or rapidly degraded, thus both transcriptional activation and synthesis of cytokines is transient. Additionally they may require transcriptional or translational processing, like proteolytic cleavage of an inactive precursor molecule into an active product. This shows that cytokine expression is a highly regulated process, but once synthesized, they are rapidly secreted, resulting in a burst of release when needed.

Cytokines can be produced both by innate and adaptive immune cells and contribute to host defense via mediating and regulating both innate and adaptive responses. They can be pleiotropic, thus one cytokine can act on a variety of cell types and have multiple biological effects, as well as redundant in their actions, meaning that different cytokines may have the same effect. After release, they can act in an autocrine manner, thus on the same cell that had released them, or in a paracrine one, acting on surrounding cells, e.g. inducing further cytokine production or recruiting neutrophils. Also, if secreted in higher amounts, they can act in the distance in an endocrine way by entering the circulation and thereby spreading over the whole body. Such systemic effects can be induction of fever, cortisol production or sickness behaviour like loss of appetite and tiredness. TNFα is an example for a cytokine that can both have local and distant effects.

The brain cytokine-system has been suggested to play a crucial role in depression, orchestrating neuronal circuits and neurotransmitters that are responsible for behaviour (Dantzer & Kelley, 2007; Dantzer, J. C. O. Connor, Freund, R. W. Johnson, & Kelley, 2008).

## Inflammation in the CNS

In the CNS there is T-cell and antibody mediated adaptive immunity as well as toll-like receptor (TLR)-stimulated innate immunity. Damage is mainly caused by non-specific reactions of the innate immunity, which mainly involves activation of effector cells such as macrophages and microglia cells, which cause tissue injury. Macrophages can produce proteases, which cleave proteins of the ECM into small peptides that can be loaded on MHC molecules and, thus, used to augment a T-cell mediated inflammatory reaction by an increase of antigen presentation within the tissue. These proteases degrade parts of the extracellular matrix (ECM) and can thereby directly damage the tissue of the CNS as well as dissolve tight junctions of the endothelial cell layer in the BBB and are essential for inflammatory cells to enter into the CNS. Macrophages can augment tissue damage and pro-inflammatory environment by production of inducible nitric oxide synthetase (iNOS), which is the key enzyme in the production of NO radicals. They can also produce cytotoxic cytokines, like TNFα, which interacts with TNF-receptors on various cell types within the CNS and can induce apoptosis and, therefore, lead to unspecific tissue damage. TNFα can bind to two different receptors: TNF-R1 and TNF-R2. TNF-R2 is mainly expressed by endothelial cells and on the vascular wall, thus stimulation of TNF-R2 signaling leads to vasculitis and ischemia. TNF-R1, however, is expressed on oligodendrocytes and, in a lower extent, on neurons and can carry out its cytotoxic effects on these cells. Despite the many negative effects of TNFα it is also important for the induction of T-cell apoptosis and, thereby, termination of an immune response and can under certain conditions have neuroprotective effects (Yirmiya & Goshen, 2010).

### The Blood brain barrier

The blood brain barrier (BBB) is a very complex structure, which consists of several components. Endothelial cells, which are the predominant barrier, form

elaborate tight junctions and prevent diffusion. Some molecules can be transported from the blood through the endothelium into the cerebrospinal fluid (CSF) through vesicular transport, but this active transport rate is very low. The second barrier is the basement membrane, which has charged pores and functions as a molecular sieve, either repelling or binding charged molecules. Astrocytes form a layer around the basement membrane with their cell processes and can take up different small molecules and ions. Thereby, they maintain the ion-concentration of the ECM absolutely constant, which is crucial for the electrical activity of neurons and their axons. Furthermore, perivascular macrophages and microglia cells patrol the surrounding of the BBB. If any foreign material managed go get through into the brain, it is taken up and degraded by them.

The BBB has several functions. It acts as a barrier for hydrophilic molecules like proteins and peptides and thus restricts entry of antibodies and inflammatory mediators into the CNS, as well as it restricts exit of CNS molecules. This is crucial, since the BBB is built up at the same time as the immune system during embryonic development. Brain antigen can´t pass the BBB and therefore never enters the thymus, where tolerance against self-antigen is built up and, with some exceptions, there are no good tolerance mechanisms against brain-proteins. Thus, every brain-specific protein from the CNS has the potential to induce an auto-immune reaction which, if induced by error of the immune-system, can cause an autoimmune disease of the brain. In case of an infection of the brain there has to be a defense mechanism to clear the brain from these pathogens. Such a reaction, as always in the immune system, then leads to activation of immune cells with toxic factors, which contribute to the elimination of pathogens but are also acting on the surrounding tissue, leading to collateral damage. In contrast to the periphery, where tissue damage can be repaired after an inflammation, these repair mechanisms are missing in the brain. Therefore, collateral damage has to be kept as small as possible within the brain.

## Mood disorders and Inflammation

Inflammation has been implicated not only in neurodegenerative diseases such as Alzheimer's and multiple sclerosis (Haider et al., 2011), but also in mood disorders and suicidal behaviour. Reports indicate that an imbalance in pro-inflammatory mediators play a role for both normal brain functions and certain psychiatric disorders. One suggested mechanism involves peripheral pro-inflammatory cytokines that activate microglia and astrocytes in the nervous system, which results in a release of pro-inflammatory cytokines such as IL-1, IL-6 and TNF in the brain (Yirmiya & Goshen, 2010). Serum levels of inflammatory mediators have been shown to be elevated in depressed patients (M Maes et al., 1997; O'Brien et al., 2004). Around 40% of patients undergoing cytokine treatment for the treatment of e.g. cancer or chronic viral infections have been reported to develop depressive symptoms as well as suicidal ideation (Lucile Capuron, Hauser, Hinze-Selch, A. H. Miller, & Neveu, 2002; Pollak & Yirmiya, 2002). Interestingly, pretreatment with the antidepressant paroxetine did not affect symptoms like fever, fatigue or pain, but alleviated depression suggesting that sickness behavior and depression induced by cytokines are mediated by different mechanisms (L Capuron, Gumnick, et al., 2002). A proposed mechanism underlying this observation is the stimulation of the enzyme indoleamine-2,3-dioxygenase (IDO) by pro-inflammatory cytokines (AM Myint, Schwarz, Steinbusch, & B. Leonard, 2009). IDO degrades tryptophan into kynurenine and quinolinic acid and, thus, decreases the bioavailability of this essential amino acid for serotonin synthesis. It has been reported that microglia in depressed patients produce an excess of quinolinic acid which is suggested to lead to the destruction of astrocytes (B. E. Leonard & Ayemu Myint, 2009).

## Endotoxin administration as an experimental model of depression

Intraperitoneal (i.p.) administration of endotoxin, such as LPS, is the most commonly used immune-depression model in rodents (DellaGioia & Hannestad, 2010). The immune response subsequently leads to symptoms similar to

depression in humans: reduced appetite, the inability to experience pleasure (anhedonia), sleep disturbance and a reduction in social exploration (Larson & A. Dunn, 2001). The peripheral production of cytokines induce the expression of inflammatory mediators in the brain, which leads to sickness behaviour (Pecchi, Dallaporta, Jean, Thirion, & Troadec, 2009). Depressive-like behaviour can continue after sickness behaviour has vanished, depending on the dose of endotoxin administrated (Frenois et al., 2007).

There are several pathways through which peripheral immune stimulation can affect the brain and cause depressive-like behaviour. Leukocytes as well as endothelial cells have toll-like receptors (TLRs) that recognize so-called pathogen-associated molecular patterns (PAMPs). The binding of the endotoxin to the TLR leads to the activation of the transcription factor NFκB, which in turn initiates the transcription of several pro-inflammatory mediators (DellaGioia & Hannestad, 2010; Gaestel, Kotlyarov, & M Kracht, 2009)
.). In this way, the signal can cross the BBB. Other possibilities are signaling through vagal afferents and by direct entry of peripheral leukocytes into the brain parenchyma (Kapás et al., 2011; M. Opp & L. Toth, 1998).

## The Forced swim test

The forced swim test (FST) was developed more than 30 years ago by Porsolt et al. (Porsolt, Le Pichon, & Jalfre, 1977) and has, ever since, widely been used to assess depressive-like behaviour in rats resp. mice (J F Cryan & Mombereau, 2004). The original test failed to detect a reliable effect of selective serotonin reuptake inhibitors (SSRIs), which is why in this thesis, we used a modified version of the test (John F Cryan & Slattery, 2007; John F Cryan, Markou, & Lucki, 2002). The test is based on the observation that animals, when placed in a cylinder filled with water, will initially try to escape but will possibly develop an immobile posture (John F Cryan et al., 2002). This immobility is believed to reflect either behavioral despair, meaning that the animal fails to persist with escape-directed behavior, or the inability to cope with stressful stimuli. The

animal is exposed to the forced swimming for 15 minutes in order to habituate it, before it is tested the following day for 10 minutes. If antidepressant treatments are applied during the two swim sessions, the time spent immobile will be shorter when compared to controls that are only vehicle-treated.

### Quantitative RT PCR and the Comparative $\Delta\Delta C_t$ method

Quantitative real time polymerase chain reaction (qRT PCR) is a widely used tool to quantify gene expression by measuring the amount of mRNA in a given tissue sample. After RNA isolation from the extracted tissue the RNA is reverse transcribed into complementary DNA (cDNA), which is much more stable than RNA. cDNA differs from genomic DNA in that it does not contain any non-coding regions, called introns, since those have been cut out during a process called splicing. qRT PCR is performed in a thermocycler using fluorescent probe sequences, which are activated during hydrolysis. The fluorescence that is detected in real-time by the machine is proportional to the amount of PCR product and is plotted against the cycle number.

In this thesis, relative quantification of gene expression was performed, which means that differences in expression of a target gene between different samples were quantified. For this, a common threshold was applied on all samples and the cycle number when this threshold $C_T$ was reached, determined. The curve that results when plotting the fluorescent intensity, which represents the amount of PCR product, against the cycle number, shows a baseline when the PCR product is low, an exponential and finally a plateau phase. It is important that the threshold is set in the exponential phase of all curves, since this represents the amplicon most accurately. The $C_T$ value is indirect proportional to the amount of starting mRNA in the sample. When there is more mRNA present in the sample, the amplification will reach the threshold after fewer cycles, thus a lower $C_T$ value means more mRNA.

Before the $C_T$ values can be compared, they need to be normalized to at least one endogenous control. This is done in order to account for differences in amount of starting material, variability in RNA quality, cDNA synthesis efficiencies or PCR. The most frequent normalization method is the use of endogenous reference genes, often "housekeeping genes", that are supposed to be expressed in the same amount in every cell, since they are needed to maintain basic, essential functions that the cell needs to survive. Since it has been shown that the expression of these genes can vary under different experimental conditions (Dheda et al., 2005) the use of more than one reference gene was proposed (B. E. Bonefeld, Elfving, & Wegener, 2008; Nelissen, Smeets, Mulder, Hendriks, & Ameloot, 2010). The following formula is used to normalize against a control gene:

$$\Delta C_T = C_{T\ \text{target gene}} - C_{T\ \text{endogenous control gene}}$$

To depict the results, the fold change between treated animal and control animal was calculated using the Comparative $C_T$ method for relative quantification (Livak & Schmittgen, 2001):

$$\Delta\Delta C_T = \Delta C_{T\ \text{target gene of treated animal}} - \Delta C_{T\ \text{target gene of untreated animal}}$$

$$\text{Fold change} = 2^{-\Delta\Delta C_T}$$

A fold change of 2 means that the treated animal has a 2-fold increase in expression of the target gene as compared to the untreated sample, meaning twice as much. A reduction in fold change, e.g. 0.5 can be calculated as the negative reverse of $2^{-\Delta\Delta C_T}$, thus $-(1/(2^{-\Delta\Delta C_T}))$, which equals -2, which means a 2-fold decrease, or half as much.

## Aims

The aim of this thesis was to investigate mechanisms related to the connection between inflammation and depression. In order to do this, mRNA levels of certain cytokines and other proteins that are involved in immune response were measured before and after peripheral immune activation in the brains of rats. Additionally, we tested whether exposure to the forced swim test (FST), a widely used animal model to test for depressive-like behaviour, might alter the expression of these proteins. The possible correlation between protein expression in the CNS as well as serum levels of certain cytokines and behaviour during the FST was investigated.

# Material and Methods

## Ethics

The animal study was approved by the Animal Ethics committee at the University of Gothenburg.

## Animals

Male Sprague-Dawley (SD) rats (260-300g at the time of the experiment) from Taconic breeding colonies, Denmark, were used in these experiments. The animals were kept 6 per cage in standard laboratory cages with sawdust bedding and free access to food and water. The housing room had a 12:12 light dark cycle (lights on at 6:30 a.m.) and all experiments were performed during the light phase. All animals were allowed to acclimatize for 7 days prior to experiments, being handled briefly every second day to get used to experimenter handling.

## Outline of the experiment

The animals were divided into four groups (n=6 per group): saline treated and no forced swim test (FST); LPS treated and no FST; saline treated and FST; LPS treated and FST. The FST was conducted exactly 6 h after the LPS resp. saline injection and all animals were sacrificed by rapid decapitation 7h after the injections, i.e. 1 h after the FST. Blood was collected and brains were removed. Blood was kept at room temperature for 1 h, thereafter kept at 4°C and spun down at 4°C for 10 min at 4000rpm. Serum was then collected and stored at -20°C until further analysis. Brains were removed from the scull and dissected on ice. Brain areas of interest (amygdala, hippocampus, hypothalamus, prefrontal cortex and striatum) were immediately submerged in 1 ml of RNA stabilization reagent (RNAlater, Quiagen, Hilden, Germany) in 1.5 ml tubes which were weighed before and after the tissue was added. The tissue samples were kept at

+8°C for 24 h so the liquid could soak through the tissue and then stored at -20°C for further analysis.

## LPS administration

Lipopolysaccharide (LPS; from *Escherichia coli* serotype 055:B5, Sigma) was dissolved in 0.9% sodium chloride (saline vehicle). Injections were administered intraperitoneally (i.p.) in a concentration of 0.5 mg/kg body weight and an injection volume of approx. 1.5 ml. Control animals were injected with 1.5ml of vehicle alone.

## Forced swim test

A modified version of the forced swim test (FST) (Porsolt et al., 1977) to screen for depressive-like behavior was conducted as described by Cryan et al.(John F Cryan et al., 2002) on two consecutive days in a transparent acrylic cylinder (60 cm high, 24 cm diameter, water depth 45 cm), which was cleaned and filled with fresh water (25±1°C) before each animal tested. The swimming sessions were conducted in a dark room with the water tank being directly surrounded by 3 black walls to avoid distraction, with a lamp illuminating only the water tank from above. On day one of the FST the animals were made to swim for 15 min in order to familiarize them to the test. On day two, the experimental day, the animals were placed in the water-filled cylinder and let swim for 10 min. The animals were dried with a towel and placed underneath an infrared lamp in a cage with towel bedding for approx. 10 min. The FST was recorded with a video camera that was set in horizontal level with the cylinder. The scoring of the animals was conducted by a researcher blind to the treatment. The 10 min swim session was divided into 5 s intervals and the predominant behavior for each interval scored with respect to immobility, swimming or climbing. The behaviour was defined as immobility as in the original Porsolt test, which is that the animal showed no additional activity than that necessary to keep its head above the water.

## Probe preparation

For total RNA extraction, the brain tissue samples were removed from stabilization reagent and homogenized with a stainless steel bead (5mm, Qiagen, Hilden, Germany) in 1ml lysis reagent each using a bead mill (Tissue Lyser, Qiagen). Disruption was followed by RNeasy Lipid Tissue Mini Kit (Qiagen) using an automated sample preparation robot (QIAcube, Qiagen) according to manufacturer´s instructions. Quality and concentration of RNA were assessed using a NanoDrop (Thermal Scientific, Odessa, TX, USA). For quantitative RT PCR 1 µg of total RNA per sample was reverse-transcribed into cDNA in a 20 µl reaction mixture using the QuantiTect Reverse Transcription Kit (Qiagen) following the manufacturer´s instructions.

## Quantitative real-time RT PCR

Real-time RT PCR was conducted on an ABI 7900HT thermocycler (Applied Biosystems) using 500 ng cDNA in a 100 µl reaction mixture using fluorescent probe sequences (TaqMan Gene Expression Master Mix, Applied Biosystems) that are activated during hydrolysis. 384-well TaqMan® Custom Array micro fluid cards had been previously configured and ordered at Applied Biosystems. The target genes were HTR-1b (Rn01637747_s1). HTR-2a (Rn00568473_m1), IL-1β (Rn01514151_m1), MMP-9 (Rn01423075_g1), TIMP-1 (Rn01430875_g1), NFKB (Rn01399583_m1), iNOS (Rn00561646_m1), and TNFα (Rn01525859_g1) and of the four endogenous controls (GAPDH: Rn99999916_s1, PPIA: Rn00690933_m1, 18S: Hs99999901_s1, ACTB: Rn00667869_m1) GAPDH and PPIA were selected.

## ELISA

IL-1β and TNFα concentrations in rat serum were measured using commercially available rat IL-1β, respectively rat TNFα, sandwich enzyme-linked

immunosorbent assay (ELISA) kits (R&D Systems Europe, Abingdon, UK). 50 µl serum were added to a 100 µl reaction and optical density measured at 450 nm (with 540nm as a correction wavelength) using a microplate reader (SpectraMax 340PC384 Absorbance Microplate Reader, Molecular Devices, Sunnyvale, CA, USA).

## Statistical Analysis and Graphs

All statistical analysis was carried out using the statistical softwear package SPSS (Version 19; SPSS, Chicago, IL, USA). The effect of LPS administration and swimming on mRNA levels in the different brain regions was assessed applying a two-way analysis of variance (ANOVA) on the $\Delta C_T$ values. The estimated effects of treatment and behaviour testing from the two-way ANOVA were expressed as up folds or down folds. To demonstrate these effects in a graph, the fold changes ($2^{-\Delta\Delta C_T}$) were calculated using the non-swimming, saline treated group as reference. Each spot in the scatterplots represents an individual rat and the horizontal lines represent the group geometrical means. A Levene´s test of Equality of Error Variances was applied in every analysis to check for unequal variances within the sample groups. The effect of LPS treatment on immobility, swimming or climbing during the FST was assessed using an independent samples T-test. Since iNOS expression was too low to reach detection limit in many of the saline-treated samples, a Chi-square analysis was conducted. iNOS mRNA expression above detection limit was compared to mRNA expression below detection limit (equals no expression) in saline-treated animals that were exposed to the FST to their non-swimming, saline-treated controls.

# Results

## Effect of LPS injection and the FST on mRNA levels in the brain

### Cytokine expression after LPS and FST

As expected, injection of LPS i.p. caused a significant up-regulation of both IL-1β (Fig. 1) and TNFα (Fig. 2) mRNA in all investigated brain regions. The stressor swimming, however, had no significant effect apart from a down-regulation of IL-1β in hippocampus and an up-regulation of TNFα in the prefrontal cortex (Fig.1). A Levene´s test of Equality of Error Variances revealed that the error variance of the dCt values for IL-1β was not equal across groups in the prefrontal cortex, suggesting that a more stringent significance level should be set for evaluating the results of the two-way ANOVA. Since the up-regulation in the prefrontal cortex had a very high significance , we decided to keep the value. The same goes for the spread of TNFα mRNA dCt values in amygdala, hippocampus and striatum, where the Levene´s test showed significance levels lower than 0.05. These results are considered to be valid due to their high significances in the two-way ANOVA ($p \leq 0.002$).

### Different effects of LPS treatment and FST exposure on BDNF expression

LPS treatment was followed by down-regulation of brain-derived neurotrophic factor (BDNF) in all brain regions, indicated in amygdala ($p=0.051$) and significant in hippocampus ($p=0.033$) and prefrontal cortex (0.032). Exposure to the FST, however, caused an up-regulation of BDNF mRNA in amygdala ($p=0.002$) and prefrontal cortex ($p=0.006$) (Fig.3).

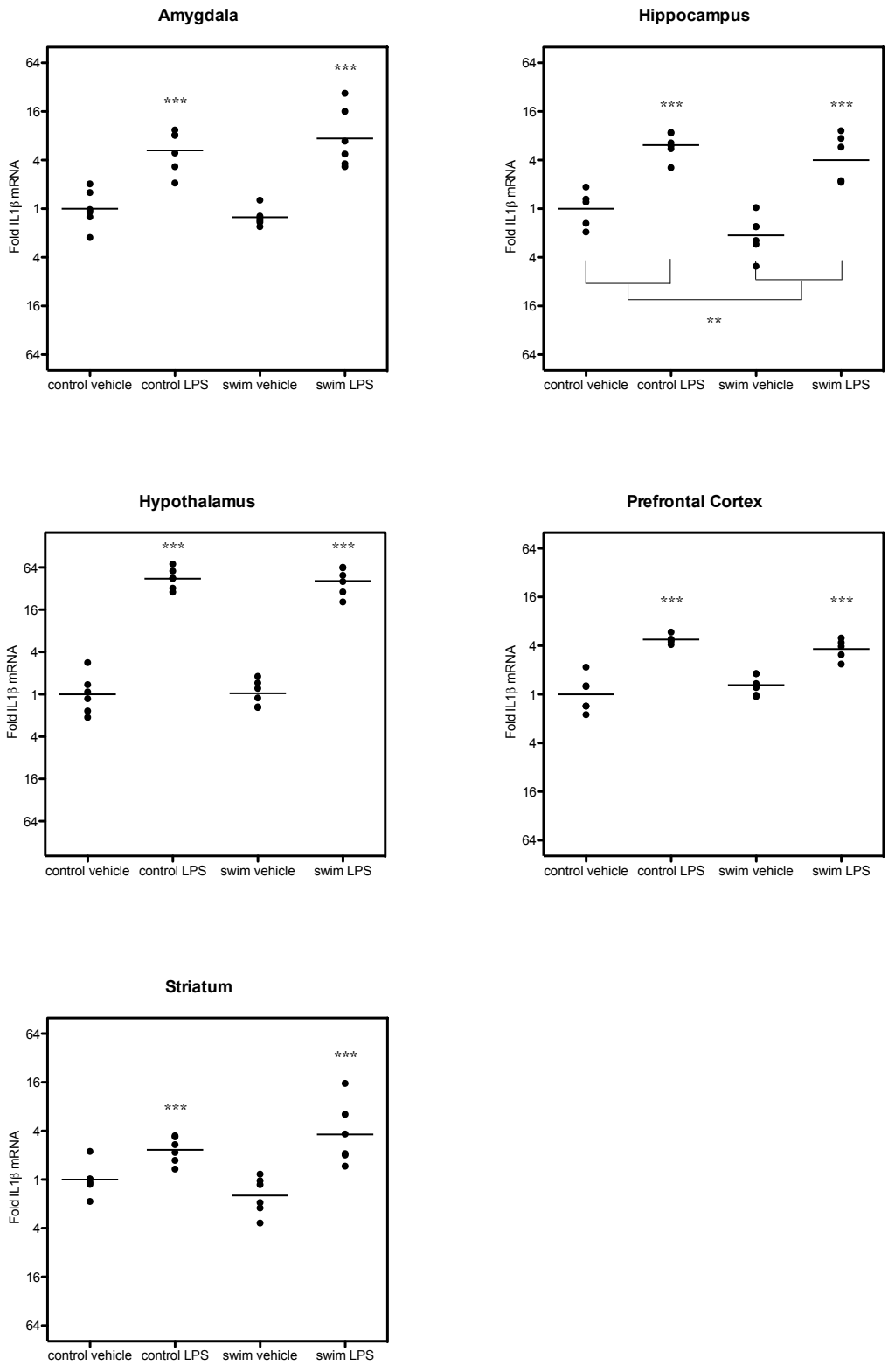


**Figure 1: mRNA fold changes of IL-1β following i.p. LPS injection and forced swim test (FST).** While LPS caused very strong up-regulation of IL-1β mRNA in all examined brain regions (p=0.000), no effect of the FST was seen apart from a down-regulation of IL-1β mRNA in the hippocampus (p=0.018). Control animals didn´t undergo the FST. Statistical significance relative to saline-treated resp. non-swimming equivalents is indicated by an asterisk (*p<0.05, **p<0.01, ***p<0.001; n=5-6 animals per group).

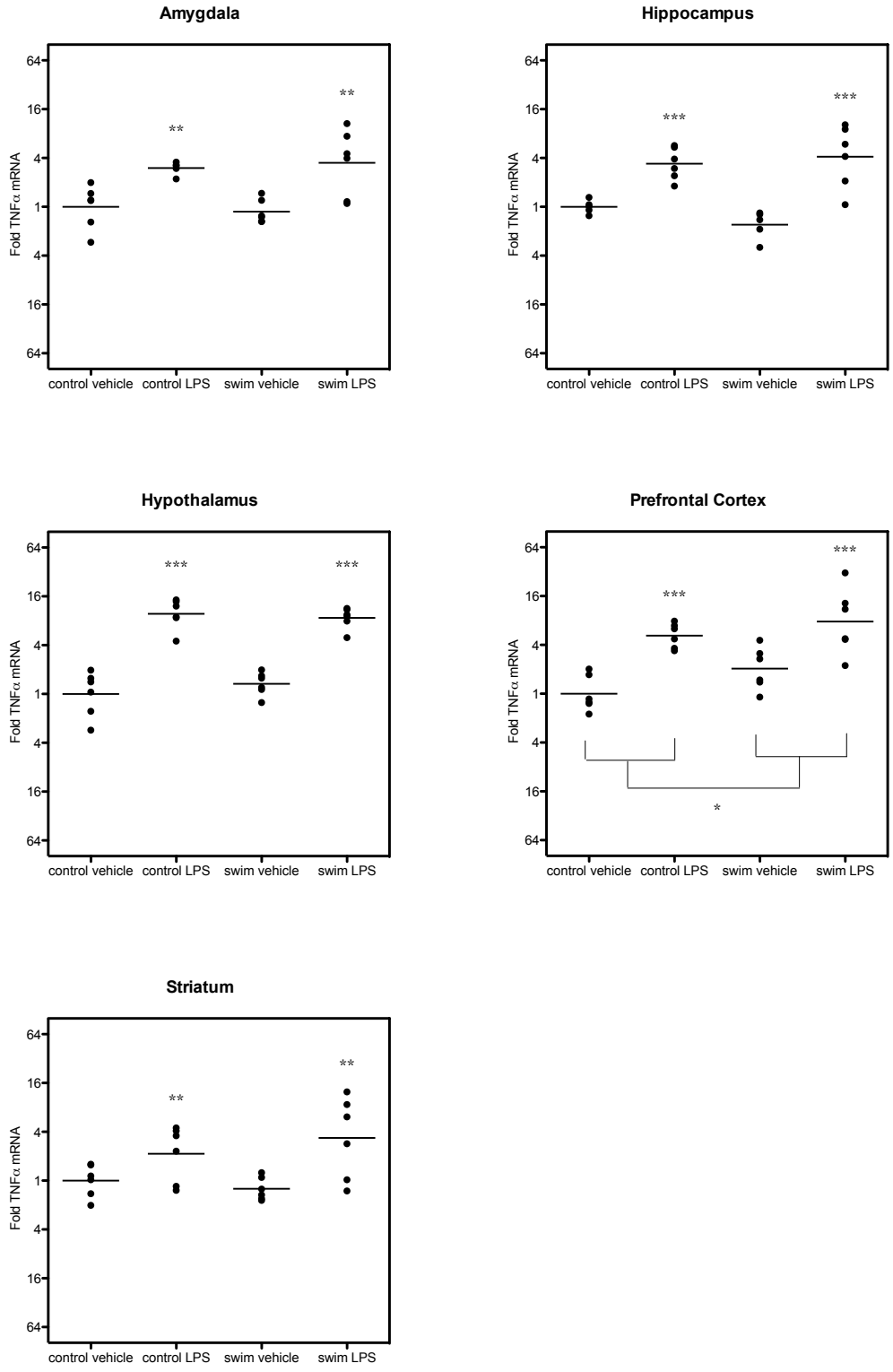


**Figure 2: mRNA fold changes of TNFα following i.p. LPS injection and forced swim test (FST).** Strong up-regulation of TNFα mRNA is seen in all brain regions (p≤0.002) after i.p. LPS but only in the prefrontal cortex of swimming animals when compared to non-swimmers (p=0.044). Control animals didn´t undergo the FST. Statistical significance relative to saline-treated resp. non-swimming equivalents is indicated by an asterisk (*p<0.05, **p<0.01, ***p<0.001; n=5-6 animals per group).

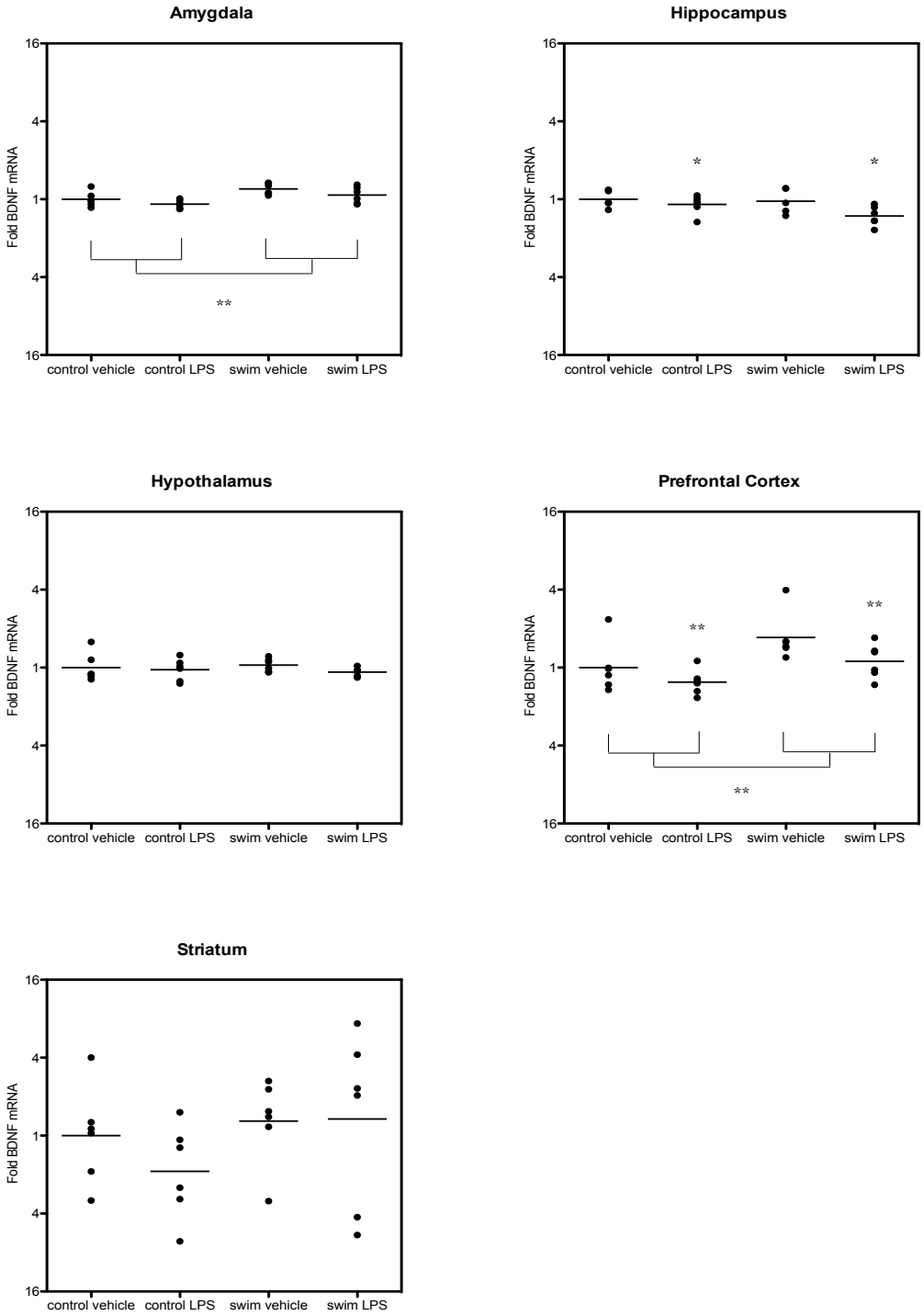


**Figure 3: mRNA fold changes of BDNF following i.p. LPS injection and forced swim test (FST).** LPS administration caused down-regulation of BDNF mRNA expression in nearly all observed brain regions, with significance in hippocampus (p=0.033) and prefrontal cortex (p=0.032) and a strong indication in amygdala (p=0.051). Exposure to the FST caused an up-regulation in amygdala (p=0.002) and prefrontal cortex (p=0.006). Control animals didn´t undergo the FST. Statistical significance relative to saline-treated resp. non-swimming equivalents is indicated by an asterisk (*p<0.05, **p<0.01; n=5-6 animals per group).

**LPS induced up-regulation of iNOS**

A strong up-regulation of iNOS mRNA following LPS treatment was observed (Fig.4). Several samples in the saline-treated groups were below detection limit of the quantitative RT PCR assay, thereby limiting analysis of the effect of the stressor swimming. When comparing detection of iNOS expression to no detection in saline-treated animals, a chi-square analysis showed no significant effect, even though 33.8% of all non-swimming saline-treated animals but 50.8% of saline-treated animals that were exposed to the FST expressed iNOS above detection limit.

**No effect of exposure to the FST on MMP-9 and TIMP-1 expression**

Administration of LPS i.p. caused a significant up-regulation of the matrix-metalloproteinase MMP-9 in the regions hypothalamus ($p=0.001$) and striatum ($p=0.000$) and a less pronounced up-regulation in the prefrontal cortex ($p=0.075$) (Fig. 5). No observable changes in MMP-9 expression was seen between animals that were exposed to the FST and those that weren´t. The endogenous inhibitor of this metalloproteinase, tissue inhibitor of metalloproteinases TIMP-1, was up-regulated in all brain regions after LPS injection ($p=0.000$ in all regions apart from the prefrontal cortex: $p=0.04$) (Fig. 6). TIMP-1 expression was unaffected by exposure to the FST in all compartments.

**No effect of LPS treatment on NFκB levels**

Administration of LPS i.p. caused no changes in NFκB mRNA levels in any of the observed brain regions (Fig.7). A slight up-regulation was seen in the prefrontal cortex when comparing swimming animals to their non-swimming controls ($p=0.007$), although a Levene´s test of Equality of Error Variances revealed that the error variance of the dCt values for NFκB was not equal across groups in the

prefrontal cortex (p=0.037), suggesting that a more stringent significance level should be set for evaluating the results of the two-way ANOVA.

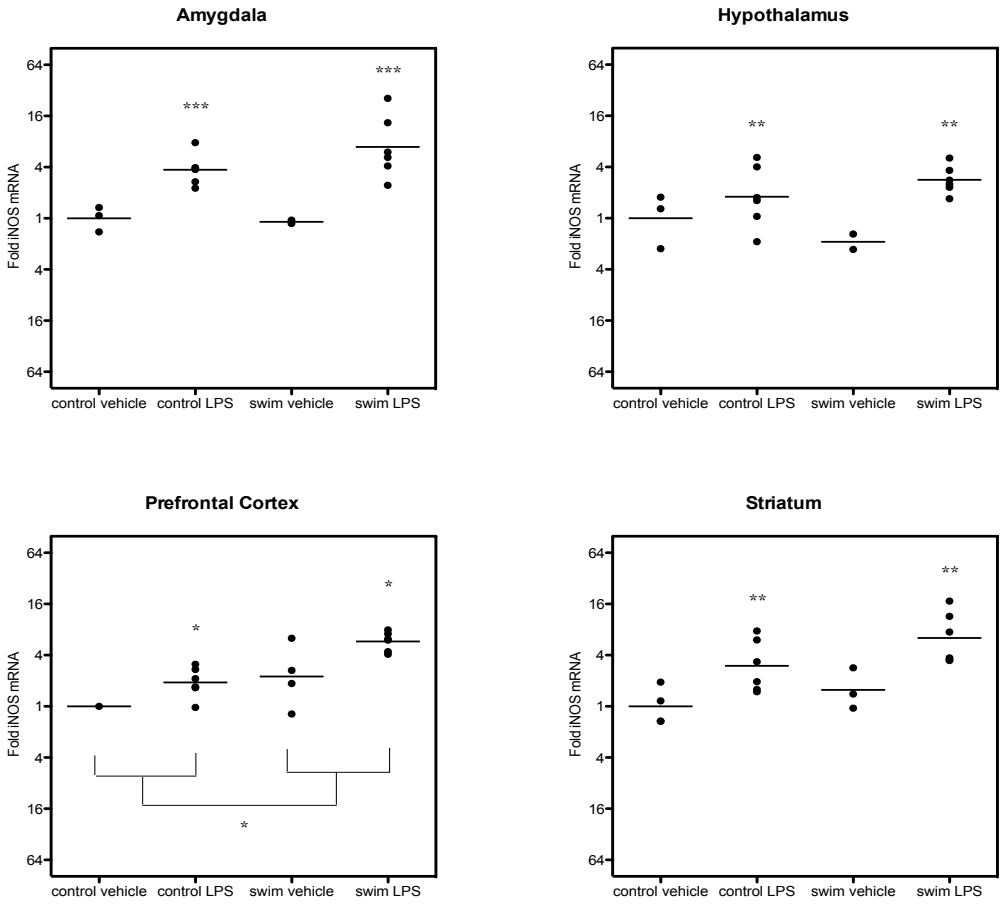


**Figure 4: mRNA fold changes of iNOS following i.p. LPS injection and forced swim test (FST).** LPS administration caused up-regulation of iNOS mRNA in all observed brain regions. Up-regulation after LPS treatment is significant in amygdala (p=0.000), hypothalamus (p=0.007) prefrontal cortex (p=0.027) and striatum (p=0.001). Exposure to the FST caused an up-regulation only in the prefrontal cortex (p=0.01). Control animals didn´t undergo the FST. Statistical significance relative to saline-treated resp. non-swimming equivalents is indicated by an asterisk (*p<0.05, **p<0.01, ***p<0.001). The low amount of animals in some groups (n=1-6 animals per group) is due to expression below detection limit in many samples. Too many samples in hippocampus were below detection limit to analyze the data.

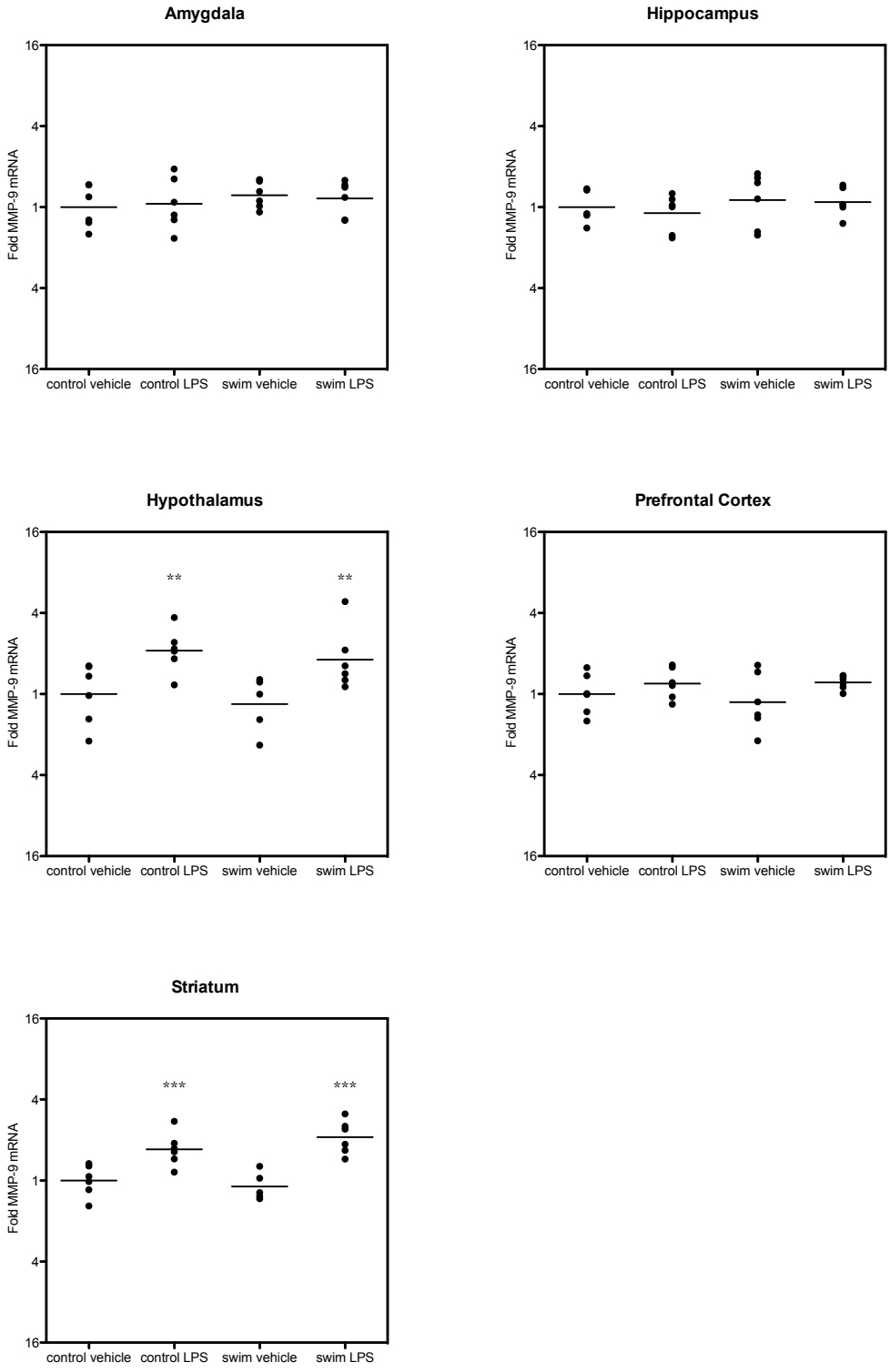


**Figure 5: mRNA fold changes of MMP-9 following i.p. LPS injection and forced swim test (FST).** Up-regulation of mRNA by i.p. LPS is significant in hypothalamus and striatum and a tendency seen in the prefrontal cortex (p=0.075). There was no effect seen when comparing swimming animals to non-swimming controls. Control animals didn´t undergo the FST. Statistical significance relative to saline-treated equivalents is indicated by an asterisk (*p<0.05, **p<0.01, ***p<0.001; n=5-6 animals per group).

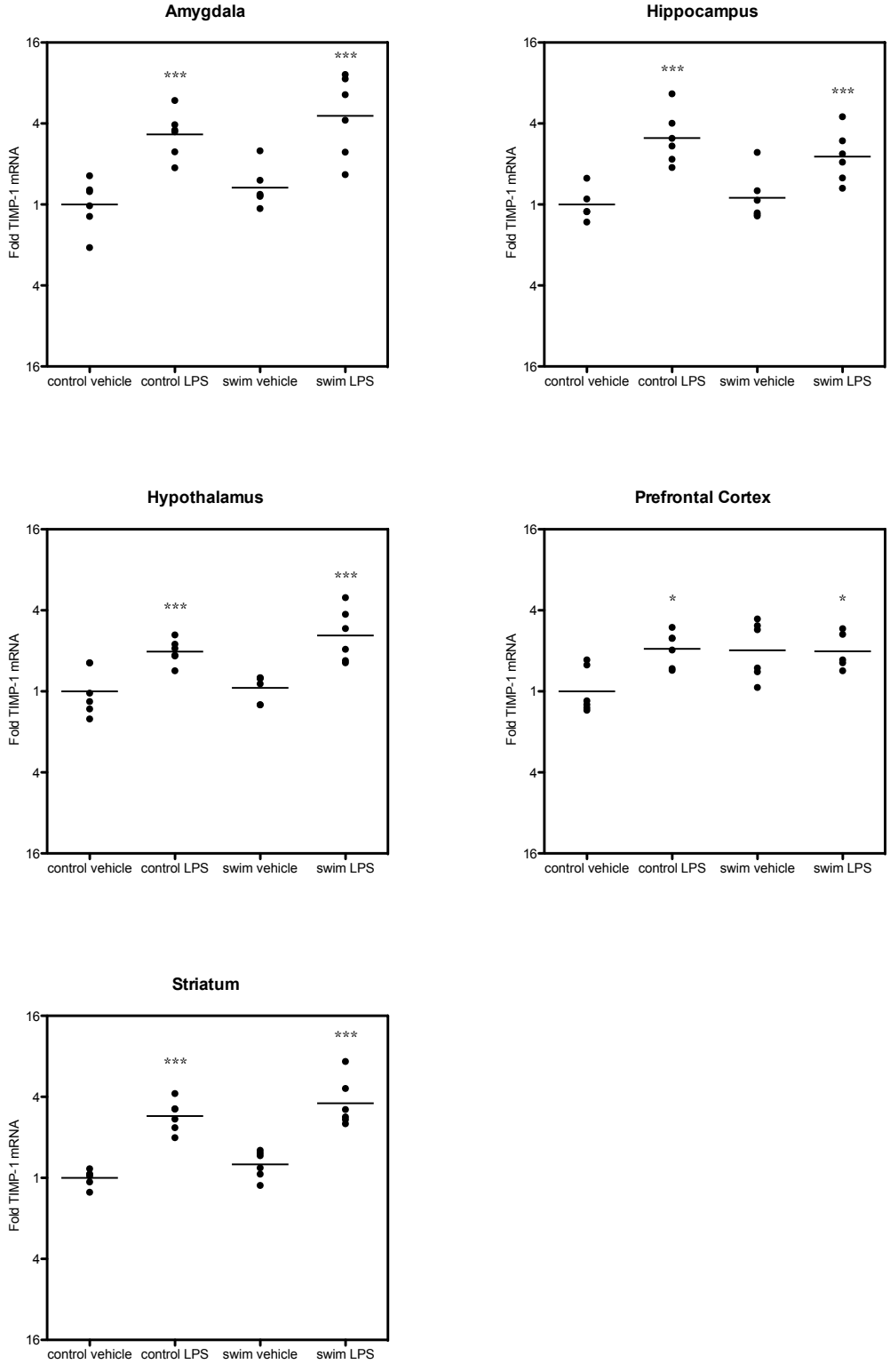


**Figure 6: mRNA fold changes of TIMP-1 following i.p. LPS injection and forced swim test (FST).** Significant up-regulation of TIMP-1 mRNA after i.p. LPS injection in all brain regions. There was no effect seen when comparing swimming animals to non-swimming controls. Control animals didn´t undergo the FST. Statistical significance relative to saline-treated equivalents is indicated by an asterisk (*p<0.05, **p<0.01, ***p<0.001; n=5-6 animals per group).

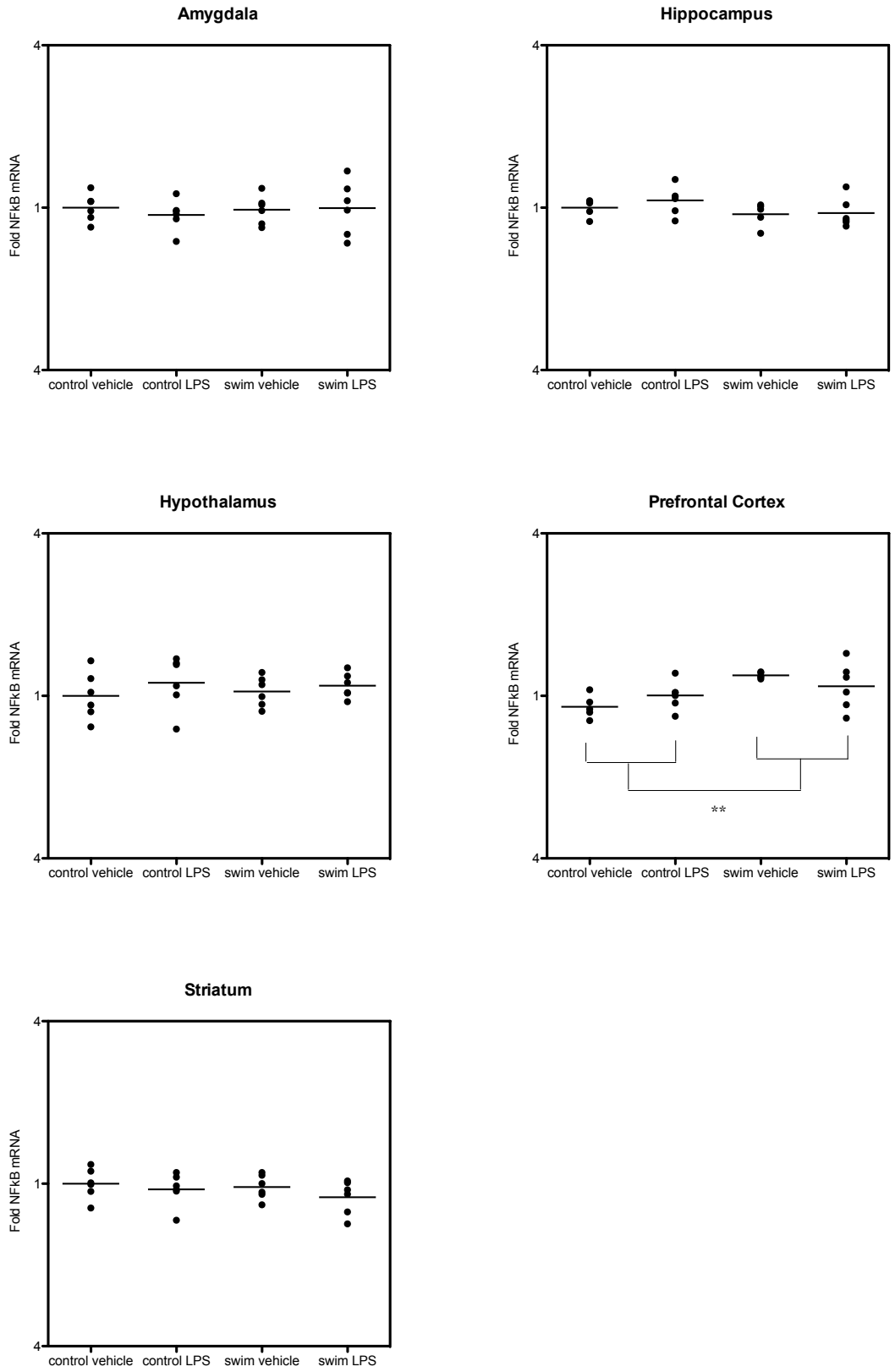


**Figure 7: mRNA fold changes of NFκB following i.p. LPS injection and forced swim test (FST).** LPS treatment had no observable effect on NFκB mRNA levels. Exposure to the FST caused an up-regulation only in the prefrontal cortex (p=0.007). Control animals didn´t undergo the FST. Statistical significance relative to non-swimming equivalents is indicated by an asterisk (**p<0.01; n=5-6 animals per group).

### Effects of LPS on the behaviour in the FST

Administration of LPS i.p. caused a 2.8 fold up-regulation of immobile behaviour and a 1.7 fold down-regulation of swimming behaviour (Fig.8), showing a strong depressive-like effect of LPS on behaviour. No observable effect of LPS treatment was seen on climbing behaviour.

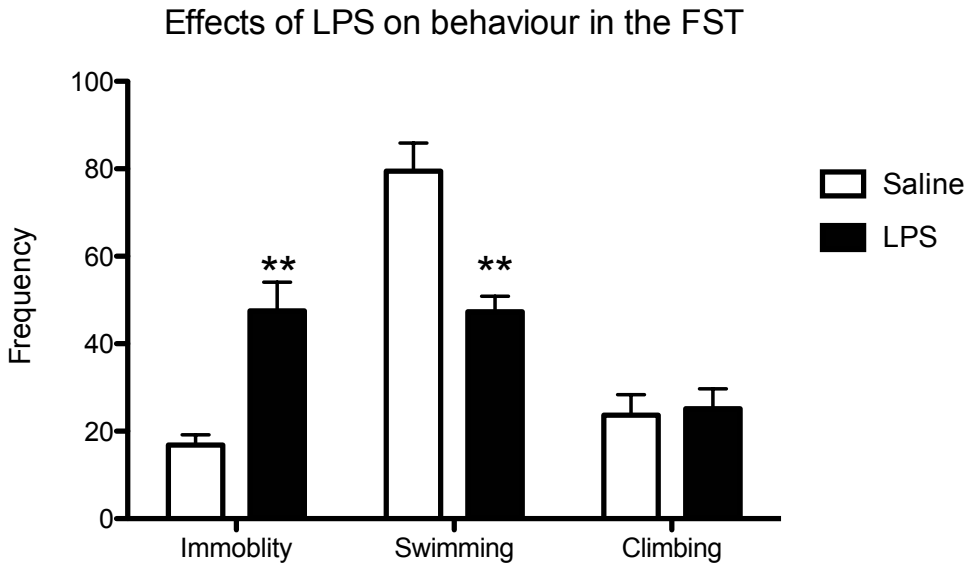


**Figure 8: Effects of LPS on the behaviour during the forced swim test (FST)**: Intraperitoneal injection of LPS (0.5mg/kg) caused a significant up-regulation of immobility resp. down-regulation of swimming during the FST. No effect of LPS on climbing behaviour was observed. Behaviour is measured as number of 5 second intervals of immobility, swimming and climbing during the 10 minute trial. Data is represented as mean ± SEM. Statistical significance relative to saline-injected controls is indicated by an asterisk (**p=0.001), n = 6 animals per group.

### Correlation between immobility in the FST and mRNA levels

A positive correlation between mRNA expression in the different brain regions and immobility scores during the FST was seen for both IL-1β (Fig.9) and TNFα (Fig.10). For IL-1β , correlation was strongest in the amygdala, hippocampus and hypothalamus, but also seen in the prefrontal cortex and striatum. Correlation between TNFα mRNA and immobility was seen as a tendency in amygdala and significant in the hippocampus, hypothalamus, prefrontal cortex and striatum.

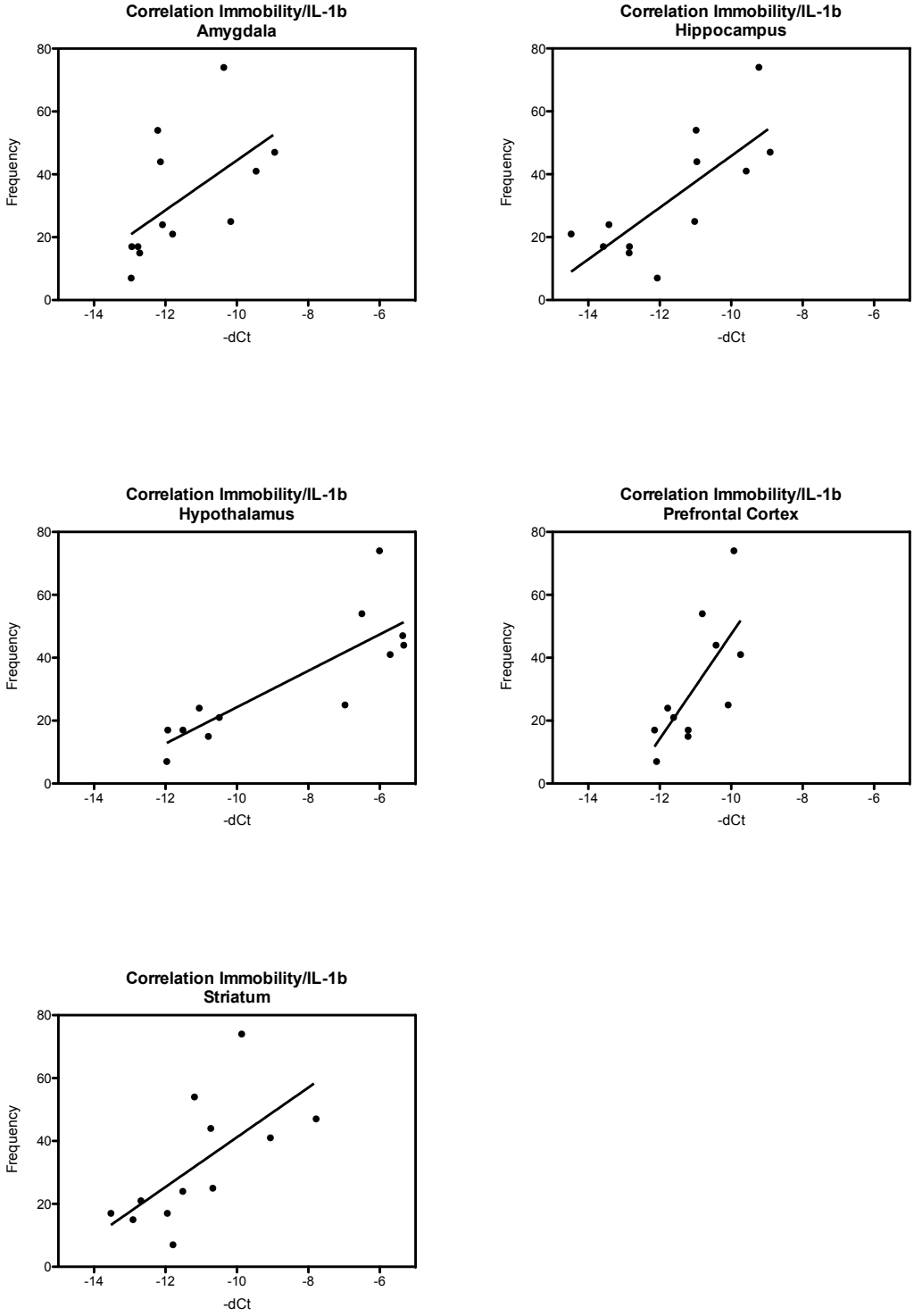


**Figure 9: Correlation between immobility scores and mRNA levels of IL-1β.** LPS induced up-regulation of IL-1β mRNA correlates significantly with increased immobility in all observed brain regions (amygdala: R=0.712, p=0.009; hippocampus: R=0.762, p=0.004; hypothalamus: R=0.832, p=0.001; prefrontal cortex: R=0.655, p=0.021; striatum: R=0.661, p=0.019). Immobility is measured as number of 5 second intervals in the FST. mRNA expression is shown as negative values of mean cycle threshold (dCt).

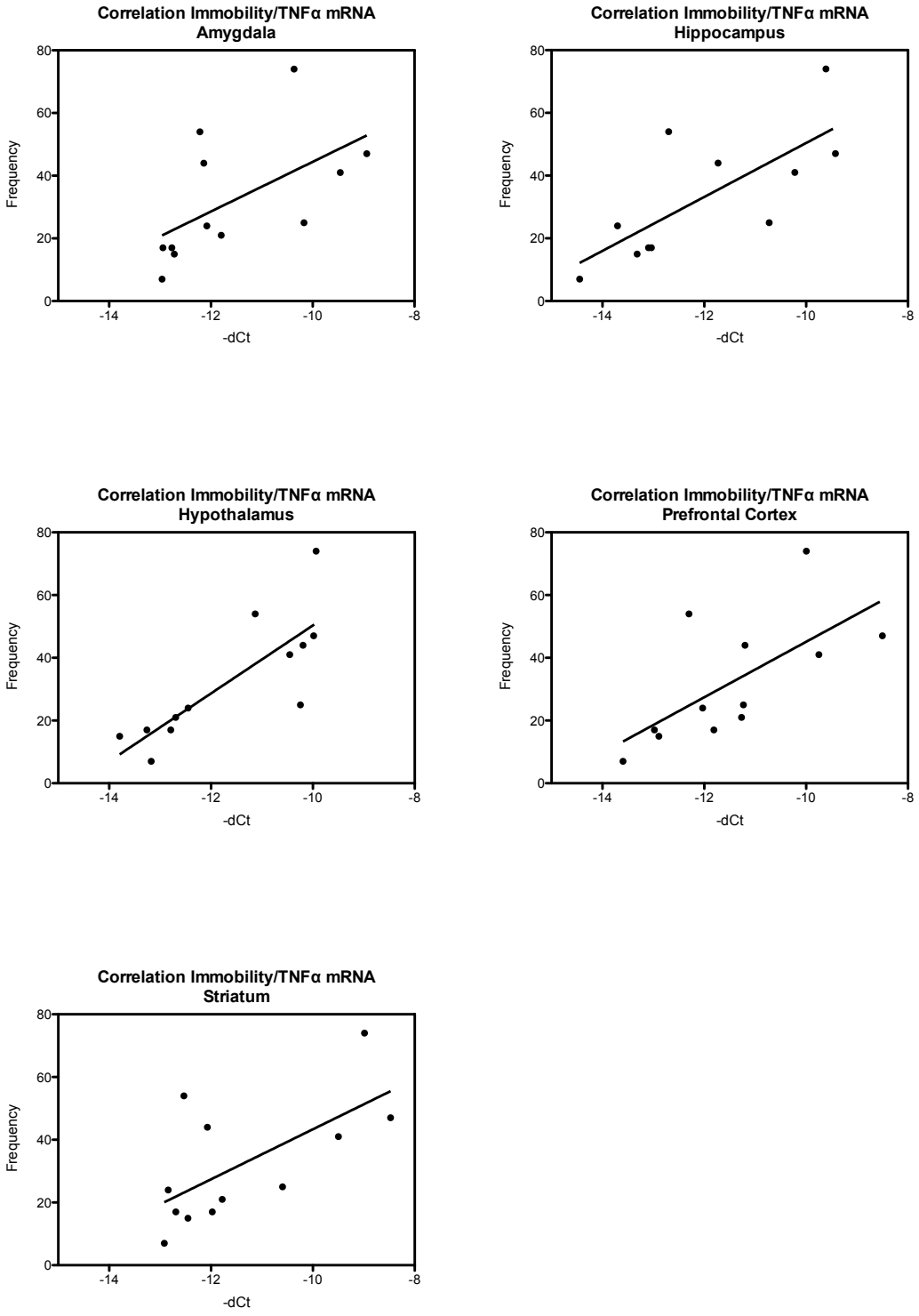


**Figure 10: Correlation between immobility scores and mRNA levels of TNFα.** LPS induced up-regulation of TNFα mRNA correlates significantly with increased immobility in all observed brain regions apart from amygdala (R=0.573, p=0.052; hippocampus: R=0.738, p=0.01; hypothalamus: R=0.811, p=0.001; prefrontal cortex: R=0.611, p=0.019; striatum: R=0.644, p=0.024). Immobility is measured as number of 5 second intervals in the FST. mRNA expression is shown as negative values of mean cycle threshold (dCt).

## Correlation between immobility during the FST and serum levels of IL-1β and TNFα

LPS caused a strong up-regulation of both cytokines in the periphery (p=0.000), but exposure to the FST didn´t have a significant effect on the peripheral levels of these cytokines (Fig.11). A Levene´s test of Equality of Error Variances revealed that the error variance of the IL-1β and TNFα protein levels was not equal across groups (p=0.009 for IL-1β and p= 0.013 for TNFα), suggesting that a more stringent significance level should be set for evaluating the results of the two-way ANOVA. To determine whether there was a direct correlation between cytokine levels in the periphery and the behaviour during the FST, correlation analysis was performed. A positive correlation between peripheral IL-1β protein levels and immobility was observed (Fig. 12A) but none between TNFα serum levels and behaviour scores (Fig.12B).

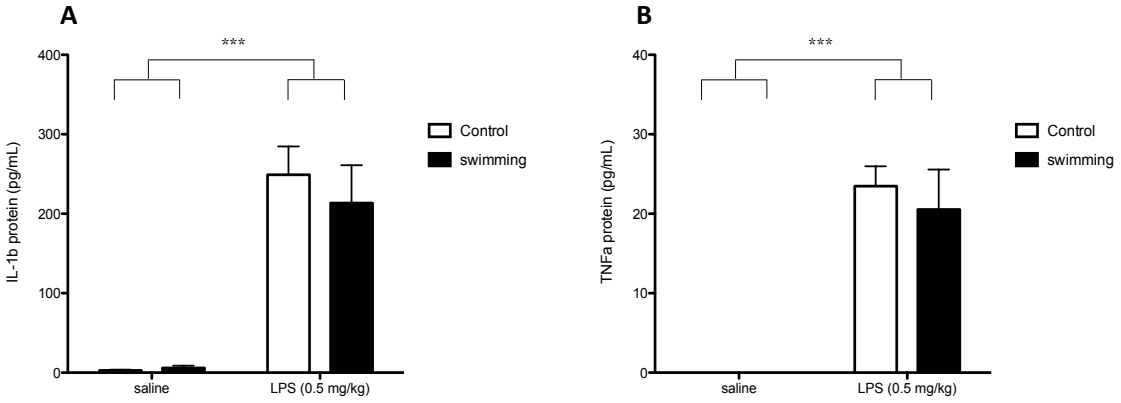


**Figure 11: Serum levels of IL-1β and TNFα.** LPS treatment causes a strong elevation of both IL-1β (A) and TNFα (B) protein levels in serum. No significant effect is seen of exposure to the FST. Control animals didn´t undergo the FST. Data is represented as mean ± SEM. Statistical significance relative to saline-treated equivalents is indicated by an asterisk ***p<0.001; n=5-6 animals per group).

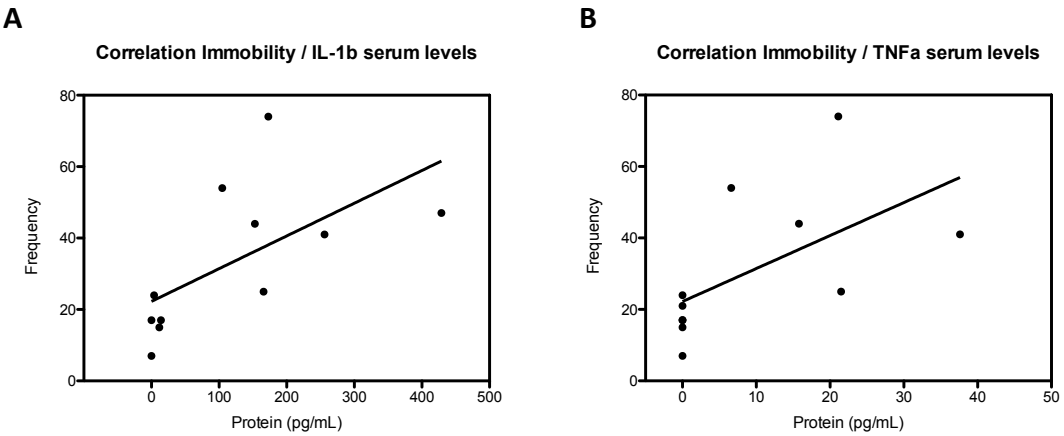


**Figure 12: Correlation between immobility during the FST and serum levels of IL-1β (A) and TNFα (B).** IL-1β serum levels show a positive correlation with immobility (R=0.612, p=0.046) but there was no significant correlation between TNFα protein levels in serum and immobility scores. Immobility is measured as number of 5 second intervals in the FST.

# Discussion

## Effects of LPS treatment on mRNA levels in the brain

In line with previous results, our experiments have shown a clear response of mRNA expression of immune related substances to the peripheral immune stimulation. Both IL-1β and TNFα mRNA levels were significantly elevated in all observed brain regions post i.p. LPS injection. Up-regulation of these cytokines after peripheral LPS is well-documented (van Dam, Brouns, Louisse, & Berkenbosch, 1992; Terrence Deak et al., 2003; M K Hansen et al., 2000; K.T. Nguyen et al., 1998; Pugh et al., 1999). This supports the concept that these cytokines are mediators in the illness-induction pathway, and shows that endotoxin administration does not only cause a systemic inflammation, but also an inflammation in the CNS, which is mediated by activated microglia that lead to chronically elevated levels of pro-inflammatory cytokines (Michael Maes et al., 2009).

We observed an up-regulation of both the matrix metalloproteinase MMP-9 and its tissue inhibitor TIMP-1 in hypothalamus and striatum after LPS. MMP-9 plays an important role in the remodeling of the extracellular matrix and facilitates the migration of immune cells such as neutrophils through the CNS, thus alleviating the induction of an immune response. The up-regulation of its inducible inhibitor, TIMP-1, is probably induced by elevated levels of IL-1β and might reflect an automatic feedback initiated by the increase in MMP-9 expression, in order to protect the tissue from damage.

Investigation of BDNF mRNA levels showed a down-regulation after LPS treatment. This effect has been proposed to play a role in the reduction of neurogenesis caused by inflammatory reactions, which in turn may contribute to depression (Michael Maes et al., 2009). Since sickness behaviour was observed only a few hours after peripheral immune stimulation, however, it is unlikely that these behavioral changes were caused by a decrease in neurogenesis but

could point to another possible mechanism of importance in depressive-like behavior involving BDNF. BDNF has been repeatedly reported to play a role in depression: lowered expression of BDNF was shown in a post-mortem study of depressed patients (Karege, Vaudan, Schwald, Perroud, & La Harpe, 2005) and direct antidepressant effects on the behavior of experimental animals were seen after injection of BDNF into the hippocampus (Shirayama, A. C.-H. Chen, Nakagawa, Russell, & Ronald S Duman, 2002). Antidepressant treatment has been shown to induce elevated levels of BDNF in hippocampus (Nibuya, Morinobu, & R S Duman, 1995). Furthermore, it was shown that chronic electroconvulsive seizures (ECS), an animal model of the antidepressant treatment electroconvulsive therapy (ECT), cause changes in the epigenetic regulation of gene expression of BDNF. Chronic ECS lead to alterations in histone modifications within the promoters for the BDNF gene, which in turn lead to an increased expression of BDNF in rat hippocampi (Tsankova et al., 2006; Tsankova, A. Kumar, & Nestler, 2004). However, no elevation of hippocampal BDNF levels but antidepressant effect in female rats has also been shown, challenging the role of BDNF in depression (Hansson, Rimondini, Heilig, Mathé, & Sommer, 2011). This points to a perhaps diverse role of BDNF in acute and chronic inflammation-mediated depression. Our data suggests that a reduction in BDNF transcription may not only underlie the detrimental effect of immune activation on memory (Yirmiya & Goshen, 2010) but also on mood.

LPS treatment was followed by significantly elevated levels of iNOS mRNA in all observed brain regions. All LPS treated animals showed iNOS expression above detection limit whereas the saline-treated controls often displayed expression below, leading to the assumption that LPS caused a strong up-regulation of iNOS expression in the brain. This leads to elevated levels of nitric oxide (NO), which is toxic for e.g. astrocytes and other cells. A role of NO in the regulation of various behavioural, cognitive, and emotional processes has been suggested, and furthermore elevated levels of NO as a major contributor to structural and functional changes in the brain, which might contribute to the pathophysiology of depression (Michael Maes et al., 2009; Wegener et al., 2010).

NFκB mRNA levels were not affected by LPS treatment at all. These observations go in line with previous findings in our group (P. Suchankova, data not shown) where no effect of LPS treatment was seen on NFκB mRNA expression in the brains of Flinders Sensitive Line (FSL) rats, an animal model of depression (Overstreet, Friedman, Mathé, & Yadid, 2005) or their controls, Flinders resistant line (FRL) rats. Since activation of NFκB on a post-transcriptional level is known to follow stimulation of TLR-mediated pathways (Schmitz, Mattioli, Buss, & Michael Kracht, 2004), our findings suggest that the significant increase in NFκB activity is mainly mediated by degradation of its inhibitor and not by up-regulation of its transcription.

### Central mRNA levels after exposure to the FST

IL-1β and TNFα mRNA in the CNS were not overall affected by exposure to the FST, suggesting that the FST as a behavioral test itself does not alter central expression of these cytokines. A down-regulation of IL-1β mRNA was seen in the hippocampus and TNFα mRNA levels were up-regulated in the prefrontal cortex. Exclusion of the FST from the class of stressors that increase central IL-1β production, and that behavioral consequences of FST exposure are not mediated by IL-1β has previously been suggested, since no observable effect of the forced swim on central or peripheral IL-1β protein levels was seen (Terrence Deak et al., 2003).

It should be mentioned, however, that exposure to the FST might have induced an up-regulation of IL-1β, but that it was inhibited by glucocorticoids. Elevated plasma cortisol levels immediately after exposure to the FST have been observed (Terrence Deak et al., 2003), and adrenal corticosterones have been reported to be modulators of stress-induced cytokine production and to suppress IL-1β production both in the brain and in the periphery. However, this was shown in adrenalectomized rats after exposure to the stressor inescapable tail shock (K.T. Nguyen et al., 1998) and the effect of glucocorticoids during the FST needs to be investigated. Furthermore, similar finding were shown in adrenal intact animals

after foot shock (K T Nguyen et al., 2000), showing that even though IL-1β up-regulation seems to be much stronger if not affected by cortisol, central IL-1β responses to stress have been seen in animals with normally functioning glucocorticoid responses to stress. However, cortisol levels following the FST and its effects on cytokine expression should be investigated in future experiments. Stress-induced up-regulation of TNFα but not IL-1β in the rat prefrontal cortex was reported after exposure to chronic variant stress (de Pablos et al., 2006). The prefrontal cortex, as well as hippocampus and amygdala, have been referred to as being especially vulnerable to the effects of stress (Andrade & Rao, 2010), which agrees with our finding that most of the effects of the FST on mRNA levels were seen in the prefrontal cortex.

No effects whatsoever of the FST on the mRNA levels of MMP-9 or TIMP-1 were seen in any of the observed brain regions, suggesting that the expression of the matrix-metalloproteinase and its endogenous inhibitor is not affected by the procedure.

In contrary to the LPS induced down-regulation of BDNF in amygdala, hippocampus and the prefrontal cortex, exposure to the FST caused an up-regulation of the neurotrophin in amygdala and the prefrontal cortex. A strong up-regulation of BDNF after LPS treatment in combination with stress in the prefrontal cortex of rats has been reported previously (de Pablos et al., 2006), and an up-regulation of BDNF in hippocampus and the prefrontal cortex has been correlated with a decrease in depressive-like behaviour during the FST (Huang et al., 2011).

NFκB expression seems to be unaffected both by LPS treatment and by exposure to the FST, apart from a slight up-regulation of NFκB mRNA levels in the prefrontal cortex. This contradicts the possible explanation that the stressor forced swim induces NFκB expression and could thereby overshadow LPS induced NFκB up-regulation. However, as mentioned above, NFκB protein is usually present in cells in an inactive state and requires proteasome-dependent

degradation of inhibitory proteins for its activation, thus, is mainly regulated after transcription and translation. (Schmitz et al., 2004)

### Changes in behaviour during the FST after LPS treatment

Peripheral immune stimulation by LPS significantly changed the behaviour during the FST, causing depressive-like behaviour expressed as an increase in immobility and a decrease in swimming. The duration of climbing behaviour was not affected by LPS treatment. This well documented effect (A. J. Dunn, Swiergiel, & de Beaurepaire, 2005; A. Dunn & Schwiergel, 2005; Fu et al., 2010; Zhu et al., 2010) was expected, even though some sources report no effect of LPS administration on immobility duration during the FST (Terrence Deak et al., 2005).

### Correlation between behaviour and cytokine expression

Immobility during the FST and cytokine mRNA levels in all investigated brain regions correlated strongly, suggesting that the levels of IL-1β and TNFα in the central nervous system may play a role in depressive-like behaviour.

Although LPS strongly up-regulated both IL-1β and TNFα protein levels in serum, no significant correlation was seen between behaviour during the FST and serum levels of TNFα. IL-1β serum levels showed positive correlations with immobility scores and negative correlations with swimming scores of the FST, but the significance level of these correlations were way below the correlations of mRNA levels in the CNS and behaviour during the forced swim, leading to the assumption that the central rather than the peripheral up-regulation of these cytokines may be related to the behavioral changes.

An up-regulation of mRNA levels of both the inhibitory serotonin receptor HTR-1b and the excitatory HTR-2a were seen in the prefrontal cortex and, only for HTR-2a, in striatum, which showed significant correlations with immobility

scores during the FST (App.1, 2). Since these receptors affect the intracellular expression of the secondary messengers cyclic adenosine monophosphate (cAMP), inositol triphosphate (IP3) and diacylglycerol (DAG) (Pytliak et al., 2011), downstream mechanisms changing the gene expression pattern of the affected cells can be expected.

## Limitations, conclusion and future aspects

Due to the short time interval between LPS injection and the FST it is possible that the increase in immobility and reduction in swimming behaviour during the FST can be classified as sickness-behaviour rather than depressive-like behaviour. In order to distinguish better between these, we could have assessed depressive-like behaviour 24h after LPS administration, when sickness-behaviour was reported to have abolished (Bay-Richter, Janelidze, Hallberg, & Brundin, 2011). Since we wanted to measure expression of mRNA in the CNS not later than 7 hours after LPS administration, this was impossible. An alternative could be to assess locomotion behaviour of the animals before exposing them to the forced swim test (Bay-Richter et al., 2011). This could be done in a locomotion box, which is considered as a relatively harmless procedure, not causing more stress to the animal. Another improvement to our study would be the testing of locomotion behaviour of each animal and subdivision into two equal groups before the beginning of the experiment, to reduce the variety in basal locomotor activity between the groups.

Another future aspect to consider might be the possible influence of clock genes on inflammatory mediators and their role in inflammation-related depression. A possible connection between the circadian clock and depression has long been postulated (Boivin, 2000). Not only links between circadian rhythm and monoamine systems, but also between clock genes and cytokine expression has been reported (Hampp et al., 2008; Imbesi et al., 2009) and IL-1β levels have been shown to follow a circadian rhythm (Terrence Deak et al., 2003; K.T. Nguyen et al., 1998).

Microglia cells are activated to produce an increased amount of pro-inflammatory mediators. Structural changes that lead to neurodegeneration are long term effects of the inflammation that is mediated by these cytokines, such as IL-1 and TNFα. Additionally, a down-regulation of neurotrophic factors such as BDNF is seen, which leads to impaired neuronal repair (B. E. Leonard & Ayemu Myint, 2009). It seems that pro-inflammatory mediators can cause depression via several pathways, all of which might be valuable therapeutic targets in anti-inflammatory-based approaches to treat patients with depressive disorders (Loftis, Huckans, & Morasco, 2010). Although inflammation as a therapeutic target for the treatment of MDD has been questioned due to the complexity of their interrelationship (Anisman, 2009), too much data points to a central role of inflammatory mediators in the development and endurance of this disease to ignore its potential for improved treatment. Therefore, the mechanisms underlying this relation have to be investigated all the more in order to elucidate the pathophysiology of depression. To do this, inducers of the cytokine cascades and possible new targets for the treatment of mood disorders should be identified.

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# References


Andrade, C., & Rao, N. S. K. (2010). How antidepressant drugs act: A primer on neuroplasticity as the eventual mediator of antidepressant efficacy. *Indian journal of psychiatry*, *52*(4), 378-386. doi:10.4103/0019-5545.74318

Anisman, H. (2009). Cascading effects of stressors and inflammatory immune system activation : implications for major depressive disorder. *Journal of Psychiatry & Neuroscience (JPN)*, *34*(1), 4-20.

Bay-Richter, C., Janelidze, S., Hallberg, L., & Brundin, L. (2011). Changes in behaviour and cytokine expression upon a peripheral immune challenge. *Behavioural brain research*, *222*(1), 193-9. Elsevier B.V. doi:10.1016/j.bbr.2011.03.060

Boivin, D. B. (2000). Influence of sleep-wake and circadian rhythm disturbances in psychiatric disorders. *Journal of psychiatry & neuroscience : JPN*, *25*(5), 446-58. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1408010&tool=pmcentrez&rendertype=abstract

Bonefeld, B. E., Elfving, B., & Wegener, G. (2008). Reference Genes for Normalization : A Study of Rat Brain Tissue. *Control* (Vol. 309, pp. 302-309). doi:10.1002/syn

Capuron, L, Gumnick, J., Musselman, D., Lawson, D., Reemsnyder, A., Nemeroff, C., & Miller, A. (2002). Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. *Neuropsychopharmacology*, *26*(5), 643-52.

Capuron, Lucile, Hauser, P., Hinze-Selch, D., Miller, A. H., & Neveu, P. J. (2002). Treatment of cytokine-induced depression. *Brain, behavior, and immunity*, *16*(5), 575-80. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2995293&tool=pmcentrez&rendertype=abstract

Chen, E., Miller, G. E., Kobor, M. S., & Cole, S. W. (2011). Maternal warmth buffers the effects of low early-life socioeconomic status on pro-inflammatory signaling in adulthood. *Molecular psychiatry*, *16*(7), 729-37. Nature Publishing Group. doi:10.1038/mp.2010.53

Chen, F., Madsen, T. M., Wegener, G., & Nyengaard, J. R. (2009). Repeated electroconvulsive seizures increase the total number of synapses in adult male rat hippocampus. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, *19*(5), 329-38. Elsevier B.V. and ECNP. doi:10.1016/j.euroneuro.2008.12.007

Chen, F., Madsen, T. M., Wegener, G., & Nyengaard, J. R. (2010). Imipramine treatment increases the number of hippocampal synapses and neurons in a genetic animal model of depression. *Hippocampus*, *20*(12), 1376-84. doi:10.1002/hipo.20718

Cryan, J F, & Mombereau, C. (2004). In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Molecular psychiatry*, *9*(4), 326-57. doi:10.1038/sj.mp.4001457

Cryan, John F, & Slattery, D. a. (2007). Animal models of mood disorders: Recent developments. *Current opinion in psychiatry*, *20*(1), 1-7. doi:10.1097/YCO.0b013e3280117733

Cryan, John F, Markou, A., & Lucki, I. (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in pharmacological sciences*, *23*(5), 238-45. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12008002

van Dam, a M., Brouns, M., Louisse, S., & Berkenbosch, F. (1992). Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? *Brain research*, *588*(2), 291-6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1393581

Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain, behavior, and immunity*, *21*(2), 153-60. doi:10.1016/j.bbi.2006.09.006

Dantzer, R., Connor, J. C. O., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression : when the immune system subjugates the brain. *Neuroscience*, *9*(January). doi:10.1038/nrn2297

Deak, Terrence, Bellamy, C., & D'Agostino, L. G. (2003). Exposure to forced swim stress does not alter central production of IL-1. *Brain research*, *972*(1-2), 53-63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12711078

Deak, Terrence, Bellamy, C., D'Agostino, L. G., Rosanoff, M., McElderry, N. K., & Bordner, K. a. (2005). Behavioral responses during the forced swim test are not affected by anti-inflammatory agents or acute illness induced by lipopolysaccharide. *Behavioural brain research*, *160*(1), 125-34. doi:10.1016/j.bbr.2004.11.024

DellaGioia, N., & Hannestad, J. (2010). A critical review of human endotoxin administration as an experimental paradigm of depression. *Neuroscience and biobehavioral reviews*, *34*(1), 130-43. doi:10.1016/j.neubiorev.2009.07.014

Dheda, K., Huggett, J., Chang, J., Kim, L., Bustin, S., Johnson, M., Rook, G., et al. (2005). The implications of using an inappropriate reference gene for real-time reverse transcription PCR data normalization. *Anal Biochem*, *344*(1), 141-3.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctôt, K. L. (2010). A meta-analysis of cytokines in major depression. *Biological psychiatry*, *67*(5), 446-57. Elsevier Inc. doi:10.1016/j.biopsych.2009.09.033

Dunn, A. J., Swiergiel, A. H., & de Beaurepaire, R. (2005). Cytokines as mediators of depression: what can we learn from animal studies? *Neuroscience and biobehavioral reviews*, *29*(4-5), 891-909. doi:10.1016/j.neubiorev.2005.03.023

Dunn, A., & Schwiergel, A. H. (2005). Effects of interleukin-1 and endotoxin in the forced swim and tail suspension tests in mice. *Pharmacology Biochemistry and Behavior*, *81*(3), 688-693. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/s0091-3057(05)00177-2

Frenois, F., Moreau, M., Connor, J. O., Lawson, Marc, Micon, C., Lestage, J., Kelley, K. W., et al. (2007). Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology*, *32*(5), 516-531.

Fu, X., Zunich, S. M., O'Connor, J. C., Kavelaars, A., Dantzer, R., & Kelley, K. W. (2010). Central administration of lipopolysaccharide induces depressive-like behavior in vivo and activates brain indoleamine 2,3 dioxygenase in murine organotypic hippocampal slice cultures. *Journal of neuroinflammation*, *7*, 43. doi:10.1186/1742-2094-7-43

Gaestel, M., Kotlyarov, A., & Kracht, M. (2009). Targeting innate immunity protein kinase signalling in inflammation. *Nat Rev Drug Discov.*, *8*(6), 480-99.

Gardner, J., & Ghorpade, A. (2003). Tissue inhibitor of metalloproteinase (TIMP)-1: the TIMPed balance of matrix metalloproteinases in the central nervous system. *Journal of neuroscience research*, *74*(6), 801-6. doi:10.1002/jnr.10835

Gomez, D., Alonso, D., Yoshiji, H., & Thorgeirsson, U. (1997). Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol*., *74*(2), 111-22.

Haider, L., Fischer, M. T., Frischer, J. M., Bauer, J., Höftberger, R., Botond, G., Esterbauer, H., et al. (2011). Oxidative damage in multiple sclerosis lesions. *Brain : a journal of neurology*, *134*(Pt 7), 1914-24. doi:10.1093/brain/awr128

Hampp, G., Ripperger, J. a, Houben, T., Schmutz, I., Blex, C., Perreau-Lenz, S., Brunk, I., et al. (2008). Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. *Current biology : CB*, *18*(9), 678-83. doi:10.1016/j.cub.2008.04.012

Hansen, M K, Nguyen, K T, Goehler, L. E., Gaykema, R. P., Fleshner, M, Maier, S F, & Watkins, L R. (2000). Effects of vagotomy on lipopolysaccharide-induced brain interleukin-1beta protein in rats. *Autonomic neuroscience : basic & clinical*, *85*(1-3), 119-26. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11189018

Hansson, A. C., Rimondini, R., Heilig, M., Mathé, A. a, & Sommer, W. H. (2011). Dissociation of antidepressant-like activity of escitalopram and nortriptyline on behaviour and hippocampal BDNF expression in female rats. *Journal of psychopharmacology (Oxford, England)*. doi:10.1177/0269881110393049

Huang, Z., Zhong, X.-M., Li, Z.-Y., Feng, C.-R., Pan, A.-J., & Mao, Q.-Q. (2011). Curcumin reverses corticosterone-induced depressive-like behavior and decrease in brain BDNF levels in rats. *Neuroscience letters*, *493*(3), 145-8. Elsevier Ireland Ltd. doi:10.1016/j.neulet.2011.02.030

Imbesi, M., Yildiz, S., Dirim Arslan, a, Sharma, R., Manev, H., & Uz, T. (2009). Dopamine receptor-mediated regulation of neuronal "clock" gene expression. *Neuroscience*, *158*(2), 537-44. IBRO. doi:10.1016/j.neuroscience.2008.10.044

Irwin, M. R. (2008). Human psychoneuroimmunology: 20 years of discovery. *Brain, behavior, and immunity*, *22*(2), 129-39. doi:10.1016/j.bbi.2007.07.013

Irwin, M. R., & Miller, A. H. (2007). Depressive disorders and immunity: 20 years of progress and discovery. *Brain, behavior, and immunity*, *21*(4), 374-83. doi:10.1016/j.bbi.2007.01.010

Jansson, L., Wennström, M., Johanson, A., & Tingström, A. (2009). Glial cell activation in response to electroconvulsive seizures. *Progress in neuro-psychopharmacology & biological psychiatry*, *33*(7), 1119-28. Elsevier Inc. doi:10.1016/j.pnpbp.2009.06.007

Jayatissa, M. N., Bisgaard, C., Tingström, A., Papp, M., & Wiborg, O. (2006). Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *31*(11), 2395-404. doi:10.1038/sj.npp.1301041

Kapás, L., Hansen, Michael K, Chang, H.-yoon, Krueger, J. M., Imeri, L., Bianchi, S., Opp, M. R., et al. (2011). Vagotomy attenuates but does not prevent the somnogenic and febrile effects of lipopolysaccharide in rats Vagotomy attenuates but does not prevent the somnogenic and febrile effects of lipopolysaccharide in rats.

Karege, F., Vaudan, G., Schwald, M., Perroud, N., & La Harpe, R. (2005). Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain research. Molecular brain research*, *136*(1-2), 29-37. doi:10.1016/j.molbrainres.2004.12.020

Kessler, R. C., Angermeyer, M., Anthony, J. C., DE Graaf, R., Demyttenaere, K., Gasquet, I., DE Girolamo, G., et al. (2007). Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. *World*

*psychiatry : official journal of the World Psychiatric Association (WPA)*, *6*(3), 168-76. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2174588&tool=pmcentrez&rendertype=abstract

Konsman, J. P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behaviour: mechanisms and implications. *Trends in neurosciences*, *25*(3), 154-9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11852148

Kumar, R. K., & Wales, S. (2010). Inflammation : Chronic. *Life Sciences*, (September). doi:10.1002/9780470015902.a0000944.pub3

Larson, S., & Dunn, A. (2001). Behavioral effects of cytokines. *Brain Behav Immun*, *15*(4), 371-87.

Leonard, B. E., & Myint, Ayemu. (2009). The psychoneuroimmunology of depression. *Pharmacology*, (January), 165-175. doi:10.1002/hup

Licinio, J., & Wong, M.-L. (2011). Advances in depression research: 2011. *Molecular psychiatry*, *16*(7), 686-7. Nature Publishing Group. doi:10.1038/mp.2011.74

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, *25*(4), 402-8. doi:10.1006/meth.2001.1262

Loftis, J. M., Huckans, M., & Morasco, B. J. (2010). Neuroimmune mechanisms of cytokine-induced depression: current theories and novel treatment strategies. *Neurobiology of disease*, *37*(3), 519-33. Elsevier B.V. doi:10.1016/j.nbd.2009.11.015

Maes, M, Bosmans, E., De Jongh, R., Kenis, G., Vandoolaeghe, E., & Neels, H. (1997). Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine*, *9*(11), 853-8. doi:10.1006/cyto.1997.0238

Maes, Michael. (2008). The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. *Neuro endocrinology letters*, *29*(3), 287-91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18580840

Maes, Michael, Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., Kubera, M., et al. (2009). The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metabolic brain disease*, *24*(1), 27-53. doi:10.1007/s11011-008-9118-1

Miura, H., Ozaki, N., Sawada, M., Isobe, K., Ohta, T., & Nagatsu, T. (2008). A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. *Stress (Amsterdam, Netherlands)*, *11*(3), 198-209. doi:10.1080/10253890701754068

Myint, AM, Schwarz, M., Steinbusch, H., & Leonard, B. (2009). Neuropsychiatric disorders related to interferon and interleukins treatment. *Metab Brain Dis*, *24*(1), 55-68.

Müller, N., & Ackenheil, M. (1998). Psychoneuroimmunology and the cytokine action in the CNS: implications for psychiatric disorders. *Progress in neuro-psychopharmacology & biological psychiatry*, *22*(1), 1-33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18280022

Nelissen, K., Smeets, K., Mulder, M., Hendriks, J. J. a, & Ameloot, M. (2010). Selection of reference genes for gene expression studies in rat oligodendrocytes using quantitative real time PCR.

*Journal of neuroscience methods*, *187*(1), 78-83. Elsevier B.V. doi:10.1016/j.jneumeth.2009.12.018

Nestler, E. J., Barrot, M., Dileone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of Depression. *Neuron*, *34*, 13-25.

Nguyen, K T, Deak, T, Will, M. J., Hansen, M K, Hunsaker, B. N., Fleshner, M, Watkins, L R, et al. (2000). Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress. *Brain research*, *859*(2), 193-201. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10719064

Nguyen, K.T., Deak, Terrence, Owens, S. M., Kohno, T., Fleshner, Monika, Watkins, L.R., & Maier, S.F. (1998). Exposure to Acute Stress Induces Brain Interleukin-1 Protein in the Rat. *The Journal of neuroscience*, *18*(6), 2239. Soc Neuroscience. Retrieved from http://www.jneurosci.org/content/18/6/2239.short

Nibuya, M., Morinobu, S., & Duman, R S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *15*(11), 7539-47. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7472505

Ongür, D., Drevets, W. C., & Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(22), 13290-5. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=23786&tool=pmcentrez&rendertype=abstract

Opp, M., & Toth, L. (1998). Somnogenic and pyrogenic effects of interleukin-1beta and lipopolysaccharide in intact and vagotomized rats. *Life Sciences*, *62*(10), 923-36.

Overstreet, D. H., Friedman, E., Mathé, A. a, & Yadid, G. (2005). The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. *Neuroscience and biobehavioral reviews*, *29*(4-5), 739-59. doi:10.1016/j.neubiorev.2005.03.015

O'Brien, S. M., Scott, L. V., & Dinan, T. G. (2004). Cytokines: abnormalities in major depression and implications for pharmacological treatment. *Human psychopharmacology*, *19*(6), 397-403. doi:10.1002/hup.609

O'Connor, J. C., André, C., Wang, Y., Lawson, Marcus a, Szegedi, S. S., Lestage, J., Castanon, N., et al. (2009). Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(13), 4200-9. doi:10.1523/JNEUROSCI.5032-08.2009

de Pablos, R. M., Villarán, R. F., Argüelles, S., Herrera, a J., Venero, J. L., Ayala, a, Cano, J., et al. (2006). Stress increases vulnerability to inflammation in the rat prefrontal cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *26*(21), 5709-19. doi:10.1523/JNEUROSCI.0802-06.2006

Parnet, P., Kelley, K. W., Bluthé, R. M., & Dantzer, R. (2002). Expression and regulation of interleukin-1 receptors in the brain. Role in cytokines-induced sickness behavior. *Journal of neuroimmunology*, *125*(1-2), 5-14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11960635

Pavlov, V., & Tracey, K. (2005). The cholinergic anti-inflammatory pathway. *BRAIN BEHAV IMMUN.*, *19*(6), 493-9.

Pecchi, E., Dallaporta, M., Jean, A., Thirion, S., & Troadec, J. (2009). Prostaglandins and sickness behavior: old story, new insights. *Physiol Behav*, *97*(3-4), 279-92.

Pollak, Y., & Yirmiya, R. (2002). Cytokine-induced changes in mood and behaviour: implications for "depression due to a general medical condition", immunotherapy and antidepressive treatment. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *5*(4), 389-99. doi:10.1017/S1461145702003152

Porsolt, R., Le Pichon, M., & Jalfre, M. (1977). *Depression: a new animal model sensitive to antidepressant treatments*. *Nature* (Vol. 266, pp. 730-732).

Pugh, C. R., Nguyen, K T, Gonyea, J. L., Fleshner, M, Wakins, L. R., Maier, S F, & Rudy, J. W. (1999). Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. *Behavioural brain research*, *106*(1-2), 109-18. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10595426

Pytliak, M., Vargová, V., Mechírová, V., & Felšöci, M. (2011). Serotonin Receptors – From Molecular Biology to Clinical Applications. *Neuropharmacology*, *1*(Peroutka 1984), 15-25.

Raison, C. L., Capuron, Lucile, & Miller, A. H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in immunology*, *27*(1), 24-31. doi:10.1016/j.it.2005.11.006

Rajkowska, G., Miguel-Hidalgo, J. J., Wei, J., Dilley, G., Pittman, S. D., Meltzer, H. Y., Overholser, J. C., et al. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biological psychiatry*, *45*(9), 1085-98. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10331101

Ressler, K., & Nemeroff, C. (2000). Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depress Anxiety*, *12*(Suppl 1), 2-19.

Schmitz, M. L., Mattioli, I., Buss, H., & Kracht, Michael. (2004). NF-kappaB: a multifaceted transcription factor regulated at several levels. *Chembiochem : a European journal of chemical biology*, *5*(10), 1348-58. doi:10.1002/cbic.200400144

Shirayama, Y., Chen, A. C.-H., Nakagawa, S., Russell, D. S., & Duman, Ronald S. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *22*(8), 3251-61. doi:20026292

Simmons, D. a, & Broderick, P. a. (2005). Cytokines, stressors, and clinical depression: augmented adaptation responses underlie depression pathogenesis. *Progress in neuro-psychopharmacology & biological psychiatry*, *29*(5), 793-807. doi:10.1016/j.pnpbp.2005.03.009

Suchankova, P, Henningsson, S., Baghaei, F, Rosmond, R, Holm, G, & Ekman, a. (2009). Genetic variability within the innate immune system influences personality traits in women. *Genes, brain, and behavior*, *8*(2), 212-7. doi:10.1111/j.1601-183X.2008.00461.x

Suchankova, Petra, Baghaei, Fariba, Rosmond, Roland, Holm, Göran, Anckarsäter, H., & Ekman, A. (2011). Genetic variability within the S100B gene influences the personality trait self-directedness. *Psychoneuroendocrinology*, *36*(6), 919-23. doi:10.1016/j.psyneuen.2010.10.017

Tsankova, N. M., Berton, O., Renthal, W., Kumar, A., Neve, R. L., & Nestler, E. J. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nature neuroscience*, *9*(4), 519-25. doi:10.1038/nn1659

Tsankova, N. M., Kumar, A., & Nestler, E. J. (2004). Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *24*(24), 5603-10. doi:10.1523/JNEUROSCI.0589-04.2004

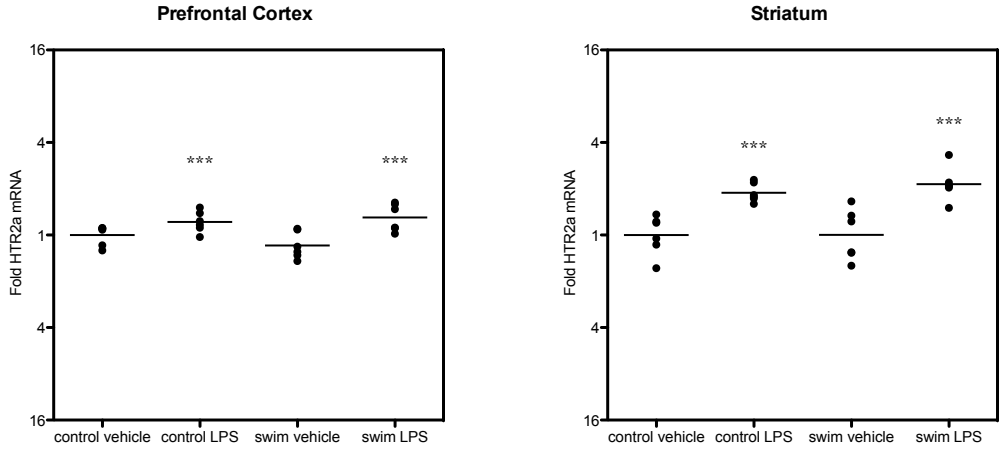
Wegener, G., Harvey, B. H., Bonefeld, B., Müller, H. K., Volke, V., Overstreet, D. H., & Elfving, B. (2010). Increased stress-evoked nitric oxide signalling in the Flinders sensitive line (FSL) rat: a genetic animal model of depression. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *13*(4), 461-73. doi:10.1017/S1461145709990241

Yirmiya, R., & Goshen, I. (2010). Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain, behavior, and immunity*, *25*(2), 181-213. Elsevier Inc. doi:10.1016/j.bbi.2010.10.015

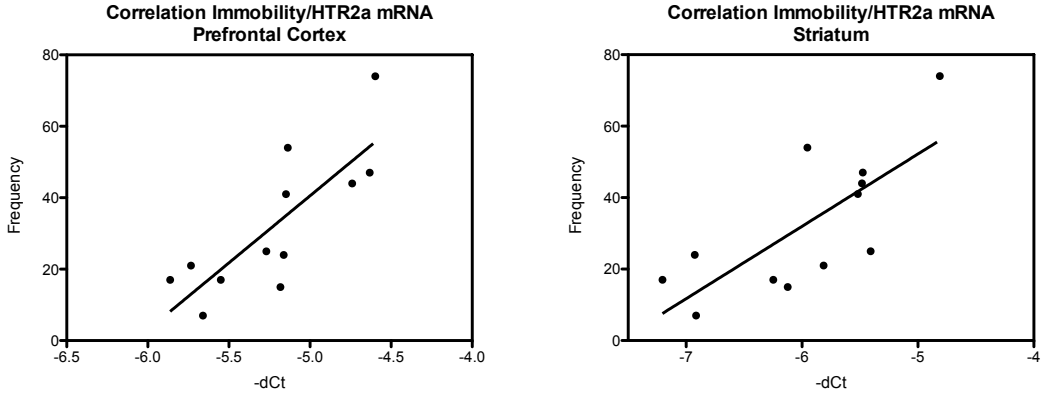
Zhu, C.-B., Lindler, K. M., Owens, A. W., Daws, L. C., Blakely, R. D., & Hewlett, W. a. (2010). Interleukin-1 Receptor Activation by Systemic Lipopolysaccharide Induces Behavioral Despair Linked to MAPK Regulation of CNS Serotonin Transporters. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*(13), 2510-2520. Nature Publishing Group. doi:10.1038/npp.2010.116

# Appendix

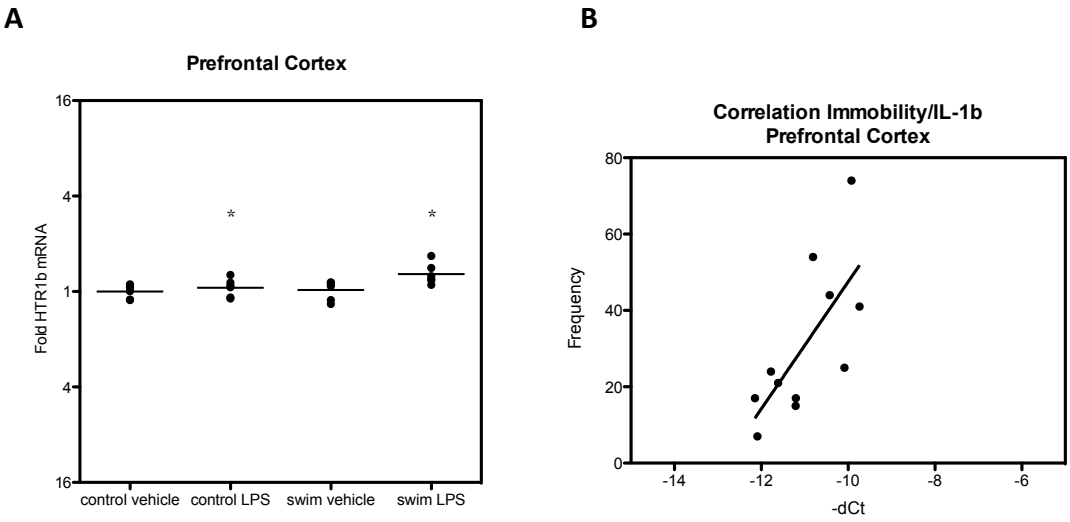
**A**




**B**




**Appendix 1:** (A) mRNA fold changes of serotonin receptor 5-HT R2A following i.p. LPS injection and forced swim test (FST). Injection of LPS i.p. caused an up-regulation of 5-HT R2A mRNA in the prefrontal cortex (p=0.000) and striatum (p=0.000). No effect of the FST was seen in any of the observed brain regions. Control animals didn´t undergo the FST. Statistical significance relative to saline-treated equivalents is indicated by an asterisk (***p < 0.001; n=6 animals per group). (B) Correlation between immobility scores measured as number of 5 second intervals in the FST and mRNA levels of 5-HT R2A. The up-regulation of 5-HT R2A mRNA following LPS treatment correlates significantly in the prefrontal cortex (R=0.803, p=0.002) and striatum (R=0.743, p=0.006). mRNA expression is shown as negative values of mean cycle threshold (dCt).




**Appendix 2:** (A) mRNA fold changes of 5-HT R1B following i.p. LPS injection and forced swim test (FST). Up-regulation of 5-HT R1B mRNA was only seen in the prefrontal cortex ($p=0.015$). No effect of the FST was seen in any of the observed brain regions. Control animals didn´t undergo the FST. Statistical significance relative to saline-treated equivalents is indicated by an asterisk (*$p < 0.05$; $n=6$ animals per group). (B) Correlation between immobility scores measured as number of 5 second intervals in the FST and mRNA levels of 5-HT R1B. LPS induced up-regulation of 5-HT R1B mRNA in the prefrontal cortex correlates significantly with increased immobility ($R=0.686$, $p=0.014$). mRNA expression is shown as negative values of mean cycle threshold (dCt).

## Abstract

Accumulating evidence suggests a role of inflammation in the pathophysiology of depression. However, despite the extensive research in this field, little is known about the underlying mechanisms. Animal experiments prove to be of great help, showing that peripheral inflammation induces central up-regulation of pro-inflammatory mediators and, at the same time, depressive-like behaviour. In this study, the effect of peripheral administration of the exotoxin lipopolysaccharide (LPS) on gene expression in the brain areas amygdala, hippocampus, hypothalamus, prefrontal cortex and striatum, as well as protein levels in the serum of Sprague Dawley rats, was investigated. Furthermore, changes in behaviour were measured in the forced swim test (FST), an animal model to test for depressive-like behaviour. The animals were injected intraperitoneal (i.p.) with LPS and 6 hours later tested in the FST. One hour later, the animals were sacrificed and their brains removed and dissected. To evaluate whether the FST itself changes gene expression in the brain, control animals that were not exposed to the FST were included in the study. mRNA was extracted from the different brain areas and differences in expression levels assessed using quantitative real-time polymerase chain reaction (qRT-PCR). LPS treatment was followed by an up-regulation in gene expression of the cytokines IL-1β and TNFα, and the tissue remodelers MMP-9 and its inhibitor, TIMP-1, which correlated significantly ($p=0.001$ in hypothalamus for both IL-1β and TNFα) with the observed significant increase in depressive-like behaviour. Exposure to the FST itself didn´t seem to have noteworthy influences on gene-expression, making this test a valuable method to investigate inflammation-related players in depression and maybe identify new targets for the treatment of mood disorders.

## Zusammenfassung

Entzündungsreaktionen werden in der Pathophysiologie von Depressionen eine immer größere Rolle zugeschrieben, doch trotz der zunehmenden Forschung in diesem Gebiet sind die zugrunde liegenden Mechanismen noch nicht geklärt. In Tierversuchen wurde gezeigt, dass eine periphere Entzündung nicht nur die zentrale Expression von pro-inflammatorischen Mediatoren erhöht, sondern auch zu Verhalten führt, dass dem depressiver Menschen ähnelt.

In dieser Arbeit wurde der Effekt einer peripheren Injektion des Exotoxins Lipopolysaccharid (LPS), das auf der äußeren Membran gramnegativer Bakterien zu finden ist, auf die Genexpression in den Gehirnarealen Amygdala, Hippocampus, Hypothalamus, Prefrontaler Cortex und Striatum in Ratten untersucht. Außerdem wurden die Proteinlevels gewisser Cytokine im Serum der Ratten gemessen. Verhaltensänderungen nach der LPS Injektion wurde in dem erzwungenen Schwimmtest (engl. “forced swim test”, FST) erfasst. Dieser Test misst depressiv-ähnliches Verhalten (engl. “depressive-like behaviour”) in Tieren.

LPS wurde den Tieren intraperitoneal injiziert und 6 Stunden später wurde ihr Verhalten im FST gemessen. Eine Stunde danach wurden die Ratten getötet und ihre Gehirne seziert. Um festzustellen, ob der FST an sich die Genexpression im Gehirn verändert, wurden Kontrolltiere, die nicht dem Schwimmtest ausgesetzt wurden, in die Studie inkludiert. mRNA wurde aus den verschiedenen Hirnarealen extrahiert und Veränderungen in der Genexpression durch quantitative Echt-Zeit Polymerasenkettenreaktion (qRT PCR) gemessen.

Die periphere Injektion von LPS löste eine erhöhte Genexpression der Cytokine IL-1β und TNFα und der Gewebsprotease MMP-9 und deren Inhibitor TIMP-1 aus. Diese Ergebnisse stehen in signifikanter Korrelation mit dem Anstieg an immobilem Verhalten während des FST ($p=0.001$ im Hypothalamus für IL-1β and TNFα). Es wurde kein nennenswerter Effekt des erzwungenen Schwimmtests auf die zentrale Genexpression festgestellt, was darauf schließen

lässt, dass dieser Test geeignet ist, um die zugrunde liegenden Mechanismen und die entscheidenden Mediatoren im Zusammenspiel von Entzündungsreaktionen und Depressionen zu untersuchen und vielleicht sogar neue therapeutische Ziele in der Behandlung von affektiven Störungen zu identifizieren.

## CURRICULUM VITAE

| | |
|---|---|
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