# The role of exercise in depression: Preclinical studies in two distinct animal models

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## Abstract

Depression is a serious and debilitating psychiatric disease for those affected, and it has a startlingly high lifetime occurrence of approximately 20%. The treatment of depression is complicated by the fact that it is a highly heterogeneous disorder that manifests with symptoms at the psychological, behavioral, and physiological levels. In fact, only about a third of patients respond to conventional antidepressant drug therapy, and novel strategies to treat depression are urgently needed. Physical exercise is a promising non-pharmacological strategy that could serve as a supplementing strategy to current antidepressant treatment. The aim of this study was to evaluate the antidepressant potential of exercise in two distinct rat models of depression, namely a genetic developed depressive rat line, the Flinders Sensitive Line (FSL) and their controls, the Flinders Resistant Line (FRL) rats, and an immunological model in which Sprague Dawley rats were treated chronically (for 8 weeks) with the pro-inflammatory agent lipopolysaccharide (LPS). Rats were either allowed long-term access to a running wheel, or treated with the conventional antidepressant drug imipramine (15 mg/kg/day) or both. Depression- and anxiety-like behaviours were assessed in the forced swim test (FST) and elevated plus maze (EPM), respectively. In addition, several metabolic markers, the mRNA expression of specific proteins suggested to be involved in depression, and the serum concentration of brain-derived neurotrophic factor (BDNF) were measured. The two last-mentioned measurements were only performed in FSL rats. Surprisingly, our main finding from the FSL/FRL model was that the single housing condition, which was required for the accurate measurement of the running activity of each individual rat, changed the characteristic phenotype of these strains in terms of the difference in immobility in the FST, while also influencing certain metabolic markers. We did not find a significant antidepressant-like effect for exercise in FSL rats, although this may be attributed to the low running activity that was observed in these rats. While imipramine alone was also ineffective as an antidepressant in this experiment, the combined exercise-imipramine treatment displayed significant antidepressant-like effects in FSL rats, suggesting that exercise may be a useful adjunct to current antidepressant drug treatment. Our main finding from the LPS-induced model of depression was that, rather than an expected depression-like state, a prolonged sickness behaviour was observed in these rats. LPS increased immobility in the FST, an effect that was reversed by both the exercise and imipramine treatments, and indicates that these treatments may have counteracted the pro-inflammatory action of LPS. Interestingly, the sickness behaviour did not affect the running activity of these rats, which may explain the apparent beneficial effects of exercise in the LPS-induced model versus the FSL/FRL rat model. Overall, these findings suggest that exercise could serve as an alternative therapy on its own, or in combination with current antidepressants in the treatment of depression. However, several confirmatory studies are required in order to support this theory.

## Resume

Depression er en alvorlig og dehabiliterende psykiatrisk lidelse for de involverede, og beklageligvis er livstidsrisikoen op imod de 20 %. Idet depression er en heterogen sygdom, der manifesterer sig på både psykologiske, adfærdsmæssige og fysiologiske niveauer, komplificerer dette behandlingen. Derudover er det ulykkeligvis kun omkring en tredjedel af patienterne der responderer på konventionel medicinsk behandling, hvormed nye behandlingstrategier for behandlingen af depression er ønskværdigt. Motion er en lovende non-farmakologisk strategi, der eventuelt kunne benyttes som et supplement i behandlingen af depression. Formålet med dette studie var at evaluere det antidepressive potentiale af motion i to forskellige rotte-modeller for depression, en genetisk udviklet depressiv rottestamme, Flinder Sensitive Line (FSL) samt deres kontrol, Flinders Resistant Line (FRL) rotter, samt en immunologisk depressionsmodel hvor Sprague-Dawley rotter blev kronisk behandlet med den pro-inflammatoriske agent lipopolysakkaride (LPS) i 8 uger. Behandlingsforløbene bestod enten af fri adgang til et løbehjul eller behandling med et konventionelt antidepressiva, imipramine (15 mg/kg/dag). Depressions- og angst-ligende adfærd blev efterfølgende evalueret i Forced Swim Test og Elevated Plus Maze. Derudover blev der målt på metabolske markører, ekspressionsniveauer af depressions-relaterede proteiner i hjernen og samt niveauet af brain-derived neurotrophic factor (BDNF) i serum. De sidstnævnte parametre blev kun målt hos FSL-rotterne. Overraskende var vores hovedfund i FSL/FRL-modellen, at ved at have rotterne gående enkeltvis i burene, som var krævet for at opnå det nøjagtige aktivitetsniveau i løbehjulene, ændrede rotte-stammernes karakteriske fænotype sig, dvs at forskellen i depressionsadfærd under FST forsvandt mellem kontrolgrupperne. Derudover influerede den individuelle opbevaring af rotterne også metabolske parametrer. Vi observerede ingen antidepressiv effekt af motion hos FSL rotterne, hvilket kunne skyldes det lave aktivitetsniveau i løbehjulene. Behandling med imipramine viste sig også at være ineffektiv i at mindske depressiv-ligende adfærd hos rotterne, hvorimod kombinationen af både motion og imipramine havde en signifikant antidepressiv effekt på FSL rotterne. Dette kunne tyde på, at motion kunne være et nyttigt supplement til den nuværende medicinske behandling af depression. Vores hovedfund fra den LPS-inducerede depressionsmodel var, at modsat en forventet depressiv adfærd, observederede vi i stedet en vedvarende sygdomsadfærd. LPS forøgede immobiliteten i FST, en effekt som kunne reverseret af både behandling med motion og imipramine, hvilket indikerer at disse behandlingsformer kan have modvirket the inflammatoriske respons fra LPS. Et interessant fund var, at sygdomsadfærden ikke påvirkede rotternes aktivitetsniveau i løbehjulene, hvilket kan forklare de gavnlige effekter fra motion i den LPS-inducerede model, modsat FSL/FRL modellen. Alt i alt, indikerer disse resultater, at motion i visse tilfælde kan benyttes som en alternativ behandlingsform eller som supplement i kombination med et tilgængeligt antidepressiva i behandlingen af depression. Dog vil det være nødvendigt med flere bekræftende studier for at støtte denne hypotese.

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# Table of content

# 1 INTRODUCTION

Depression is one of the most prevalent psychiatric disorders, affecting 340 million people worldwide. Moreover, depression is postulated to become the leading cause of disability measured in *Years Lived with Disability* (a time-based measure that sums the years of life lost due to premature mortality and years of life lost due to a disease or health condition) and the second leading contributor to the global burden of disease by the year 2020 (WHO, 2009; Murray and Lopez, 1996). The prevalence of depression in the Danish general population is 3-4% correlating to 100.000-200.000 people (Olsen et al., 2004). More generally speaking, the risk of getting a depression during our lifetime is about 20% (Kessler et al., 1994). From an economic view, expenses for brain diseases constitute 3% of the gross national product in Denmark, and the total expenses for all investigated brain diseases are 37.3 billion DKK (Olesen et al., 2008). Among brain disorders, affective disorders including depression are among the most costly diseases, and anxiety disorders among the most prevalent (Olesen et al., 2008). When taking all these data into consideration, it becomes evident that major depression is a massive burden for the general human population, and therefore that new research on the pathophysiology and treatment of depression is urgently needed.

## 1.1 THE DIAGNOSIS OF MAJOR DEPRESSION

Major depression disorder (MDD) is a heterogeneous disorder that manifests with symptoms at the psychological, behavioural, and physiological level (Rabe-Jablonska and Bienkiewicz, 1994). This makes the diagnosis of depression complex because brain scans or laboratory tests cannot be used. In Denmark the diagnosis is determined by using the World Health Organisation (WHO) criteria, namely *The International Statistical Classification of Diseases and Related Health Problems 10th Revision* (ICD-10) (WHO, 1992). This test consists of an interview in which the patient subjectively describes his/her own feelings, which are then compared to the specific ICD-10 criteria. The ICD-10 specify that at least one core symptom along with accompanying depressive symptoms must be present for at least 2 weeks in order to diagnose a major depressive episode (see Table 1-1). The severity increases with the addition of symptoms and the status of the disorder is then graded as mild, moderate or severe (Goldberg, 2006).

**Table 1-1 The ICD-10 criteria for diagnosing depression**

| A – fundamental criteria | B – core symptoms | C – accompanying symptoms |
|---|---|---|
| Symptoms lasting more than 2 weeks | Depressed Mood<br>Loss of interest and enjoyment<br>Reduced energy and decreased activiy | Reduced self esteem and confidence<br>Ideas of guilt and unworthyness<br>Ideas of self harm and death/suicidal thoughts<br>Reduced concentration<br>Pessimistic thoughts<br>Disturbed sleep<br>Diminished/increased appetite and weight |
| Mild: A + at least 2 of B + at least 2 of C<br>Moderate: A + at least 2 of B and at least 3 of C<br>Severe: A + all 3 of B and at least 4 of C | | |

Despite the high prevalence of depression, the knowledge about the aetiology of this disorder remains limited compared with knowledge of other common chronic and multifactorial conditions, such as type 2 diabetes or heart disease. One explanation is that the brain is markedly more difficult to examine than other organs. In addition, not only one area seems to be involved in the pathogenesis of depression, but several regions in the brain are implicated in mediating the diverse symptoms (Nestler et al., 2002a). This is primarily based on post-mortem as well as imaging studies of depressed individuals where structural brain changes have been found in the extensively interconnected hippocampus, amygdala, striatum and frontal cortex (Manji et al., 2001, Lorenzetti et al., 2009). For example, the prefrontal cortex and hippocampus may mediate the cognitive aspects of depression, such as impairment of memory and feelings of worthlessness, guilt and suicidal thoughts, whereas the amygdala is likely involved in emotional processes such as dysphoric emotions and anxiety (Nestler et al., 2002a). However, the published findings are not consistent and imply that caution should be exercised against a simplistic localization of function in the brains of both normal and depressed humans. Adding to the complexity of the diagnosis of depression is that it most often occurs idiopathically, and it is now generally believed that depression is caused by a complex interaction between genetic and environmental factors, hereby predisposing vulnerable individuals.

## 1.2 THE AETIOLOGY OF DEPRESSION

Depression is a complex disorder with many possible aetiologies. However, several lines of evidence suggest that MDD in most people is caused by interactions between a genetic predisposition and environmental factors. Epidemiologic studies suggest that genetic factors account for approximately 40 % of the risk for developing depression (Sullivan et al., 2000), whereas the environmental factors, such as early life stress, account for the remaining 60 %. This implies that depression is a highly heritable disorder, although no specific “depression genes” have been identified as yet. Furthermore, a strong gender-dependency exists for the risk of developing MDD, with twice as many women as men developing the disorder (Young, 1998).

A schematic method of illustrating the aetiology of depression can be created by illustrating the interaction between a genetic predisposition and environmental risk factors for depression that can create a vulnerable phenotype, with alterations in the central nervous system (see Figure 1-1) (Heim et al., 2008). This phenotype can then be modified and affect the individual in either a positive or negative way. For instance, stressful events and trauma, including grief over deceased loved ones or chronic stress can trigger the onset of depression. Depressive episodes can also be induced by endocrine abnormalities, cancers or side-effects from medical treatment. On the other hand, social support and pharmacological treatment may have a positive outcome, thereby relieving depressive symptoms.

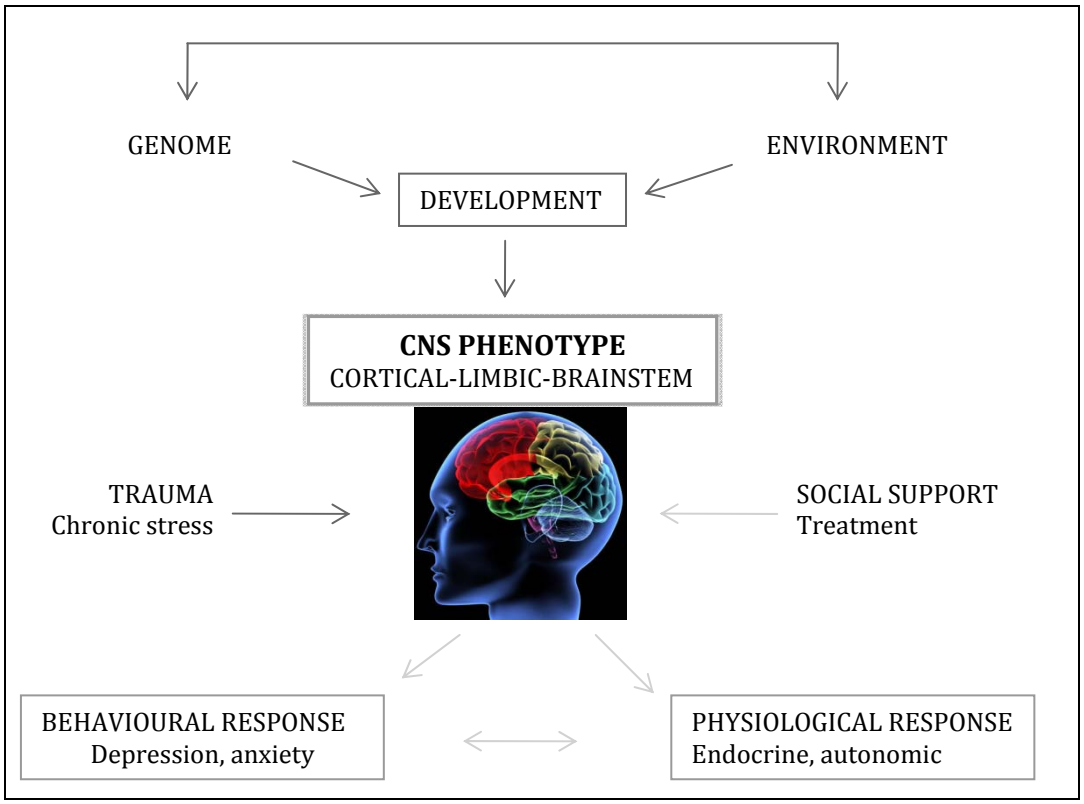


**Figure 1-1: An illustration of a putative model of depression in which a genetic disposition and environmental factors interact in shaping a vulnerable phenotype with changes in cortical-limbic-brainstem circuits.** Stress, trauma or maladaptation in the neuronal circuitries can lead to behavioural and physiological changes. Modified from (Heim et al., 2008)

There exist several neurobiological hypotheses about neuronal circuitries involved in the regulation of mood and the pathophysiology of depression.

## 1.3 THE NEUROBIOLOGY OF DEPRESSION

The pathophysiology of depression is both complex and multi-factorial. Several hypotheses have been suggested during the last few decades, the most popular ones being the monoamine hypothesis, the HPA axis dysregulation hypothesis, the neuroplasticity hypothesis and the cytokine hypothesis (see Table 1-2). However, it is now becoming increasingly evident that none of these theories are fully able to explain the multifaceted clinical picture, but that the different physiological systems interact on several levels to produce a collective neurobiological state that underlies the depressed condition (see Figure 1-2).

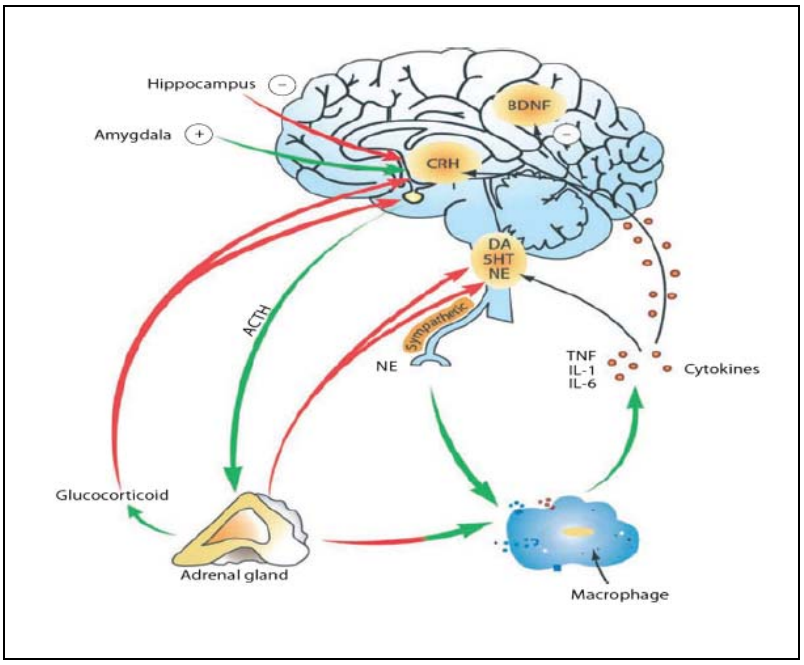


**Figure 1-2**: **The interactions of different physiological systems in producing the neurobiological state of depression.** Several hypotheses exist trying to elucidate some of the mechanisms in the pathophysiology of depression. These include reduced brain monoamine transmission, dysregulation of the HPA-axis, reduced neurotrophic support, increased pro-inflammatory cytokine (Maletic, Robinson et al. 2007)

### 1.3.1 THE MONOAMINE HYPOTHESIS

Although a detailed description of the monoamine hypothesis is beyond the scope of this project, this hypothesis will be briefly discussed here given its historic relevance and its relevance to the tricyclic antidepressant imipramine, which was used in this study. The monoamine hypothesis of depression was the first biological theory of depression, and it was formulated more than 30 years ago. The hypothesis proposed that the main symptoms of depression were due to a functional deficiency of the brain monoaminergic transmitters serotonin (5-HT), noradrenaline (NA) and/or dopamine (DA) (Schildkraut, 1967), and it was based on the early clinical findings that two structurally unrelated compounds (developed for non-psychiatric conditions) showed potent antidepressant effects in humans (Chopra et al., 2011). It was later shown that these drugs enhance central 5-HT and NA transmission. These monoamine systems are widely distributed in the central nervous system and are involved in the regulation of many aspects of behaviour, such as mood, cognition, appetite and anxiety. Despite its age, the monoaminergic hypothesis of depression is still being explored today, with studies focusing on altered synthesis, storage and release of monoamines as well as a disturbed sensitivity of monoamine receptors. In fact, the mechanism of action of all clinically available antidepressant drugs today is still the increase of monoamine neurotransmission in various ways (see Section 1.3.1), with the only substantial improvements of drug design being improved side effect profiles (Nestler et al., 2002b). However, a problematic drawback of this

hypothesis is that it fails to explain an important temporal observation following treatment with monoaminergic antidepressants. Specifically, the changes in synaptic monoamine concentrations occur almost immediately following the administration of these drugs, whereas the therapeutic antidepressant response requires continuous administration for several weeks (Schildkraut, 1967, Baldessarini, 1989). This lead to the suggestion that monoaminergic antidepressants may have secondary downstream effect(s) on, for example neuroplasticity processes, and that this may ultimately underlie the clinical antidepressant response (Nibuya et al., 1996).

### 1.3.2 THE HPA-AXIS HYPOTHESIS

One of the most consistent physiological findings in people suffering from major depression is a dysregulation of the hypothalamic- pituitary-adrenal (HPA) axis (Gibbons and Mc, 1962, Nestler et al., 2002a). This is confirmed by glucocorticoid resistance being one of the most reproducible biological findings and is observed in up to 80% of depressed patients (Pace et al., 2007). The HPA-axis represents the major endocrine stress response system that serves to adapt the organism to changes and responses to threats, thereby maintaining stability and health. Briefly, stressful stimuli activate the hypothalamus to release corticotropin-releasing factor (CRF), which stimulates the secretion and release of adrenocorticotropin (ACTH) from the anterior pituitary gland. Subsequently the hormonal end product of the HPA axis, corticosteroid (cortisol in humans, corticosterone in rodents) is released from the adrenal cortex, and normally to terminate the stress response it exerts a negative feedback effect on itself by acting on the pituitary and hypothalamus. Cortisol acts on many organs including the brain, and exerts its effects via two types of receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) (Nemeroff, 1996).

A dysfunctional HPA axis in depression is believed to be related to a sustained activation of the HPA axis caused by impairment of the negative feedback response, resulting in hypercortisolemia. Furthermore, it has been shown that long-term exposure to excess corticosteroids can lead to hippocampal damage, by triggering apoptotic death in hippocampal neurons and reduce neurogenesis in the hippocampal dentate gyrus (Hoschl and Hajek, 2001, Krishnan and Nestler, 2008). Under normal circumstances the hippocampus has an inhibitory action on glucocorticoid release. However, following hypercortisolemia-induced hippocampal damage, the inhibitory control on the HPA axis is impaired, and creates a vicious cycle that ultimately leads to high circulating glucocorticoid levels and, evidently, hippocampal toxicity and depression.

### 1.3.3 THE NEUROPLASTICITY HYPOTHESIS

Newer theories regarding the pathophysiology of depression and downstream molecular events of antidepressant actions gave rise to an increasing interest in brain neuroplasticity. Neuroplasticity refers to the brains ability to undergo functionally relevant adaptations following external and/or internal stimuli (Fuchs et al., 2004). These dynamic processes depend on the neuronal circuitries, neurons, synapses and neurotrophins to adapt in response to specific stimuli. Neurotrophins are secreted peptides that are essential for the differentiation and survival of neurons. Loss of neurotrophic support or impairment of neurotrophic mechanisms can occur as a result of stress and lead to cell death (Calabrese et

al., 2009). Results from both clinical and preclinical investigations have demonstrated that stress and depression lead to a reduction of the total volume of the hippocampus as well as cell loss in the limbic pathways (Czeh and Lucassen, 2007). Moreover post-mortem studies have revealed reductions in the numbers and/or sizes of glia and neurons in cortical and limbic brain areas (Rajkowska, 2000), which might contribute to this volume loss. Furthermore, it has been reported that stress-induced decreases in neurotrophin expression are normalised following chronic antidepressant treatment (Nibuya et al., 1996). Together, these findings form the basis of the neuroplasticity hypothesis of depression, that states that depression is caused by an impairment of structural plasticity and cellular loss, and that antidepressant medications act by normalising this deficiency (Duman et al., 1999).

One of the most well studied neurotrophins is brain-derived neurotrophic factor (BDNF). BDNF is a crucial mediator of neuroplasticity, as it inhibits cell death cascades and increases cell survival proteins that are responsible for proliferation and maintenance of CNS neurons (Schinder and Poo, 2000). BDNF and its receptor, TrkB, are the most widely and abundantly expressed neurotrophins in the brain (Huang and Reichardt, 2001). By stimulating TrkB, this neuropeptide activates several intracellular cascades, including the Ras/Raf/ERK (extracellular-signal regulated kinase), PI3K and PLCg (phospholipase Cg) pathways (Hashimoto et al., 2004). These distinct pathways diverge by activating the cAMP response element binding protein (CREB). CREB is a transcription factor that regulates the transcription of numerous genes, including many of the neurotrophins (Chopra et al., 2011). Both chronic stress in animals and depression in humans have been associated with reductions in BDNF and CREB, whereas chronic antidepressant treatment has been shown to restore these reductions (Manji et al., 2000).

Other important mediators in neuroplasticity are the growth factors vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1). VEGF was previously mostly known for its role in angiogenesis, but is now becoming an increasingly interesting player in depression (Viikki et al., 2010) and stress-related diseases (Asberg et al., 2009). It has been shown that chronic antidepressant treatment increase VEGF expression (Fournier and Duman, 2011). As is the case for BDNF, studies indicate that antidepressant-induced increase in VEGF expression corresponds to the time needed to achieve therapeutic effectiveness, suggesting a role in the long-term adaptations required for the action of antidepressants (Warner-Schmidt and Duman, 2007). IGF-1 plays an important role in cell growth and development, is up-regulated after antidepressant treatment, and elicits some of the same antidepressant behaviour as BDNF (Miskowiak et al., 2008).

However, not all studies have been able to confirm the neuroplastic changes that have been reported inducible by stress and antidepressants, and this emphasises that the neuroplasticity hypothesis alone cannot completely account for depression and the alleviation thereof. Therefore, the investigation of other theories of depression is warranted.

### 1.3.4 THE IMMUNOLOGICAL HYPOTHESIS

The first suggestions of the involvement of immunological parameters in the neurobiology of depression occurred following clinical observations in patients treated for infectious diseases or cancer with interferon (IFN) and interleukin (IL)-2 (Miller et al., 2009). These patients

frequently displayed nonspecific neuropsychiatric symptoms, some of which represented clinical depression. In further support of this theory, the depressive symptoms induced by INF could be reduced with conventional antidepressant medication (Musselman et al., 2001, Capuron et al., 2002). There is now accumulating evidence that connects increased levels of pro-inflammatory cytokines with depression. Some of the most frequently observed pro-inflammatory cytokines in patients with depression are IL-6 and TNF-α (Dowlati et al., 2010).

Cytokines are multifunctional immune cell secreted proteins, which help orchestrate the complex immune responses. Functionally, cytokines are classified as being either pro-inflammatory or anti-inflammatory depending on the final balance of their effects on the immune system (Seymour and Henderson, 2001). Following tissue damage, or in response to pathogens, pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α are released by activated immune cells. These help organise the cellular responses to immune challenges, and subsequently aid in coordinating the behavioural and/or physiological adaptations necessary for recovery. The organism's initial response to infection is a *sickness behaviour* which is an adaptive mechanism for conserving energy and coping with illness (Hart, 1988) (described in detail further below). It has been suggested, that depression can develop as a late phenomenon over a background of early sickness, however, it still remains unclear whether it is a processes originating from the periphery or central processes that cause inflammatory responses within the brain (Kelley et al., 2003, Miller et al., 2009). Cytokines can affect the brain both from peripheral immune organs and cross the blood brain barrier e.g. through leaky areas or be produced by neuronal cells within the CNS (Miller et al., 2009). Cytokines within the brain interact with a variety of cells, including neurons, microglia and astrocytes, that are able to produce additional cytokines, express cytokine receptors and thereby amplify cytokine signals. As a consequence, cytokines can then interfere with some of the most important pathophysiological processes that are relevant to depression, including neurotransmitter metabolism, activation of the HPA axis, and neuroplasticity (Raison et al., 2006).

It is suggested that cytokines might affect the HPA-axis by impairing its negative feedback regulation. This functional impairment is believed to be mediated, at least in part, by alterations of the glucocorticoid receptor (GR) (Pace et al., 2007). Pro-inflammatory cytokines also impair BDNF signalling in neurons, leading to a condition referred to as 'neurotrophin resistance' (Tong et al., 2008). This implies the development of a resistance toward neurotrophins in the brain, predisposing neurons to dysfunction and placing them at increased risk for functional defects and degeneration. Therefore, these changes may ultimately lead to at least some of the pathological changes associated with depression.

Cytokines alone, or through stimulation of other inflammatory signalling pathways, can interfere with the kynurenine pathway (see Figure 1-3). The kynurenine pathway is the major metabolic pathway of tryptophan (TRP), the primary amino acid precursor of serotonin. Cytokines can activate the enzyme indoleamine 2,3 dioxygenase (IDO), that normally play a small role in TRP oxidation. When activated, IDO break down TRP into kynurenine (KYN). This is believed to contribute to a lower level of 5-HT (Dantzer et al., 2008), which is in line with the monoamine hypothesis of depression. In support of this, decreased TRP and increased KYN in the peripheral blood have been associated with the development of depression in patients that have been treated with IFN-α (Bonaccorso et al., 2002).

Low grade inflammation (LGI) has been investigated in most detail in the disorder known as metabolic syndrome (MS), might be a common periphery risk factor in declining cognition in both depression and MS. A systemic inflammation exacerbates CNS inflammation that correlates with cognitive decline (Yaffe et al., 2004).

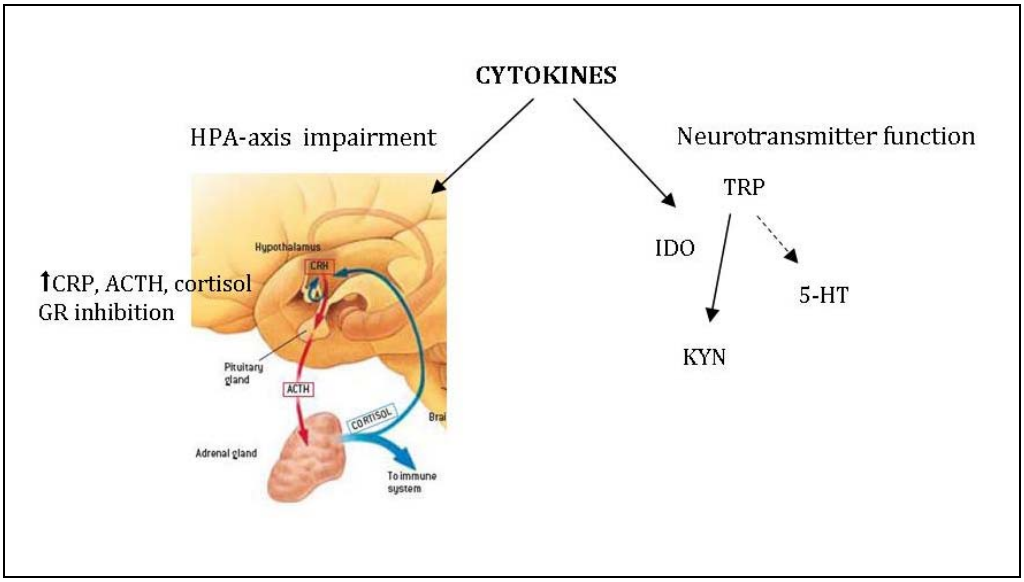


**Figure 1-3: Cytokines influence the HPA-axis as well as neurotransmitter function.** Abbreviations used: HPA: hypothalamic-pituitary-adrenal, CRH: corticotrophin-releasing hormone, ACTH: adrenocorticotrophic hormone, TRP: tryptophan, IDO: indoleamine 2,3 dioxygenase, KYN: kynurenine, 5-HT: serotonin.

Some of the most popular neurobiological hypothesis and their main findings are summarised in the table below:

**Table 1-2: Neurobiological hypotheses of depression with their main findings**

| Hypothesis | Main finding | Main reference |
|---|---|---|
| **Monoamine** | Reduced serotonine | (Schildkraut, 1967) |
| **HPA-axis** | Hyperactive HPA axis:<br>Hypercortisolemia | (Gillespie and Nemeroff, 2005) |
| **Neuroplasticity** | Decreased neurotrophic support<br>Decreased neurogenesis | (Duman and Monteggia, 2006) |
| **Immunological** | Increased pro-inflammatory cytokines:<br>Increased TNF-α, IL-1, IL-6 | (Raison et al., 2006) |

## 1.4 PHARMACOLOGICAL TREATMENT OF DEPRESSION

The selection of treatment for depression is based upon its severity. Psychotherapy is often used as a first-line treatment for mild depression, whereas pharmacological treatment is usually reserved for cases of moderate and severe depression. Examples of the current clinically available antidepressant drugs are outlined in Table 1-3. These can be grouped into tricyclics antidepressants (TCA), selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (NARIs), Serotonin noradrenaline reuptake inhibitors (SNRIs) and monoamine oxidase inhibitors (MAOIs). The primary mechanism of action of all these drugs is to increase the synaptic concentrations of monoaminergic neurotransmitters, namely 5-HT, NA and/or DA, and thereby enhance neurotransmission in these circuits (Manji et al., 2001, Lanni et al., 2009).

**Table 1-3: Some currently available antidepressants classified by their mechanism of action**

| Group | Actions | Examples |
|---|---|---|
| **Tricyclics** | Inhibits the reuptake of serotonin and noradrenaline by blocking the serotonin and noradrenaline transporters | Imipramine<br>Desipramine |
| **SSRI** | Selectively inhibits the reuptake of serotonin by blocking the serotonin transporter | Escitalopram<br>Fluoxetine<br>Sertraline |
| **SNRIs** | Selectively inhibits the reuptake of serotonin and noradrenaline by blocking the SERT and noradrenaline transporter | Duloxetine<br>Venlafaxine |
| **NARI** | Selectively inhibits the reuptake of noradrenaline by blocking the noradrenaline transporter | Reboxetine |
| **MAOI** | Inhibit the activation of monoamine oxidase, thus preventing the breakdown of monoamine neurotransmitter | Selegiline |

Convincing clinical evidence exist for the efficacy of these antidepressant drugs above placebo (Price et al., 2011). However, there are also substantial problems with these treatments. For example, only approximately 30-35% of patients respond to treatment, and furthermore there is a 4-6 week delay period before symptoms are alleviated (Baldessarini et al., 2002, Trivedi et al., 2006). For that reason, there is currently an urgent need in the healthcare profession for other forms of treatment as an alternative or adjunct to drug therapy in the treatment of depression. Cognitive behavioural therapy and counselling are current alternatives or adjuncts to medication, but access to therapists can involve long waiting times. For these reasons, there is a need to identify other effective non-pharmacological interventions for the management of depression. In this project we explore exercise as a possible alternative approach in the treatment of depression.

## 1.5 EXERCISE AS AN ALTERNATIVE ANTIDEPRESSANT STRATEGY

It is well known that physical activity has beneficial effects on illnesses such as diabetes, hypertension and cardiovascular diseases. For example, the level of physical activity is inversely linked to high blood pressure, body mass index as well as glucose intolerance

(Hansen et al., 2010). However the effect of exercise extends beyond the periphery to the brain, where it also improves learning and memory, delay age-related cognitive decline, reduce risk of neurodegeneration, and there is also some indication that exercise can alleviate depression (discussed below) (Cotman et al., 2007). Subsequently, several health agencies around the world have made guidance statements to encourage people to increase their daily physical activity for the benefit of their own health. In Denmark, adults are recommended a daily physical activity of at least 30 minutes of moderate intensity (for example cycling, gardening work, jogging etc.) according to national health guidelines (Sundhedsstyrelsen, 2003).

Although the beneficial effects of exercise on depression already began to emerge in the 1980s, it was not until 1999 that it was concluded in a meta-analysis of 14 papers (Lawlor and Hopker 2001), that the antidepressant-like effect of exercise is equivalent to that of cognitive therapy. Several other reports describing the role of exercise in depression have subsequently been published. It should, however, be noted that some trials fail to show any benefit from increasing physical activity (De Moor et al., 2008). This discrepancy may be explained by the fact that the different trials have diverse experimental layouts, which has also been indicated by a more recent meta-analysis examining the effects of exercise on depression (Mead et al., 2009). These authors compared 25 randomized controlled trials in which exercise was compared to standard treatment, no treatment or placebo in adults with depression. However they concluded that most of these trials had inadequate experimental procedures, and only three trials fulfilled the desired level of accuracy. This stresses the need for more methodologically robust trials to obtain more accurate estimates of the effect of exercise.

The mechanism(s) underlying the hypothetical beneficial effects of exercise on major depression remains largely unknown, but given the wide range of biological systems implicated in depression (see Section 1.3), several lines of investigation are currently being pursued. These include effects on monoamines, the HPA axis, neuroplasticity and immunological markers. The last two processes mentioned will be discussed in somewhat more detail, given the relevance of these to the current project.

### 1.5.1 EXERCISE AND THE MONOAMINES

It has been reported that physical activity stimulates the monoamine system by increasing several neurotransmitters including 5-HT, DA and NA (Deslandes et al., 2009). Furthermore, exercise increases the activity of tryptophan hydroxylase, which is the rate-limiting enzyme in serotonin biosynthesis (Malek et al., 2007). This said, there remain many discrepancies between the experimental protocols used, which add confusion to the total picture. However, the involvement of monoamines in depression is beyond the scope of this project.

### 1.5.2 EXERCISE AND THE HPA AXIS

The HPA axis is a major adaptive mechanism in reaction to stress (see Section 1.3.2). Although exercise activates the HPA axis, the response reacts in a different manner compared to stress caused by a physical threat (Stranahan et al., 2008). From preclinical studies, predictable and controllable stressors, such as wheel running, have in fact shown to enhance cellular function

and moreover, it appears that wheel running is perceived by rodents as a pleasurable activity, since they will readily learn to press a bar for wheel access (Iversen, 1993).

Running, like stressors, activates the sympathetic nervous system with a resulting increase in the hypothalamic-pituitary-adrenal (HPA) axis and an increased glucocorticoid production (Droste et al., 2003). This is attributed to an increased demand for energy in peripheral tissues. Furthermore, although running has been associated with increased levels of proopiomelanocortin (POMC), a precursor for the synthesis of ACTH (Makatsori et al., 2003), exercise has also been shown to attenuate the stress response by decreasing mRNA expression of CRF in the paraventricular nucleus (Droste et al., 2003). This indicates one discrepancy between the actions of detrimental stress and exercise on the HPA-axis. In addition, whereas GRs will normally be down-regulated following prolonged exposure to increased glucocorticoids, no change in GR expression was observed following exercise (Zheng et al., 2006). Higher levels of glucocorticoids are usually associated with hippocampal damage with retraction of dendrites in the hippocampus, however, interestingly, following exercise-induced increases in glucocorticoids, dendritic branching has been reported to be increased in hippocampal neurons (Eadie et al., 2005). Taken together, these data indicate that the effects of exercise-induced stress response are different from those of the threat-induced activation of the HPA-axis.

### 1.5.3 EXERCISE AND NEUROPLASTICITY

The effects of exercise on neuroplasticity-related processes may especially be important for the cognitive impairment that some depressed patients experience. A number of these beneficial effects of exercise may be mediated by a complex interplay between enhanced hippocampal neurogenesis, increased synaptic plasticity, increased neurotrophic support and angiogenesis (Cotman et al., 2007, van Praag, 2009). From preclinical studies, one of the most reproducible effects of exercise seems to be an enhanced hippocampal neurogenesis (van Praag et al., 1999). Exercise has been found to stimulate proliferation of neural progenitor cells, increase the number of new neurons, and promote survival of these new cells (van Praag et al., 1999, Fabel et al., 2003). From a genetic developed rat model of depression, running was found to increase hippocampal cell proliferation which was further associated with an antidepressant effect (Bjornebekk et al., 2005). Adult hippocampal neurogenesis is not only affected by external stimuli but also regulated by internal growth factors including BDNF. BDNF is considered to be one of the most important factors in regulating the positive effect of exercise, because of its important role in neurogenesis, cell growth and survival as well as the ability to modulate synaptic plasticity in the adult brain (Cotman et al., 2007). Exercise increases both the synthesis and release of BDNF, with increased levels reported in both the brain (hippocampus) (Berchtold et al., 2005) and plasma and serum (Gold et al., 2003). In addition, recent studies show that BDNF is also expressed in peripheral tissues such as skeletal muscle (Matthews et al., 2009), and electrical stimulation of muscles increases the mRNA and protein expression of BDNF in this tissue (Pedersen et al., 2009).

Adequate nutrient and energy supply is required for neurogenesis and other exercise-induced neurobiological alterations to occur, which, in turn, is supported by increased metabolism and blood flow (Cotman et al., 2007). Indeed, exercise influences brain vasculature, and it has been shown that physical activity increases the proliferation of brain endothelial cells and

angiogenesis throughout the brain (Bullitt et al., 2009). Accordingly, VEGF, which regulates endothelial cell proliferation and angiogenesis, was found to be increased following exercise (Ding et al., 2006). Another growth factor that has been shown to be affected by exercise is IGF-1 (Trejo et al., 2001), which acts in concert with BDNF and VEGF to regulate hippocampal neurogenesis and angiogenesis, and hereby may produce complementary functional effects that modulate both overlapping and unique aspects of exercise-related benefits in brain plasticity, function and health (Cotman et al., 2007).

**Table 1-4: The effect of exercise on neurogenesis and mechanisms of neuroplasticity**

| Mechanisms affected | Main finding | Reference |
|---|---|---|
| **Enhances neurogenesis:** | Stimulate proliferation of neural progenitor cells | (van Praag, 2009) |
| | Increases the number of new neurons | (Cotman et al., 2007) |
| | Promotes survival of new cells | (van Praag et al., 1999) |
| **Enhance neurotrophic support** | Increase BDNF | (Cotman and Berchtold, 2002) |
| | Increase VEGF | (Fabel et al., 2003) |
| | Increase IGF-1 | (Llorens-Martin et al., 2008) |

In summary, it seems that the neurobiological bases of exercise on the brain include several areas including enhanced neurotrophic factor expression (Neeper et al., 1995), increased blood vessel growth (Van der Borght et al., 2009), hippocampal neurogenesis (Ernst et al., 2006) and cell proliferation (van Praag et al., 1999).

### 1.5.4 EXERCISE AND THE IMMUNE SYSTEM

There is growing evidence that regular physical activity also has beneficial effects on immune function. The relationship of physical activity and susceptibility to infection can be represented by an inverted U-shaped curve, with moderate activity enhancing immune function, whereas a low activity level or excessive amounts of prolonged, high intensity exercise may impair immune function (Gleeson, 2007).

Recent evidence has provided substantial support for exercise-induced reductions in circulating inflammatory markers (Goldhammer et al., 2005). Acute exercise is known to increase the level of IL-6, a cytokine classified as both a pro- and anti-inflammatory cytokine (Petersen and Pedersen, 2005). Exercise-induced IL-6 exerts inhibitory effects on the production of anti-inflammatory cytokines such as IL-10, while decreasing the level of pro-inflammatory cytokines such as TNF-α (Starkie et al., 2003).

Increasing evidences further point to a role of exercise in modulating the level of T lymphocytes (Gleeson, 2007). T helper ($T_h$) cells can be classed as type 1 ($T_h1$) or type 2 ($T_h2$) cells, depending on which cytokines they predominantly produce. $T_h1$ cells produce mostly TNF-α, and are involved in activation of macrophages, whereas $T_h2$ cells mainly produce the anti-inflammatory cytokine IL-10 (among many others) and can inhibit the production of $T_h1$ cytokine production. It has been shown that exercise increases the plasma concentrations of adrenalin and cortisol, which both suppress $T_h1$ cell cytokine production (Gleeson, 2007). In

addition, exercise-induced IL-6 directly stimulates $T_h2$ cell cytokine production, and exercise may hereby shift the balance of T lymphocyte production in favour of the anti-inflammatory type.

Additionally, exercise has been shown decrease the expression of the Toll-like receptors (TLRs) 1, 2, and 4 on monocytes (Gleeson et al., 2006). The TLRs are highly conserved trans-membrane proteins that play an important role in whole body inflammation by detection and recognition of microbial pathogens. A downstream effect of TLR activation is an increase in the pro-inflammatory response, an effect that acute exercise can decrease.

**Table 1-5: The effects of exercise on immune function**

| Mechanisms affected | Action on immune modulators | Reference |
|---|---|---|
| **Increase IL-6 →** | Increase anti-inflammatory cytokines such as IL-1ra and IL10<br>Decreased TNF-α | (Petersen and Pedersen 2005) |
| **Increase adrenaline and cortisol → Decreased TRL expression →** | Decreased $T_h1$ cell cytokine production | (Gleeson et al., 2006)<br>(Gleeson, 2007) |

Unfortunately, the role of cytokines as neuromodulators in the brain has not been studied in as much detail as in the periphery, and subsequently the specific mechanisms by which exercise may induce central anti-inflammatory effects are currently poorly understood. This stresses the need for further research in this field in order to address these shortcomings.

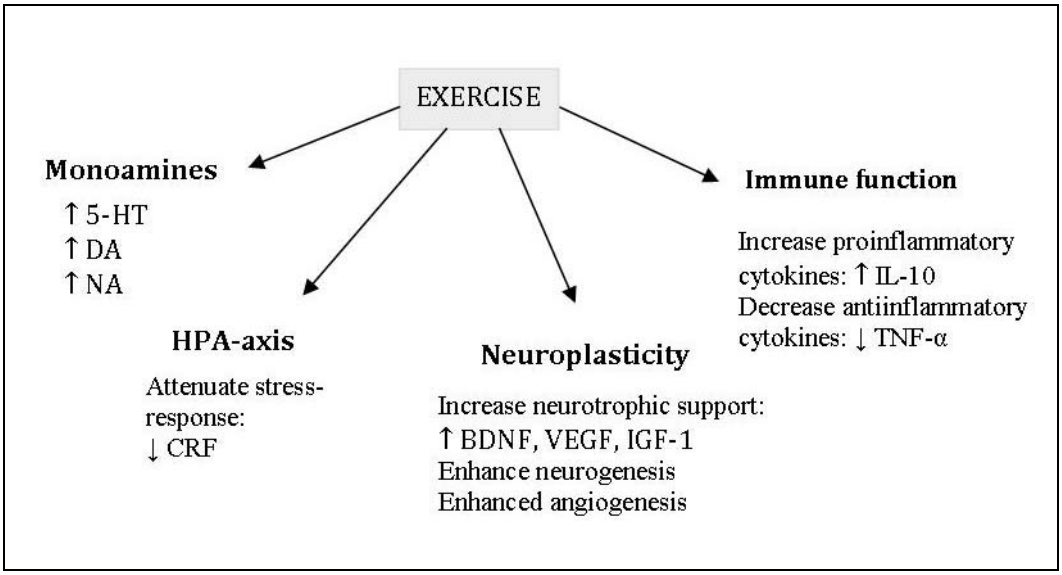


**Figure 1-4: A schematic illustration of the wide-spread beneficial effects of exercise on physiological mechanisms that are associated with a number of hypotheses of depression.**

## 1.6 MODELING DEPRESSION IN ANIMALS

Modelling depression in animals is an especially complicated challenge compared to the modelling of other disorders. Depressed patients show a range of symptoms (see Section 1.1), many of which are difficult or impossible to reproduce in animals, such as excessive thoughts of guilt or suicidal thoughts. However there remains a strong need for animal models in elucidating the neurobiology of depression and to study the mechanism of action of established and candidate antidepressants, since obvious ethical issues would arise if brain parameters were to be examined in living humans. An ideal animal model of depression would offer the opportunity to understand molecular, genetic and environmental factors that may lead to depression. Today, despite the difficulties mentioned above, there exist several models that offer insight into different aspects of depression. The models differ in the degree to which they produce features that resemble a depressive-like state, and include paradigms that employ acute, sub-chronic, or long-term stress as well as genetic models. Such models need to be validated for comparison to the human disease, and should be assessed on the basis of three major criteria (Willner, 1984):

- Face validity: how well the model resembles the disease/condition, thus similar pathophysiology and symptomatology,
- Construct validity: how well the model is consistent with theoretical rationale, and
- Predictive validity: how well the model responds favourably to clinically established drugs.

An ideal model would fulfil all of these criteria. However, the model can still be useful even if it does not fulfil all criteria, and good predictive validity is considered to be the most important criteria to validate an animal model of depression (Geyer, 1995).

In this section only the animal models relevant to this project will be discussed.

### 1.6.1 A GENETIC MODEL OF DEPRESSION: THE FSL/FRL LINE RATS

The Flinders Sensitive Line (FSL) rats are an inbred rat strain that display characteristics that in many ways resemble those in depressed humans. The strain was originally developed in an attempt to create a line of rats that was genetically more resistant to the effects of the cholinesterase inhibitor, diisopropyl fluorophosphate (DFP) (Overstreet and Russell, 1982). Instead, the selective breeding of Sprague-Dawley rats lead to a strain that was genetically more sensitive to the effects of this cholinergic agent, the Flinders Sensitive Line (FSL) rats and a strain that was more resistant, the Flinders Resistant Line (FRL) rats, which became the controls of the "depressed" line. The FSL rats were found to be more sensitive to drugs targeting the cholinergic system, and to have a higher muscarinic receptor density in several brain regions (Overstreet and Russell, 1984). It was later suggested that the rats could be used as a model of depression, since depressed patients were more sensitive to cholinergic agonists than normal controls (Janowsky et al., 1980, Wegener et al., 2011).

The FSL rats present with several behavioural, neurochemical, and pharmacological features that have been reported in depressed individuals. In line with the diagnostic criteria for depression as stated in the ICD-10, the FSL rats display several "depressive" characteristics, including lower weight, altered sleep pattern (increased REM sleep), reduced locomotor activity and cognitive (learning) difficulties (Overstreet, 1993), see Table 1-6. Hence, the FSL

strain shows good face validity. The monoamine and cholinergic hypotheses can also be partially modelled in the FSL, with serotonergic and cholinergic abnormalities, respectively (Overstreet et al., 2005). In addition, newer research has shown that FSL rats have impairments in neurotrophic factors (Elfving et al., 2010a, Elfving et al., 2010b), supporting the neurotrophic factor hypothesis of depression. This gives the model some degree of construct validity for these theoretical traits. The "state of depression" in FSL rats is routinely assessed using the forced swim test (see below), since FSL rats inherently present with increased immobility (depression-like behaviour) compared to FRL controls (Overstreet, 1993). This deficit has been shown to be normalised following chronic, but not acute, treatment with several antidepressant drugs including tricyclic antidepressants (e.g. imipramine) and SSRIs (e.g. sertraline) (Pucilowski and Overstreet, 1993) reducing the exaggerated immobility in FSL rats.

### 1.6.2 AN IMMUNOLOGICAL MODEL OF DEPRESSION: THE LPS-INDUCED MODEL

An inflammation-associated model of depression stands out compared to other animal models of depression because its underlying factors are evident and it is easy to mimic in an animal model (Dantzer et al., 2011). Depicting an inflammatory model of depression is most often based on the administration of a central or periphery pro-inflammatory mediator such as LPS, IL-1β, TNF-α or inoculation with a microbial pathogen. The timeframe and dose are varied among the studies, with both the induction of depressive like behaviour following one injection of long-term treatments. A shared feature is systemic inflammation, initially observed as sickness behaviour. Sickness behaviour manifests with numerous behavioural and physiological alterations and include decreased appetite and weight loss, social withdrawal, decreased motor activity and altered cognition (see Table 1-6). Sickness behaviour is believed to be transiting state, that following long-term administration of a pro-inflammatory mediator will create the foundation of a depressive model (Dantzer et al., 2011). Sickness behaviour and the depressive-like state may share some similar pathological mechanisms including overproduction of endogenous pro-inflammatory cytokines and a dysregulation of the HPA axis, which could explain some of the overlapping symptoms, such as decreased appetite and increased sleep (Ericsson et al., 1994, Loftis et al., 2010). However, studies have shown that sickness behaviour usually disappears after 24 to 48 h indicated by locomotor activity returning to normal whereby depressive-like behaviour could be evaluated (Frenois et al., 2007, Moreau et al., 2008). Furthermore, antidepressant treatment has been shown to relieve some of the cytokine-induced symptoms (Yirmiya et al., 1999)

In this study, a putative immunological model of depression was attempted with eight weeks of daily injections with lipopolysaccharide (LPS). LPS is a cell wall component of the Gram-negative bacteria and has long been considered a potent activator of the innate immune machinery (Beutler and Rietschel, 2003). When the immune system detects even minute amounts of LPS from invading bacteria, the organism respond to counteract the infection. The physiological recognition happens when LPS activates TLR4 and myeloid differentiation factor 2 (MD-2), which is recognized by macrophages (Triantafilou and Triantafilou, 2002). Stimulation of TLR4 leads to activation the NF-κB pathway to activate several cytokines, including IL-1 and TNF-α.

From pilot studies with four weeks of either daily injections or by a surgical implanted pump for four weeks, animals were tested for recovery from initial sickness behaviour and no signs of decreased locomotor activity and fever were found, indicating that rats had recovered from sickness behaviour (Buhl, CS unpublished data). This was in line with previous studies were repeated administration of rodents developed tolerance towards LPS and the severity of sickness behaviour diminishes, indicated by food intake (Langhans et al., 1991) and hypoactivity (Engeland et al., 2001) returning to baseline levels. Thus it seems that with continued administration of LPS, sickness behaviour is replaced by a chronic low-grade inflammation state that may be used as an animal model of depression. In addition, results from the pilot studies showed that animals increased their weight and fasting glucose levels (Buhl, CS, unpublished data), indicating that that in addition to an animal model of depression, there might be a dysmetabolic association as well. Chronic activation of the immune system has been hypothesized to underlie the link between depression and the associated risk factors with the metabolic syndrome (Capuron et al., 2008). On the basis of these assumptions an experimental model of depression with a possible dysmetabolic state was investigated.

To summarise, these two putative animal models of depression each have varying degrees of face, construct, and predictive validity and can each contribute differently to our understanding of the pathology of depression and antidepressant action. A routinely used method to assess the behavioural depression-like state in rodents as well as the efficacy of antidepressant drugs is the forced swim test (FST).

**Table 1-6: Comparative depression-like symptoms of FSL rats versus LPS-induced rats**

| Manifestation in humans | Behaviour in the FSL | Behaviour after LPS-induction |
|---|---|---|
| **HPA-activation** | No activation | Activation |
| **Altered appetite** | Lower body weight and reduced appetite | Decreased appetite in sickness behaviour, increased after long term |
| **Altered sleep pattern** | Increased REM sleep | Increased slow-wave sleep |
| **Decreased cognition** | Inconclusive | Decreased cognition |
| **Anxiety of some types** | Anxiety in some tasks | Anxiety in some tasks |
| **Circadian rhythm abnormalities** | Abnormal, with shortened period for temperature and drinking rhythms | n/a |
| **Antidepressant response** | Respond to antidepressants | Respond to antidepressants |

### 1.6.3 THE FORCED SWIM TEST – THE RAT 'INTERVIEW'

The forced swim test (FST) was originally developed by Porsolt and colleagues in the 1970's (Porsolt et al., 1977) to detect antidepressant-like activity in rats. The FST is based on the observation that rats, following initial escape-directed movements, develop an immobile state when exposed to an inescapable cylinder of water. The immobility behaviour is believed to underlie a failure in actively trying to escape the cylinder, also referred to as behavioural despair (Lucki, 1997). The test was originally shown to successfully detect the

antidepressant-like properties of tricyclic antidepressants and monoamine oxidase inhibitors, but was proven to be unreliable in detecting the antidepressant-like activity of SSRIs. In the face of this the model was modified by distinguishing between three different types of behaviour (Cryan et al., 2002), (see Figure 1-5). The first behaviour, which is typically observed shortly after placing the animal into the cylinder, is climbing behaviour, and is defined as vigorous upward-directed movements against the walls of the cylinder with the forepaws. Swimming behaviour is defined as horizontal, less vigorous, movements throughout the cylinder, whereas immobility is defined as when the rat makes only those active movements that are necessary to keep the rat's head above the water. A significant advantage of the modified FST is that the antidepressant-like activity of drugs can be categorised for having either a serotonergic-like or noradrenergic-like antidepressant action, based on increases in swimming or climbing behaviours, respectively (Cryan et al., 2002). This theory is based on the circumstantial evidence surrounding the actions of selective serotonergic or noradrenergic antidepressants in the FST.

Prior to the FST, the open field test is routinely carried out to screen for false positives, that is, drugs that cause a general increase in locomotor activity and therefore not produce "true" antidepressant-like effects.

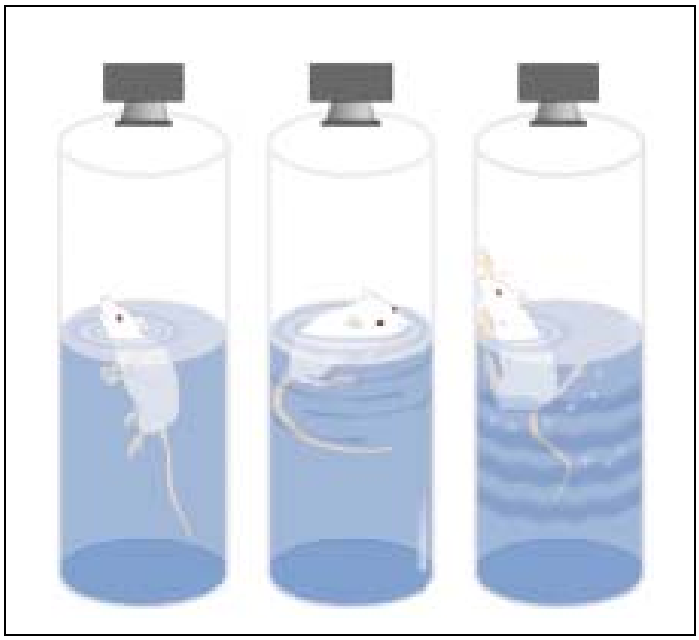

**Figure 1-5: Behavioural components in the modified FST.** The three different behaviours that can be evaluated are climbing (left), swimming (middle) and immobile (right). (Cryan, Markou et al. 2002)

In summary, depression is a disease that poses serious problems for the Danish population as well as the rest of the world. Depression is a complex, multifaceted disease that most likely involves various neuronal circuits and regions of the brain. Though evidence exists for the efficacy of the clinical available antidepressant drugs above placebo, there are still a

substantial amount of patients that do not respond to pharmacological treatment. For this reason, an unconventional treatment approach might serve as a supplementary and/or alternative strategy. Exercise has many advantageous effects throughout the body and evidence suggests that regular physical activity might further have positive effects on the brain and on mood.

# 2 STUDY OBJECTIVES

The main objective of this study was to evaluate the efficacy of an alternative antidepressant strategy, namely voluntary exercise, in two distinct rat models of depression that each mimics a distinct pathophysiological component of depression. This study layout was chosen based on the multi-factorial nature of major depression, and enabled us to investigate the effect of our putative antidepressant treatment on several facets of this disorder. Firstly, we studied the effect of exercise in a well-validated genetic model of depression, namely the FSL and FRL rats, and secondly, also studied these effects in one experimental immunological model, namely an LPS-induced model of depression. The LPS-induced model was mainly based on unpublished results from pilot studies (Buhl, 2010), from which we hypothesised that chronic treatment of rats with LPS would induce a low-grade inflammation that would initially present as a state of sickness behaviour, but then dissipate within approximately one week after which a depression-like state would be attained. Furthermore, the initial studies indicated that the model could mimic some of the features observed in the metabolic syndrome, which, as mentioned above, also displays a high co-morbidity with depression.

The evaluation of the putative antidepressant-like activity of exercise using these two models was performed in two separate experiments that were carried out consecutively, namely Experiment 1 and Experiment 2. The specific objectives for these two phases will be provided separately

**Objectives for Experiment 1 - The FSL/FRL model of depression**

The specific aims in this first experiment were to:

1. Evaluate the effects of exercise on several behavioural parameters in FSL rats by using a number of behavioural tests, namely:
    - the forced swim test (FST) for the evaluation depression-like behaviour,
    - the elevated plus maze (EPM) for the evaluation anxiety-like behaviour,
    - the object recognition task (ORT) for the evaluation memory function, and
    - the open field (OF) test for the evaluation of locomotor activity.
2. Examine the effects of exercise on a number of metabolic markers in FSL rats, namely:
    - weight gain,
    - food consumption,
    - blood glucose levels at fasting and following a glucose load in the oral glucose tolerance test (OGTT).
3. Investigate the effects of exercise on the mRNA expression of a selection of proteins that may be involved in depression by using real-time qPCR,
4. Measure the effect of exercise on serum BDNF levels in FSL rats by using an ELISA method, and

5. Evaluate the effects of the conventional antidepressant drug imipramine alone or in combination with exercise, in FSL rats on all of the abovementioned tests and measurements.

**Objectives for Experiment 2 – An LPS-induced model of depression:**

The specific aims in the second experiment were to:

1. Evaluate the effects of exercise on several behavioural parameters in LPS-treated rats by using a number of behavioural tests, namely:
    - the forced swim test (FST) for the evaluation depression-like behaviour,
    - the elevated plus maze (EPM) for the evaluation anxiety-like behaviour, and
    - the open field (OF) test for the evaluation of locomotor activity, and
2. Examine the effects of exercise on a number of metabolic markers in LPS-treated rats, namely:
    - weight gain,
    - food consumption,
    - blood glucose levels at fasting and following a glucose load in the oral glucose tolerance test (OGTT)
3. Evaluate the effects of the conventional antidepressant drug, imipramine, in LPS-treated rats on all of the abovementioned tests and measurements.

# 3 MATERIALS AND METHODS

All animals were housed at a constant temperature of 20°C and maintained on a 12 hour light/dark cycle (lights on at 07:00 a.m.) with ad libitum access to food and water. All animal procedures were approved by the Danish Animal Experiments Inspectorate (permission id 2007/561-1378). This project consisted of two separate experimental set-ups, and will be discussed accordingly.

## 3.1 EXPERIMENT 1: THE FSL/FRL RATS

Male Flinders Line rats (38 FSL and 16 FRL) were supplied from the Centre for Psychiatric Research, University of Aarhus (Risskov, Denmark). Handling of the rats took place twice a week, namely when animals and chow were weighed and when cages were cleaned. The rats were on a standard laboratory diet for rodents.

### 3.1.1 EXPERIMENTAL DESIGN

This part of the project consisted of five groups, namely two control groups that did not receive any treatment (FRL-CON, n=8 and FSL-CON, n=8), and three treatment groups of FSL rats that received either exercise (FSL-EXE, n=8), imipramine (FSL-IMI, n=8) or both imipramine and exercise (FSL-IMI+EXE, n=6). Rats were evenly distributed among the different groups on the basis of their age, weight and immobility in the FST that were assessed prior to the start of the experiment. One animal from the FSL-IMI group had to be terminated during the experiment due to an inappropriate physical condition.

**Table 3-1: The age, weight and immobility of rats prior to the start of the experiment**

| Group | FRL-CON | FSL-CON | FSL-EXE | FSL-IMI | FSL-IMI+EXE |
|---|---|---|---|---|---|
| **Group size** | 8 | 8 | 8 | 8 | 6 |
| **Age** (days) | 73.50 ± 1.02 | 70.63 ± 1.57 | 70.88 ± 1.09 | 71.13 ± 1.27 | 73.33 ± 1.20 |
| **Weight** (g) | 308.1 ± 15.54 | 249.9 ± 20.70 | 255.5 ± 26.64 | 255.3 ± 14.11 | 263.0 ± 14.00 |
| **Immobility** (sec)[a] | 73.75 ± 6.99 | 185.6 ± 15.94[a] | 179.4 ± 17.71 [a] | 180.0 ± 14.70 [a] | 180.0 ± 10.88 [a] |
| **Weight at T0** (g) | 333.0 ± 8.8 | 294.0 ± 17.2 | 302,6 ± 15,9 | 288,3 ± 10,1 | 309,2 ± 14,2 |

Data were analysed using one way ANOVA followed by Tukey post-tests; [a]indicates a significant difference between the immobility of the FRL-CON group and the different FSL groups ($p < 0.001$). Values are expressed as the mean ± SEM.

In order to accurately measure the running activity for each animal, those rats that were subjected to exercise were housed individually. To control for the effect of this housing condition, we also included two groups of pair-housed animals (FRL-P, n=8, FSL-P, n=8) (Data can be found in Table 8-1 and Table 8-2 in the Addendum).

**Exercise paradigm:** The two groups that were subjected to exercise were housed in cages connected to a running wheel (34.5 cm diameter, one revolution corresponding to 1.07 m) and attached to mechanical counters that were connected to a CLOCKLAB data collection system (Lafaytte Instrument Company, USA). Using this system we continuously recorded the wheel-running activity throughout the duration of the experiment. The rats had free access to the running wheel at all times, making the exercise of a voluntary form. This type of exercise was selected based on reports that voluntary exercise has a more beneficial effect compared to forced exercise, with the latter being associated with symptoms of chronic stress, such as elevated basal corticosterone levels or immunosuppression (Moraska et al., 2000). An exercise period of four weeks was decided upon since previous results have shown that 3 – 6 weeks of wheel running produces the most beneficial effects on brain and behavioural changes (Pietropaolo et al., 2008). A long-term running schedule has the additional advantage of obtaining a stable level of wheel running for the animals. Running distance was evaluated for from the beginning of the experiment, T0 until the day of the first behavioural test, namely T29.

**Drug treatment***:* Imipramine was added to the food of the rats to achieve a daily dose of 15 to 20 mg/kg. This was done by mixing the imipramine powder into crushed chow pellets, and following the addition of water, making small pellets that were kept frozen until needed. This oral administration route was selected to avoid stressful injections.

Of note, the group that received imipramine and exercise (FSL-IMI+EXE) was initiated on imipramine treatment 2 weeks prior to the start of the exercise, i.e. T(-)14 days. The rational behind this design was to ensure that imipramine has had enough time to exert its antidepressant effects, given the known delay in onset of action of antidepressants. In other words, we wanted to be able to compare whether chronic imipramine treatment would modify the response of FSL rats to voluntary exercise, relative to that of untreated FSL rats. This design resulted in FSL-IMI+EXE receiving a total of 6 weeks of imipramine treatment.

The experimental design is illustrated in Figure 3-1. Animals were after proximal 4 weeks subjected to a set of behavioural paradigms, namely the EPM, ORT, OF and FST. For evaluation of metabolic parameters, fasting blood glucose was measured at T24+25 and an oral glucose tolerance test was performed at T36+37.

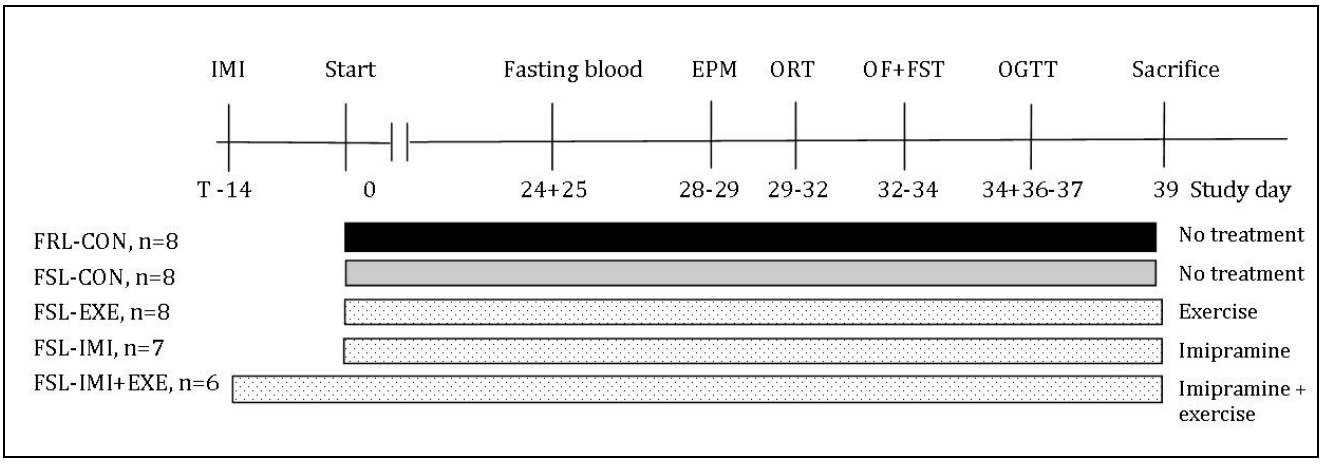


**Figure 3-1 Experimental design with study groups and time line for Experiment 1**. IMI: imipramine, CON: control, EXE: exercise, EPM: elevated plus maze, ORT: object recognition task, OF: open field, FST: forced swim test, OGTT: oral glucose tolerance test.

### 3.1.2 MEASUREMENT OF METABOLIC MARKERS

To examine the effects of exercise and imipramine treatment on metabolic parameters in FSL rats, we noted the weight and food intake of the animals on a weekly basis. In addition, we measured fasting blood glucose levels at T24-25. This was done by collecting blood from the tail vein after a fasting period of 12 hours, and determining the blood glucose levels (the mean of two measurements) using a "Precision Xtra Plus" glucose monitor (Abbott, Denmark). Fasting blood glucose gives an immediately indicating of glucose and insulin sensitivity, and is used as a predictor of insulin resistance. Furthermore, we measured blood glucose changes during an oral glucose tolerance test (OGTT), which was conducted on day T36-37. The OGTT was carried out by measuring plasma glucose levels (blood also collected from the tail vein) at 0, 30, 60 and 120 minutes after the oral administration of a glucose solution (g glucose/kg body weight). The OGTT further assist in evaluating insulin production and gives an indication of whether there exists an impaired glucose tolerance, as assessed by determining area under the curve. Additional fasting and OGTT blood samples were collected in heparin-coated tubes for the determination of insulin levels. However, these measurements have not been carried out as yet.

### 3.1.3 BEHAVIOURAL EVALUATIONS

All behavioural testing took place between 9:00 and 13:00 a.m. in an area of the laboratory that was free from noise and other disturbances. The animals were moved to the experimental room 2 hours prior to the start of each test to habituate. All behavioural experiments were carried out by the same individual.

#### 3.1.3.1 THE OBJECT RECOGNITION TASK:

Clinical evidence indicates that depression can lead to the deterioration of cognition including deficits in learning and memory (Marazziti et al., 2010). Therefore, in this project we investigated behaviour related to learning and memory by subjecting the rats to a test of

spontaneous object recognition. The object recognition task (ORT) is a memory test for declarative memory, referring to the type of memory that can consciously recalled such as facts and events (Bevins and Besheer, 2006).

The ORT consists of three phases (see Figure 3-2), namely a training phase and two testing phases. In the training phase, the rat is only placed in the testing arena for 10 min to habituate to the environment. Twenty-four hours later the learning phase (or sample phase) is carried out, in which the rat is placed in the testing arena that contains two distinct objects, with its head facing away from the objects, for 6 min. In this phase the rat familiarises itself with the objects, and the exploration time for each object is measured. The last phase is conducted 90 minutes later in the recognition phase, (or choice phase), in which the rat is again placed in the arena for 6 min, but with one of the objects replaced with a new object. Normally, rats will spend more time exploring the novel object compared to the familiar object.

The testing apparatus consisted of a square arena (50 x 50 x 40 cm), with an open top, dark walls and a dark floor. The objects used were children's toys, manufactured from plastic, with different colours and shapes. The objects were fixed to the floor with adhesive tape to ensure that the object could not be moved by the rat. Exploration behaviour was defined as directing the nose to the object or touching the object with the nose. Sitting on the object was not considered as exploratory behaviour. In order to avoid the presence of olfactory trails, the objects were thoroughly cleaned between trials with 50 % ethanol.

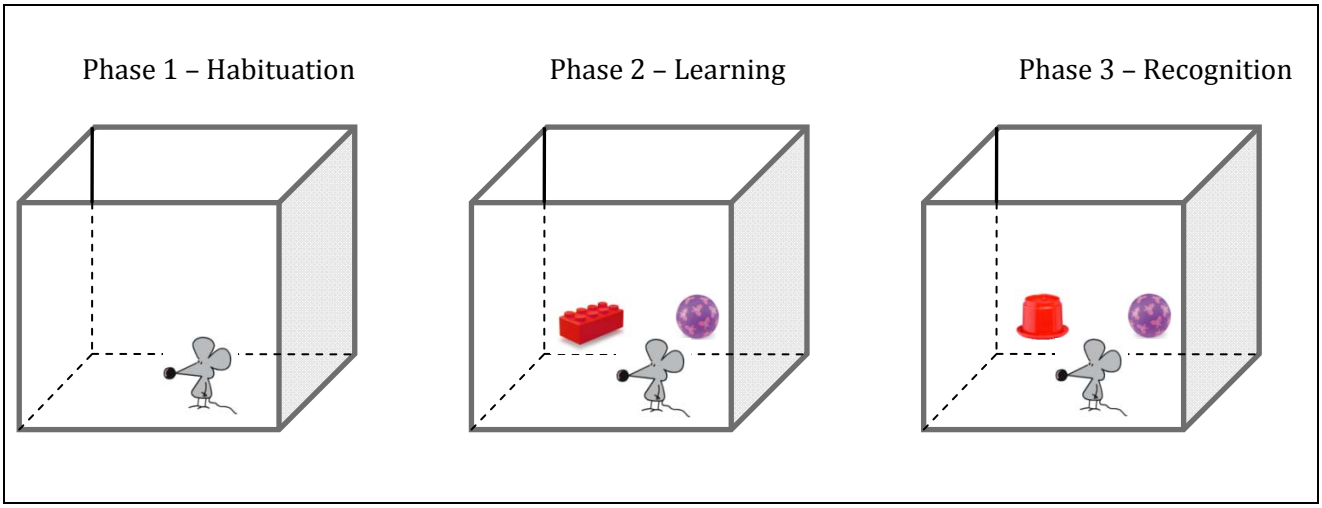


**Figure 3-2: The object recognition task**. The test consists of 3 phases, namely the habituation, learning and recognition phases. In the first phase, rats become habituated to the environment. In the second phase 24 h later, two objects are placed in the testing apparatus. In the third phase, recognition memory is measured when a novel object is placed in the testing apparatus and the ratio between the novel and familiar object is evaluated.

In the learning phase, total exploration time and the exploration time of each object was measured. Rats were excluded from the recognition phase if they during the second phase did not explore both objects, hence they would not be able to recognize the novel object from the familiar one (one rat was excluded on this basis). In the recognition phase, total exploration time and novel object recognition time relative to the first five seconds was measured. If rats

explored the objects for less than 5 seconds, they were excluded from the latter measurement. In addition, the novel object:known object exploration time ratio was evaluated, however if rats did not explore both objects, this ratio could not be determined.

#### 3.1.3.2 THE ELEVATED PLUS MAZE:

As mentioned previously, there exists a significant co-morbidity between depression and anxiety in humans (Kessler et al., 1994; Zimmerman et al., 2000; Kessler et al., 2005). Therefore, in this project we investigated the effects of the treatment conditions on anxiety-like behaviour in the Elevated Plus Maze (EPM), a test in which the innate fear of a rat to be in an bright, open and elevated area is used to evaluate its "state of anxiety" (Pellow et al., 1985, Hogg, 1996).

The EPM arena consists of a plus-shaped, black acrylic maze, elevated 80 cm above the ground, with four (50 cm x 10 cm) arms, two of which are open (devoid of any wall) and two of which have dark walls. The light intensity was adjusted so that the open arms had an intensity of 80-90 lux and the closed arms an intensity of 20 lux.

The test is conducted by placing the rat in the centre of the testing apparatus, with its head facing a closed arm, and recording its behaviour for 5 minutes. We used a camera mounted on the ceiling above the maze that was connected to a computer. The maze was swiped clean after each trial with an ethanol solution to avoid excessive odour disturbances.

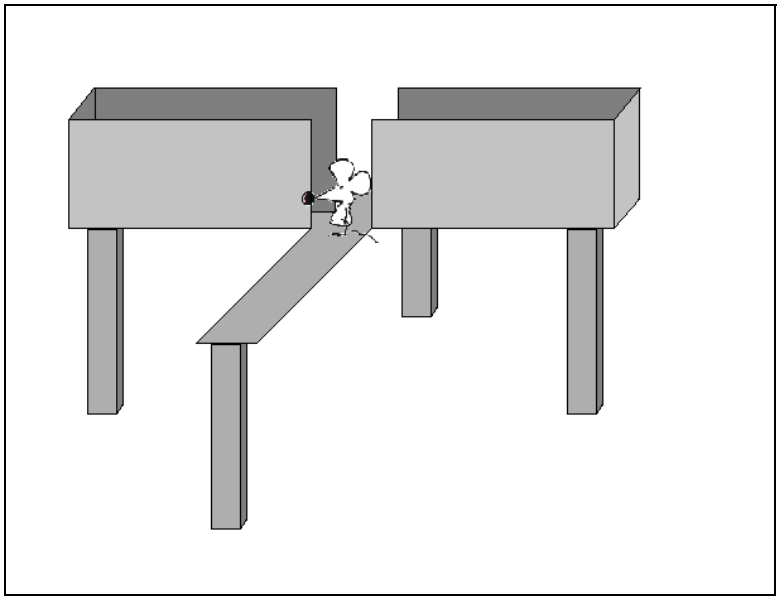

**Figure 3-3: The elevated plus maze (EPM)**. The plus-shaped apparatus consists of two closed arms and two open arms. An anxiolytic effect is indicated by an increase in time spend in the open arms relative to the closed arms.

The normal behavioural tendency of rats is to spend more time in the closed arms of the maze, and a reduction in anxiety is associated with more time spent in the open arms. It is possible to measure several parameters in the EPM. These include the percentage of time spend in the

open arms and the percentage of entries into open arms, as well as the total number of entries which also gives an estimation of the general activity level of the animal during the test. Two rats were excluded from the experiment due to freezing behaviour, a state where the animal stays frozen in the one position in the maze. Scoring was done blindly after the trial.

#### 3.1.3.3 MEASUREMENT OF LOCOMOTOR ACTIVITY:

Locomotion activity was measured in an open field arena (100 cm x 100 cm), under a light intensity of 40 lux. Rats were placed in the arena and their behaviour recorded on video for 5 minutes. Locomotor activity level was measure in terms of the distance moved (cm) and was measured automatically using specialised software (Noldus Ethovision XT, The Netherlands).

#### 3.1.3.4 THE FORCED SWIM TEST (FST):

The FST was originally developed by Porsolt and colleagues (Porsolt et al., 1977) to detect antidepressant-like activity in rats, and is used as a screening tool for antidepressant action as well as to assess the depressive-like state of rodents (Cryan et al., 2002) (discussed in detail in Section 1.6.3). The conventional protocol for the FST involves a pre-swim session 24 hours prior to the final swim test, to induce a state of "behavioural despair" which may then be modified by antidepressant interventions. In this part of the project, the pre-swim was conducted prior to the start of the experiment in order to group animals so the baseline behaviour was similar in the FSL and FRL groups.

Specifically, the test was carried out by placing the rats in transparent cylinders (diameter 24 cm, height 60 cm), filled with 40 cm of water at a temperature of 25°C ± 1°C, for 15 min, after which the rats were removed and dried with towels before being returned to their home cages. Each trial was recorded using a video camera, and later scored by an observer blind to the treatment groups. Scoring was carried out for the 7 first minutes by evaluating the total time each rat engaged in immobility, swimming or climbing behaviour.

### 3.1.4 TISSUE EXTRACTION

The animals were sacrificed by means of decapitation after which arterio-venous blood for the measurement of serum BDNF was collected in anticoagulant-free tubes with gel (Terumo, Venosafe™, VF-054SAS). The brain was removed and the left and right frontal cortex and hippocampus regions were quickly dissected out on a cold tile. The tissue was immediately frozen on powdered dry ice and stored at -80°C until further analysis.

### 3.1.5 DATA ANALYSIS

All values are expressed as mean ± SEM and an alpha level (p-value) of 0.05 or lower was used as the threshold for statistical significance. Normality in a data set was assumed when two or more normality tests (KS normality test, D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test) were passed with equal variances. In case of a non-Gaussian distribution within the groups, the data were logarithmically transformed. This was performed for the data set of percentage of time spent in open arms in the EPM.

Area under the curve (AUC) was calculated from the data for weight gain, food consumption and running distance. A one-way analysis of variance (ANOVA) followed by Tukey post-hoc analysis was used to reveal differences between treatment groups. To compare the data from the pair- and individually housed rats, a two way ANOVA was used with the main factors being defined as strain and housing condition. A Student's t-test was used to compare the running data of the two groups that were subjected to exercise.

A value was defined as an outlier and excluded from the analysis when it deviated more than 3 times the standard deviation (SD) of that dataset.

All statistical analyses were performed using GraphPad Prism 5 (version 5.00 for Windows, Graphpad Software, San Diego, CA, USA), and Microsoft Office Excel 2007 (for Windows).

### 3.1.6 QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION (Q-RTPCR)

We used real-time qPCR in order to amplify and simultaneously quantify a selection of target genes (see Table 3-2).

**Tissue homogenization:** The day before the analysis, the weight of each of the right dissected hippocampi was determined while taking care to keep the tissue frozen. On the day of analysis, the tissue was submerged in a mixture of 800 µl Lysis buffer (Applied Biosystem, CA) and 800 µl PBS. The tissue was homogenized by adding the sample to a tube containing a 4 mm stainless steel ball which was then placed in a mixer-mill (Retsch) and agitated three times for 1 min at 30 Hz). The, samples were placed on ice for 20 min before commencing with the RNA extraction step.

**RNA extraction:** Total RNA was isolated using the commercially available RNA Tissue-Filtr-DNA kit (includes a genomic DNA removal step) on the ABI PRISMTM 6100 Nucleic Acid Prep station (Applied Biosystems), and following the manufacturer's instructions.

**RNA characterization:** The purity and concentration of the extracted RNA were determined using a NanoDrop 1000 Spectrometer (Thermo Fisher Scientific). The RNA purity was measured by determining the optical density (OD) of the RNA extracts at 260 nm and 280 nm. Since nucleic acids and protein have absorption maximum at 260 nm and 280 nm, respectively, the $OD_{260}/OD_{280}$-ratio can be used to obtain a measure of the purity of the RNA extract. An RNA solution is considered of good purity when it has an $OD_{260}/OD_{280}$-ratio of 1.9 to 2.2. Secondly, the RNA integrity was assessed using RNA StdSens microfluidic chips and the Experion Automated Electrophoresis System (BioRad, CA, USA). Assessment of RNA integrity is made by this system by inspecting the clarity of the 18S and 28S ribosomal RNA bands on the chip following electrophoresis. The purity, concentration and integrity of the RNA samples were evaluated to ensure that there were not any significant differences between the samples regarding the parameters mentioned, and also that the samples were of sufficient quality to proceed to the cDNA synthesis step. The RNA concentrations of all samples were adjusted to match the concentration of the most diluted sample (namely 27.1 ng/ml). The extracted RNA was evaluated to ensure that the experimental groups were not statistically different with respect to 18S/28S ratio, concentration and RNA purity.

**cDNA synthesis:** Before complimentary DNA (cDNA) synthesis the RNA concentration of the samples was adjusted to match the sample with the lowest concentration.

The cDNA was synthesized from the mRNA in each sample by reverse transcription by using a mixture of random primers and the Superscript III Reverse Transcriptase kit (Invitrogen, CA, USA) following manufacturer´s instructions, with a RNA concentration per reaction of 27,10 ng/µl (for hippocampus). Appropriate volumes of sample solutions were mixed with 1 µl of random primer solution and 1 µl of 10 mM dNTP mix, whereafter DEPC-treated water was added to give a total volume of 13 µl. The mixture was heated at 65° C for 5 min and allowed to cool on ice for 1 min. Next, 7 µl of a mixture consisting of 4 µl 5X first strand buffer, 1 µl 0.1 M DTT, 1.8 µl double distilled water, 0.2 µl RT superscript III (Invitrogen), was added to each well. The mixture was reheated to 25° for 5 minutes, then to 50° C for 50 minutes wherafter the reaction was inactivated by heating it to 70° C for 15 minutes. The cDNA samples were stored undiluted at -80°C until further analysis. All samples were diluted 1:15 with DEPC-treated water prior to real-time qPCR analysis.

**Real-time qPCR:** Real-time qPCR was carried out on 3 µl of cDNA solution for each gene in 96-well plates by using the Mx3000P (Stratagene, USA) and the SYBR® Green method. A mixture of 5 µl SYBR® Green PCR Master Mix (Applied Biosystems, CA, USA), 0.5 µl of a solution containing 10 µM of each primer and 1.5 µl of DEPC-treated water was added to each sample. The gene expression of eight selected reference genes (i.e. 18s rRNA, ActB, CycA, Gapd, Hmbs, Hprt1, Rpl13A, Ywhaz) and 8 genes for investigation (i.e. BDNF, CREB, VEGFA, VEGFR2, NRP1-2, MR and IL-1β) were determined as previously described (Bonefeld 2008). The characteristics of the primers for these genes are given in Table 3-2. The thermal cycling conditions for the PCR were as follows: 3 minutes at 95°C to activate the hot-start iTaq DNA polymerase followed by 40 cycles of denaturation for 10 seconds at 95°C, annealing for 30 seconds at 60°C and extension for 60 seconds at 72°C. Following each run a melt curve was obtained to confirm the amplification specificity and the absence of primer dimers. Each sample was run on the plate as a single reaction and a standard curve was generated on each plate.

**Data analysis:** The mRNA levels of the reference genes were used for data normalization by doing a stability comparison of the expression of the reference genes using Normfinder software (http://www.mdl.dk). P3 Actb and P5 Hmbs were determined to be the best combination of reference genes. Therefore, values from each individual sample were normalized with the geometric mean of the reference genes. The results were analysed using one-way ANOVA followed by Bonferroni`s multiple comparison tests. A value of $p<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using GraphPad Prism 5 (version 5.00 for Windows, Graphpad Software, San Diego, CA, USA)

**Primer design:** Primers were designed to be intron-spanning to avoid amplification of genomic DNA. Primer3 software (http://frodo.wi.mit.edu) was used to generate primers and genomic and mRNA sequences obtained from NCBI (http://www.ncbi.nlm.nih.gov/Tools). Exon-intron structure was predicted using Spidey (http://www.ncbi.nlm.nih.gov/spidey/). The most optimal primer set was selected according to both the rating obtained with NetPrimer (http://www.premierbiosoft.com/netprimer) and experimental testing. Amplicons were 80-350 bp long. To verify the specificity of the amplification, following a run the product was analysed by 1% EtBr agarose gel electrophoresis. Primers were made by Betina Elfving and the PCR study was supervisored by Betina Elfving.

**Table 3-2: The sequences and lengths of the primers for the genes investigated in this project**

| Gene symbol | Gene name | Primer Sequence | Amplicon size[a] |
|---|---|---|---|
| **18s rRNA** | 18s subunit ribosomal RNA | (+) acggaccagagcgaaagcat<br>(-) tgtcaatcctgtccgtgtcc | 310 |
| **ActB** | Beta-actin | (+) tgtcaccaactgggacgata<br>(-) ggggtgttgaaggtctcaaa | 165 |
| **CycA** | Cyclophilin A | (+) agcactggggagaaaggatt<br>(-) agccactcagtcttggcagt | 248 |
| **Gapd** | Glyceraldehyde-3-phosphate dehydrogenase | (+) tcaccaccatggagaaggc<br>(-) gctaagcagttggtggtgca | 168 |
| **Hmbs** | Hydroxy-methylbilane synthase | (+) tcctggctttaccattggag<br>(-) tgaattccaggtgagggaac | 176 |
| **Hprt1** | Hypoxanthine guanine phosphoribosyl transferase 1 | (+) gcagactttgctttccttgg<br>(-) cgagaggtccttttcaccag | 81 |
| **Rpl13A** | Ribosomal protein L13A | (+) acaagaaaaagcggatggtg<br>(-) ttccggtaatggatctttgc | 167 |
| **BDNF** | Brain derived neurotrophic factor | (+) gaaagtcccggtatcaaaag<br>(-) cgccagccaattctctttttg | 187 |
| **CREB** | cAMP response element-binding | (+) cgtcatctgctcccactgta<br>(-) ccttcgtttttgggaatcag | 194 |
| **VEGFA** | Vascular endothelial growth factor A | (+) aatgatgaagccctggagtg<br>(-) tttcttgcgctttcgttttt | 210 |
| **VEGFR2** | Vascular endothelial growth factor receptor 2 | (+) gacaccgatgtctcctccat<br>(-) gtcactgacagaggcgatga | 75 |
| **NRP1** | Neuropilin 1 | (+) ggagctactgggctgtgaag<br>(-) atgtcgggaactctgattgg | 203 |
| **NRP2** | Neuropilin 2 | (+) acacaaggagccatttccag<br>(-) cggatcctgatgaaacgagt | 200 |
| **MR** | Mineralocorticoid receptor | (+) tgagttccttcccacctgtc<br>(-)aagcctcatctccacacacc | 192 |
| **IL-1a** | Interleukin 1 alpha | (+) aagacaagcctgtgttgctgaagg<br>(-) tcccagaagaaaatgaggtcggtc | 85 |

[a]Amplicon length in base pairs.

### 3.1.7 SERUM BDNF LEVEL MEASUREMENT

The blood was collected and kept at room temperature for between for at least 30 min and were afterwards centrifuged (3000xg, 10 min, 20˚C) whereafter the supernatants were stored at -80˚C.

**ELISA method:** Quantification of plsasma BDNF was performed with ELISA kits (R & D Systems, Switzerland). The standard curves and the samples were run in duplicates. Serum was diluted to 1:15. The standard curves ranged from 22.9 to 733 pg/ml. Briefly, 96-well immunoplates (NUNC, Denmark) were coated with 100 µl/well of the diluted Capture Antibody and incubated overnight at room temperature. Non-specific binding was blocked with Reagent Diluent. Then the samples and standards in duplicates were added to the coated wells (100 µl each) for 2 h at room temperature. The antigen was incubated with mouse anti-human BDNF for 2 h at room temperature and incubated with Streptavidin HRP for 20 min at room temperature. The addition of 3,3'-5,5'-tetramethylbenzidine initiated the colour reaction. The reaction was stopped 20 min later with 2 M $H_2SO_4$ solution and the absorbency was immediately measured at 450 nm on a plate reader (EL 800 Universal Microplate reader, Bio-Tek instruments Inc., USA). According to the manufacturer, the BDNF ELISA kit has <3% cross-reactivity with other related neurotrophic factors. Determination of BDNF levels in serum was performed by Pia Plougmann.

**Data analysis:** A one-way ANOVA followed by a Tukey post-hoc analysis was used to compare the serum BDNF levels between groups, and $p<0.05$ was considered to be statistical significant.

## 3.2 EXPERIMENT 2: LPS-INDUCED MODEL OF DEPRESSION

In this experiment we used 60 male Sprague-Dawley rats (supplied by Taconic MB A/S, Denmark), weighing approximately 250 g at the time of arrival. Rats were housed two per cage and were left undisturbed for six days to allow them to habituate to our laboratory.

### 3.2.1 EXPERIMENTAL DESIGN

This part of the project consisted of 6 groups. Of these, 3 groups received daily injections of LPS whereas the other 3 groups received vehicle. In each of these sub-groups, one group was subjected to exercise (CON-EXE and LPS-EXE), one group received daily imipramine treatment (CON-IMI and LPS-IMI) and one group served as a control (CON and LPS). Rats were randomly assigned to the groups with 10 rats in each group. Table 3-3 summarises the group sizes and weights at the start (T0) of this experiment.

**Table 3-3: The group sizes and weights at the onset of Experiment 2**

| | **CON** | | | **LPS** | | |
|---|---|---|---|---|---|---|
| Group | Vehicle | EXE | IMI | Vehicle | EXE | IMI |
| Size (n) | 10 | 10 | 10 | 10 | 10 | 10 |
| Weight (T0) | 296.5 ± 2.17 | 292.8 ± 6.49 | 289.6 ± 2.31 | 295.7 ± 1.79 | 293.2 ± 2.09 | 294.1 ± 1.52 |

**Exercise paradigm**: Two groups in this experiment were subjected to voluntary exercise from T1 until the day of sacrifice, T56-57 and the same exercise protocol was used as in Experiment 1 of this study and discussed in Section 3.1.1. In this experiment, however, the rats subjected to exercise were housed in pairs as the rest of the groups, in order to avoid the problematic effects of single housing that was observed in Experiment 1, and that has also been shown by others (Lapiz et al., 2003). Therefore, the mean value for running distance for a pair of rats was divided by two to obtain an estimate activity value for each rat per day. Running distance is evaluated from the beginning of the experiment, T0 until the day of the first experiment, T45, the day of the first behavioural test. In this experiment we also calculated the hourly running activity in order to explore the involvement of circadian rhythm in the physical activity of the animals.

**Drug treatment**: Bacterial lipopolysaccharide (LPS, 600 μg/kg/day) (*Escherichia coli* serotype 055:B5) and imipramine (15 mg/kg/day) were purchased from Sigma (St. Louis, MO, USA). Drug doses were based on previous studies (Schiller et al., 1992)(Buhl, 2010 unpublished). Both LPS and imipramine were dissolved in saline and were administered via daily intraperitoneal (i.p.) injections for a total of 56-57 days. All injections were given at approximately the same time of day (13:00 with the exception of testing days, were injections were given two hours after end trial.

The experimental design is illustrated in Figure 3-4. Animals were after T45 subjected to a set of behavioural paradigms, namely the elevated plus maze, open field and forced swim test. In order to determine metabolic parameters an oral glucose tolerance test was performed at T49-T51 and fasting blood glucose was measured on T56-T57 at least 2 h prior sacrifice.

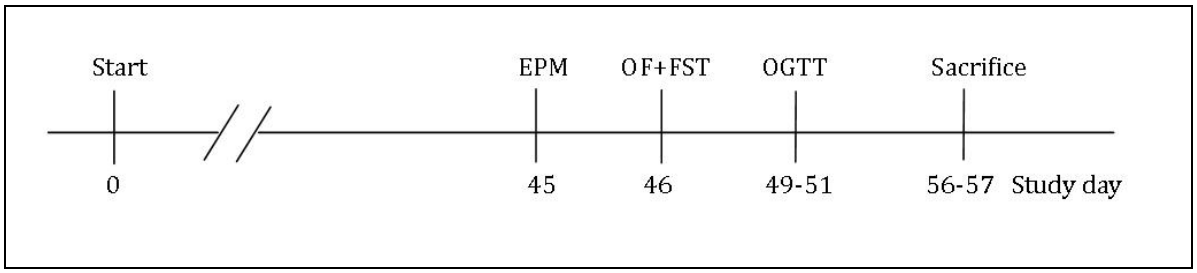


**Figure 3-4: The experimental design of Experiment 2, showing the different behavioural models used in the study**. EPM: elevated plus maze, OF: open field, FST: forced swim test, OGTT: oral glucose tolerance test.

### 3.2.2 METABOLIC PARAMETERS

We also examined the effects of LPS, exercise and imipramine on metabolic parameters in this putative immune model of depression. Specifically, we evaluated fasting blood glucose on T56 and glucose tolerance by conducting an OGTT on T49-T51. The test was carried out using the same protocol as discussed in Section 3.2.2. However, an additional blood sample time-point was inserted in this experiment, namely 15 minutes following the ingestion of glucose. Additional fasting and OGTT blood samples were also collected in heparin-coated tubes for the determination of insulin levels in these rats, but these measurements have not been carried out as yet.

### 3.2.3 BEHAVIOURAL TESTING

In this part of the study, animals were subjected to a similar set of screening tools as was used in Experiment 1. Specifically, to investigate the effects of LPS, exercise and imipramine on depression- and anxiety-like behaviour, the FST and EPM was used, respectively, and we also assessed locomotor activity in an open field arena. The same protocols described in Section 3.1.3 were used.

### 3.2.4 TISSUE EXTRACTION

After being fasted overnight, the animals were sacrificed by decapitation on T56. Trunk blood was collected directly afterwards in anticoagulant-free tubes with gel (Terumo, Venosafe™, VF-054SAS). Trunk blood was collected for the measurement of LPS and imipramine levels. This has yet to be performed. The brain was quickly removed and left and right frontal cortex, left and right hippocampus and hypothalamus were quickly isolated on a cold tile. The tissue was frozen on powdered dry-ice and stored at -80° C. The liver as well as epididymal fat pads were dissected out and weighed, whereafter it was frozen in liquid nitrogen. In addition, musculus quadriceps were removed from the left side of the animals and also frozen in liquid nitrogen. Tissue was stored at -80° C. Analysis from tissue has yet to be performed.

### 3.2.5 DATA ANALYSIS

All values are expressed as mean ± SEM and an alpha level (p-value) of 0.05 or lower was used as the threshold for statistical significance. Normality in a data set was assumed when two or more normality tests (KS normality test, D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test) were passed with equal variances. Area under the curve (AUC) was calculated from the data for weight gain, food consumption and running distance. Two way analyses of variance (ANOVA) was used to evaluate the two main factors in this experiment, namely LPS administration and treatment, and were followed by a Bonferronni's multiple comparison test. A Student t-test was used to compare the running data of the two groups that were subjected to exercise.

A value was defined as an outlier and excluded from the analysis when it deviated more than 3 times the standard deviation (SD) of that dataset or if an animal's behaviour was estimated to be unusual. All statistical analyses were performed using GraphPad Prism 5 (version 5.00 for Windows, Graphpad Software, San Diego, CA, USA), and Microsoft Office Excel 2007 (for Windows).

# 4 RESULTS

This results section will be divided into two parts, according to the two different experiments in this study.

## 4.1 EXPERIMENT 1: THE FSL/FRL RAT MODEL

### 4.1.1 METABOLIC MARKERS

#### 4.1.1.1 WEIGHT GAIN

There were no significant differences in weight between the groups at the start of the experiment ($F_{4,36}$=1.64, p=0.36) (see Table 3-1). An analysis of the weight gain during the 5 weeks of the experiment revealed a strain difference by showing that FRL control rats gained significantly more weight than the FSL controls ($F_{4,32}$=4.45, p<0.05) (see Figure 4-1). However, none of the treatment conditions had a significant effect on weight gain in FSL rats.

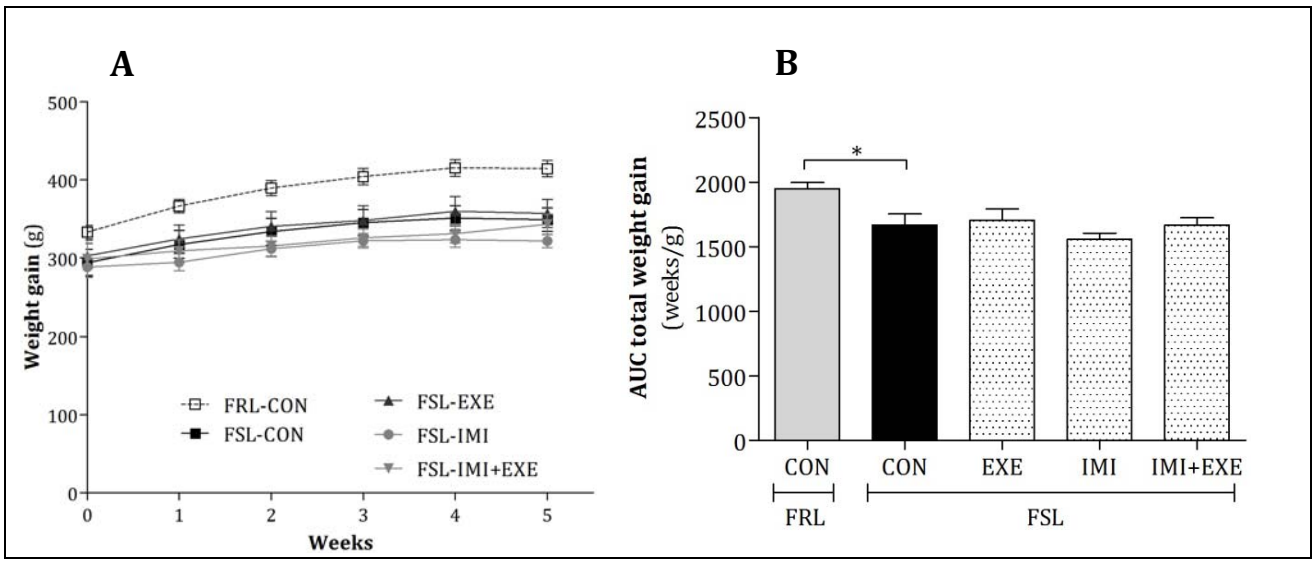


**Figure 4-1: The effect of strain, imipramine and/or exercise treatment on weight gain, plotted as (A) the weekly mean of daily weight gain from T0 to T42 (week 0 to 5), and as (B) the area under the curve (AUC) as calculated from (A) from T0 to T42 (week 0 to 5)**. The number of rats in each group are as follows: FRL-CON(8), FSL-CON(8), FSL-EXE(8), FSL-IMI(7), FSL-IMI+EXE(6). *p<0.05. Values are shown as the mean ± SEM. Abbreviations used: FRL (Flinders resistant Line), FSL (Flinders sensitive line), CON (control), EXE (exercise), IMI (imipramine).

#### 4.1.1.2 FOOD CONSUMPTION AND DRUG DOSAGE CALCULATION

An analysis of the overall food consumption revealed a strain difference by showing that FRL rats have an increased food consumption compared to FSL rats ($F_{4,36}$=12.64, p<0.0001).

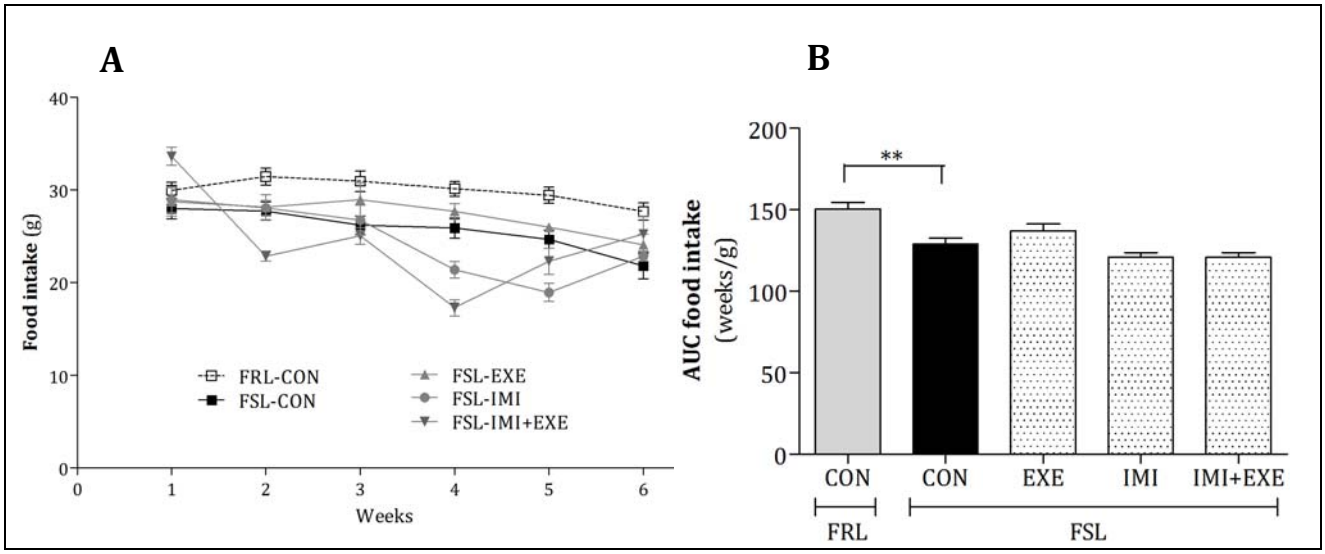


**Figure 4-2: The effect of strain, imipramine and/or exercise treatment on food consumption, plotted as (A) the weekly mean of daily food intake from T0 to T42 (week 1 to 6) and (B) the area under the curve (AUC) as calculated from (A) from T0 to T42 (week 1 to 6).** The number of rats in each group are as follows: FRL-CON(8), FSL-CON(8), FSL-EXE(8), FSL-IMI(7), FSL-IMI+EXE(6). **p<0.01. Values are shown as the mean ± SEM. Abbreviations used: FRL (Flinders resistant Line), FSL (Flinders sensitive line), CON (control), EXE (exercise), IMI (imipramine).

The dose of imipramine received by the rats was also calculated from the amount of food consumed (see Figure 4-3). The dose ranged from 9.99 mg/kg to 22.30 mg/kg in the imipramine group, and 9.03 mg/kg to 20.44 mg/kg for the imipramine plus exercise group. The exact dose was not measured because of technical reasons.

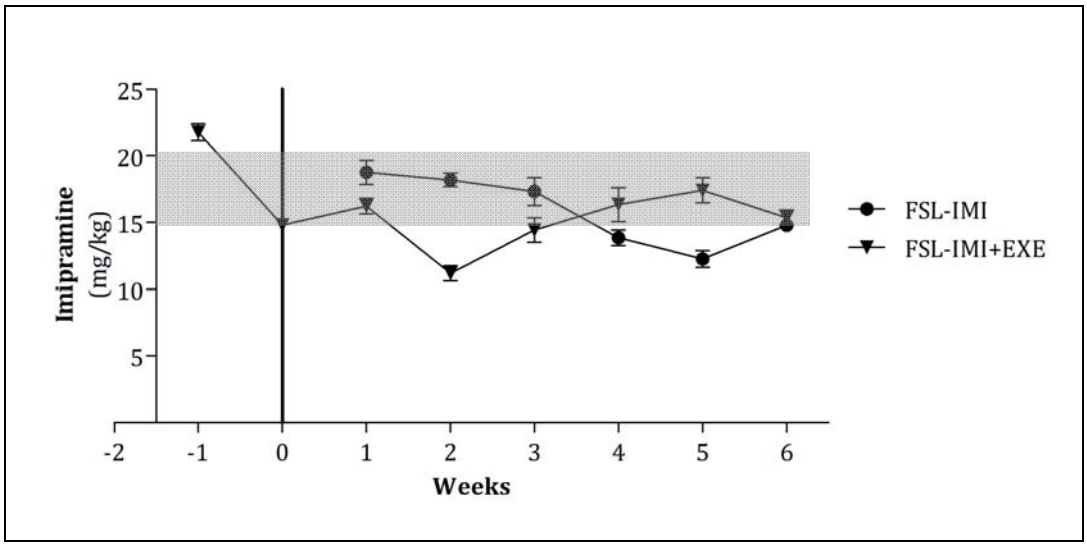


**Figure 4-3**: **The weekly means of the imipramine dose received as calculated from the food intake, and plotted from T(-)14 to T42.** The graded area shows the intended dose range, namely 15–20 mg/kg. Values are shown as the mean ± SEM. Abbreviations used: FSL (Flinders sensitive line), CON (control), EXE (exercise), IMI (imipramine).

### 4.1.2 RUNNING BEHAVIOUR

The running activity, in terms of distance run (in meter) per day is shown in Figure 4-4A. The FSL rats treated with exercise alone ran on average 157.2 m per day, whereas the FSL rats treated with imipramine plus exercise rats had an average running distance of 3642.5 m per day. When evaluating the AUC for the distance run during the first 4 weeks, we found that FSL rats treated with imipramine displayed a significant increase in running distance (p=0.015) compared to rats that received exercise alone.

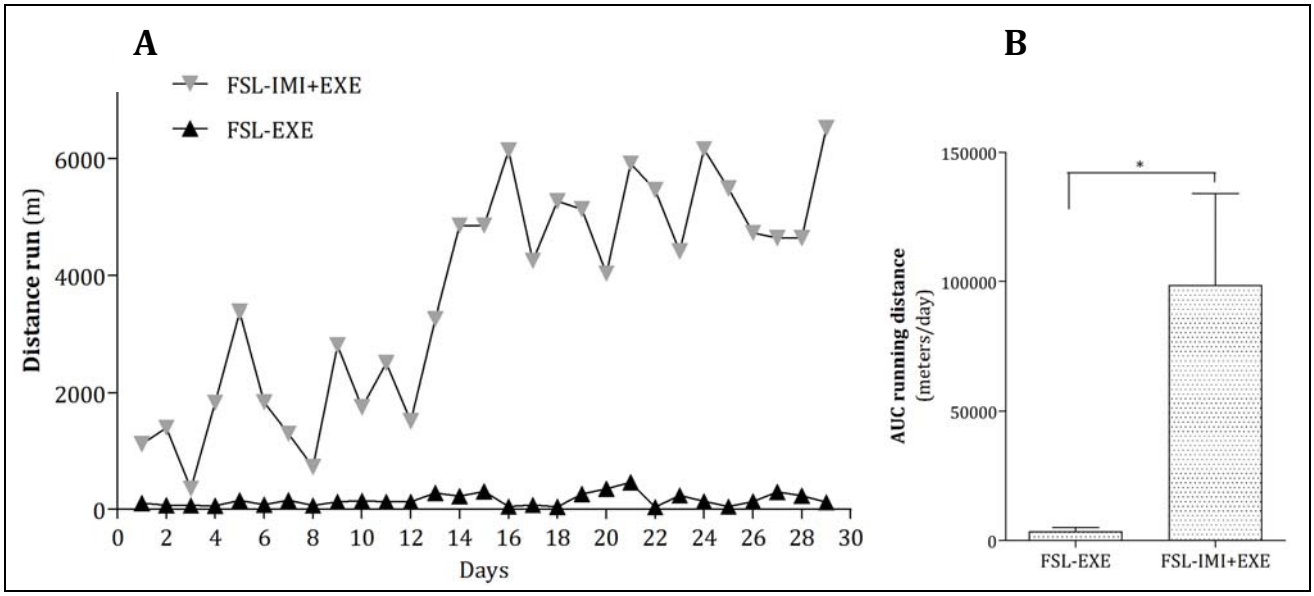


**Figure 4-4: Mean running distance of FSL rats allowed voluntary access to running plotted as (A) daily run from T0 to T29 wheels and (B) area under the curve (AUC) calculated from (A)**. The number of rats in each group are as follows: FSL-EXE(7), FSL-IMI+EXE(6). *p<0.05. One animal from the FSL-EXE group was excluded as an outlier. Abbreviations used: FSL (Flinders Sensitive Line), EXE (exercise), IMI (imipramine).

By doing an hour-by-hour analysis of the running behaviour in weeks 1 and 4 (see Figure 4-5) we found that both groups displayed similar running patterns, with most of their activity taking place in the dark phase (FSL-EXE: week 1, p=0.03 and week 4, p=0.0002. FSL-IMI+EXE: week 1, p=0.0002, week 4, p=0.0003).

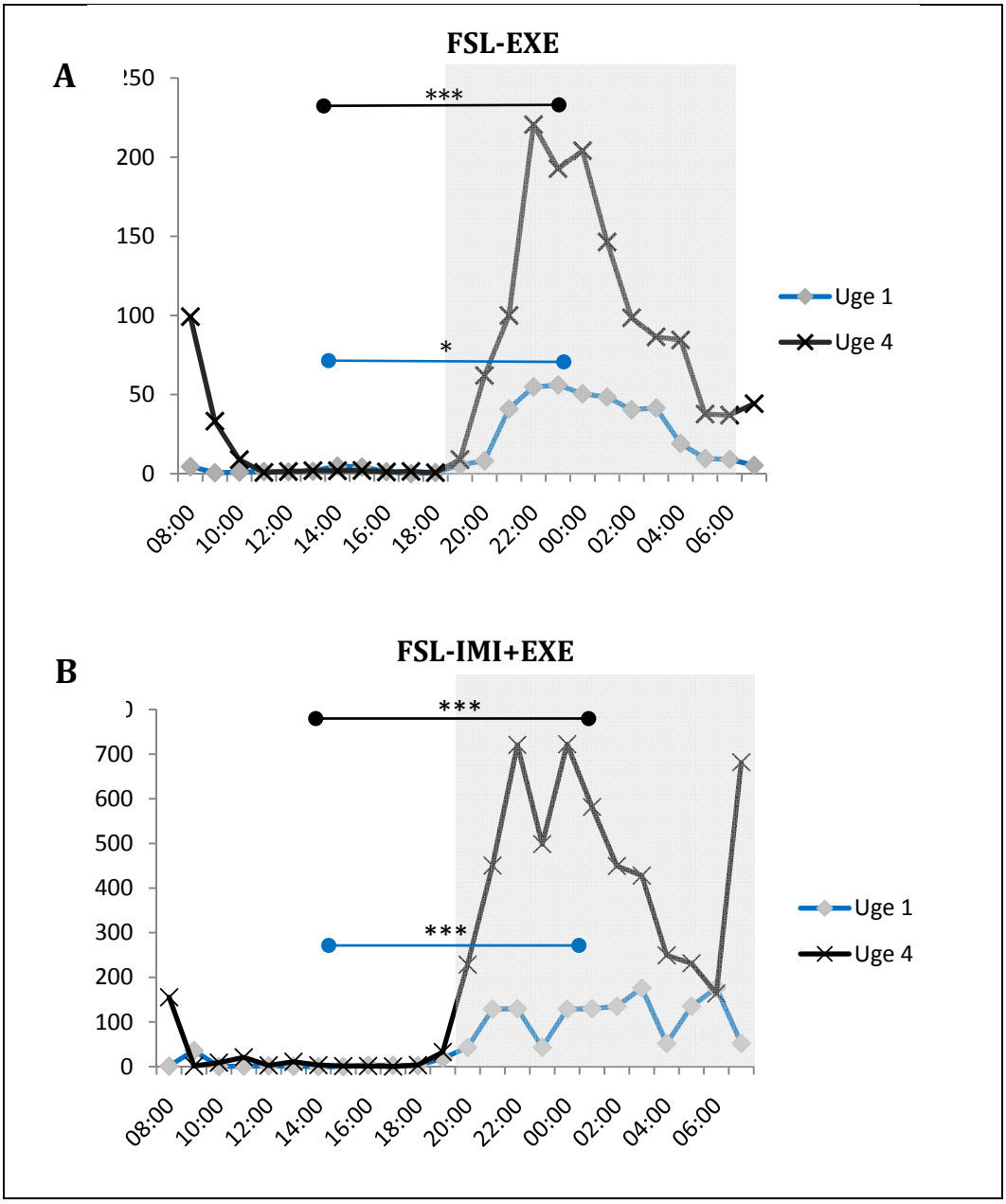


**Figure 4-5 Hour-by-hour evaluations of the mean running distances in week 1 and 4 for the two groups that were subjected to exercise, namely FSL-EXE (A) and FSL-IMI+EXE (B).** Both groups had most running activity during the dark cycle. The number of rats in each group are as follows: FSL-EXE (7), FSL-IMI+EXE (6). *p<0.05, ***p<0.01. Values represent the weekly mean average distance per hour in each group. Abbreviations used: FSL (Flinders sensitive line), CON (control), EXE (exercise), IMI (imipramine).

### 4.1.3 METABOLIC PARAMETERS

#### 4.1.3.1 FASTING BLOOD GLUCOSE LEVELS AND THE OGTT

Fasting plasma glucose was measured on study days T24 and T25 (see Figure 4-6A). Although we did not find any significant strain differences regarding this parameter, the FSL rats treated with imipramine plus exercise displayed a significantly lower fasting plasma glucose relative to the FSL controls ($F_{4,36}$=3.87, p=0.011).

An evaluation between single- and pair-housed rats revealed that fasting glucose was increased in single-housed rats ($F_{1,28}$=4.77, p=0.038) (see Figure 4-6B).

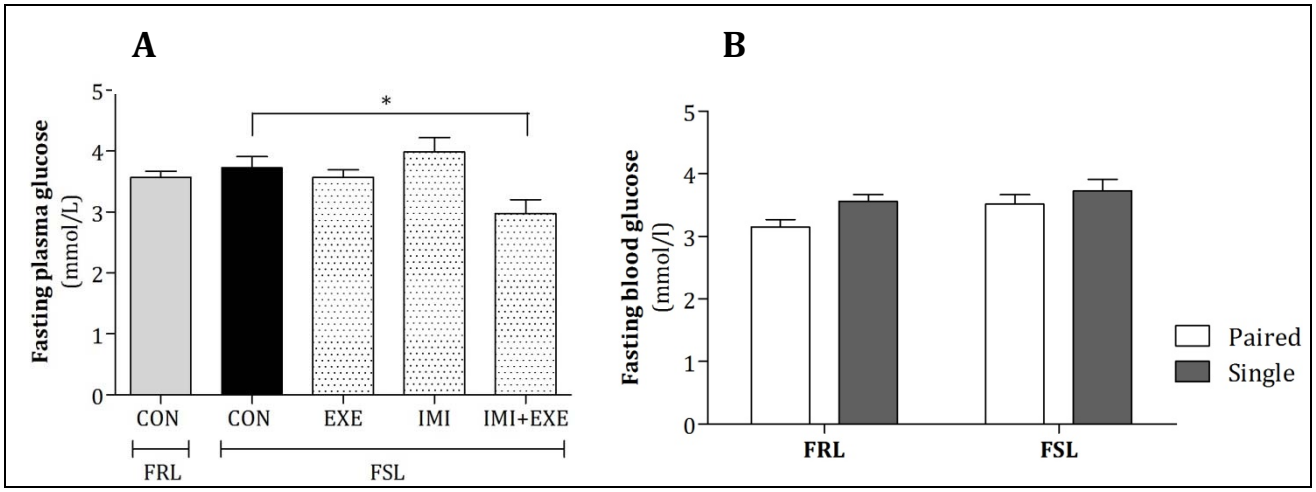


**Figure 4-6: The effects of (A) strain, imipramine and/or exercise treatment and (B) single or paired housing conditions on fasting plasma glucose levels in FSL and FRL rats as measured on T24+T25**. The numbers of rats in each group are as follows: FRL-CON(8), FSL-CON(8), FSL-EXE(8), FSL-IMI(7), FSL-IMI+EXE(6). *p<0.05. Values are shown as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line), CON (control), EXE (exercise), IMI (imipramine).

After performing the OGTT on T24-T25, the AUC was calculated for the graph of glucose level plotted against the time following glucose administration (data shown in Table 8-1). A significantly lower AUC ($F_{4,36}$=4.52, p=0.0052) was found for FRL rats compared to FSL rats treated with imipramine (p<0.05) or imipramine plus exercise (p<0.01).

### 4.1.4 BEHAVIOURAL ASSESSMENT

Prior to the FST, we also measured locomotor activity in the OF. No differences were observed between the groups ($F_{4,36}$=2.46, p= 0.065) was assessed in the open field. No differences were found between groups (data shown in Table 8-2).

#### 4.1.4.1.1 THE FORCED SWIM TEST

In the first FST, which was carried out on T(-)7, we found a significant strain difference between the FRL and FSL animals (see Figure 4-7). There were no large variances between the different FSL groups at the onset of the experiment. However, in the second FST, which was carried out on T32-34, the higher immobility displayed by the control FSL rats compared to the FRL strain was no longer significant.

After 6 weeks of treatment, rats receiving imipramine plus exercise showed a significantly reduced immobility relative to FSL controls ($p<0.01$) (see Figure 4-7), while no significant differences were found for rats treated with either imipramine or exercise alone. Furthermore, rats treated with imipramine plus exercise displayed significantly lower immobility compared to animals treated with imipramine alone ($p<0.05$).

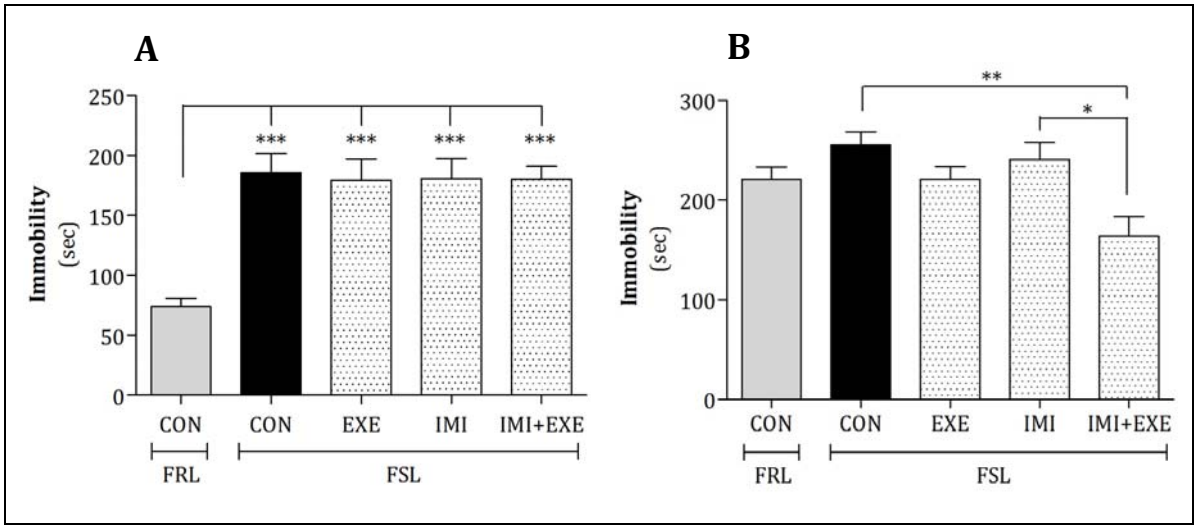


**Figure 4-7**: **The effects of strain, imipramine and/or exercise treatment on immobility in FSL and FRL rats during an FST on study days (A) T(-)7 and (B) T32-34.** The number of rats in each group are as follows: FRL-CON(8), FSL-CON(8), FSL-EXE(8), FSL-IMI(A:8, B:7), FSL-IMI+EXE(6)**.** *p<0.05, **p<0.01. ***p<0.001 Values are shown as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line), CON (control), EXE (exercise), IMI (imipramine).

After considering the somewhat surprising finding that the FRL/FSL strain difference in immobility was no longer evident at T32-34, we included additional FRL and FSL control groups that were housed in pairs to evaluate the housing effect on immobility (see Figure 8). As expected, FSL rats displayed a significantly higher immobility than FRL rats ($p>0.05$) when both of these groups were housed in pairs. However, single housing conditions for the same time period, increased immobility in FRL rats by 22%, whereas immobility in FSL rats were increased only by 6% compared to the respective groups housed in pairs.

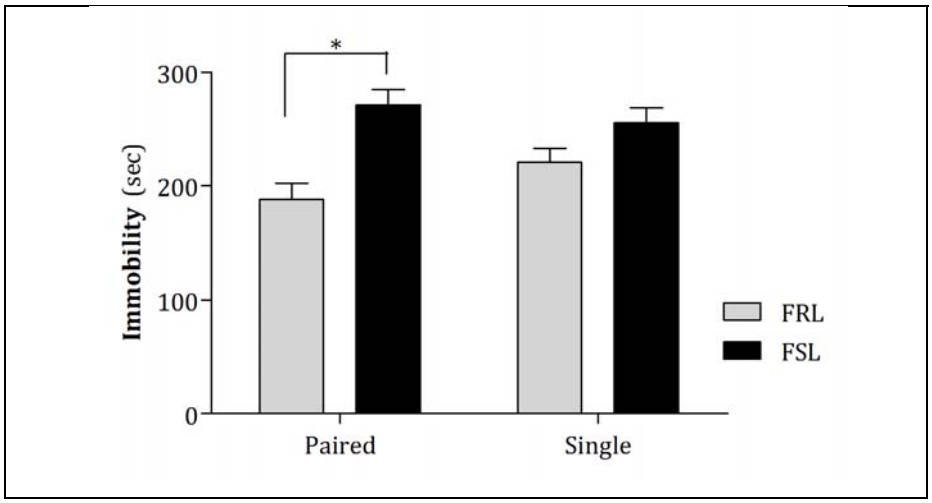


**Figure 4-8: The effect of single- or paired housing conditions on immobility in FSL and FRL rats during an FST on T32-34.** The singly housed FSL and FRL groups are also shown in **Figure 4-7**. n=8 for all groups. *p<0.05. Values are given as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line)

#### 4.1.4.1.2 THE ELEVATED PLUS-MAZE

We did not find any significant strain difference in anxiety-like behaviour in the EPM, nor did we observe any significant treatment effects (see Figure 4-9A+B). The parameters evaluated in the EPM were percentage of time spent in open arms ($F_{4,36}$=1.52, p= 0.22), percentage of open arm entries ($F_{4,36}$=1.21, p=0.33) (data not shown) and full entries ($F_{4,36}$=2.23, p=0.088).

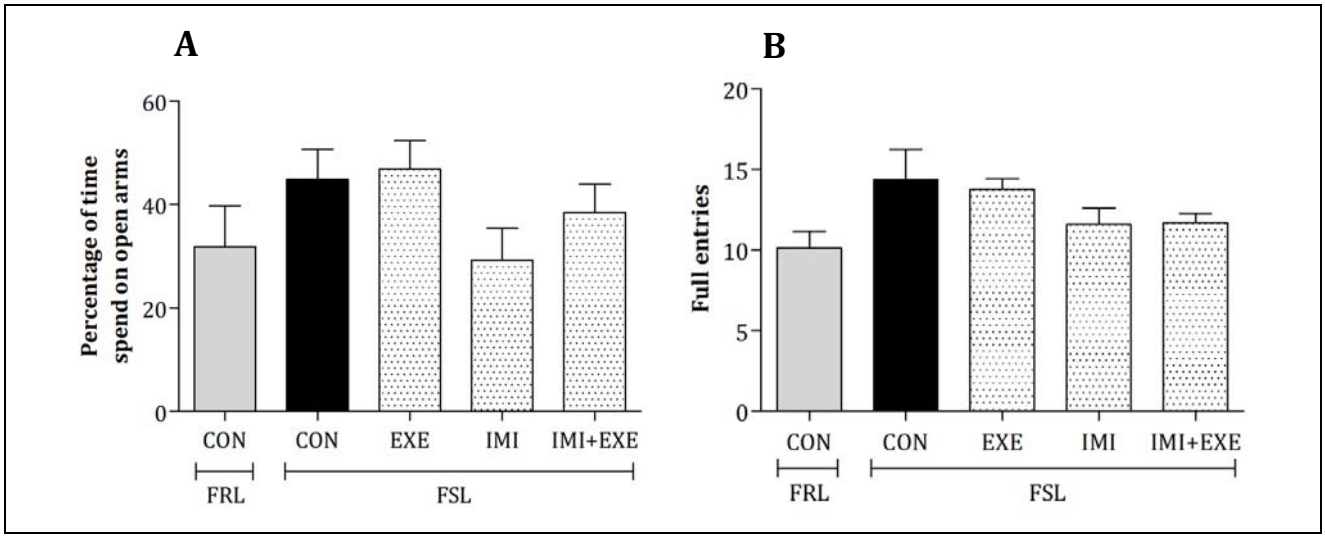


**Figure 4-9: The effects of strain, imipramine and/or exercise treatment on anxiolytic parameters in FRL and FSL rats in the EPM on T28-T29, namely (A) percentage of time spent in open arms, and (B) full entries.** The number of rats in each group are as follows: FRL-CON(8), FSL-CON(8), FSL-EXE(8), FSL-IMI(7), FSL-IMI+EXE(6). Values are shown as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line), CON (control), EXE (exercise), IMI (imipramine).

#### 4.1.4.1.3 THE OBJECT RECOGNITION TASK

One half of the animals in this test were tested twice, due to technical problems during experimental setup. Animal behaviour was investigated and dividing groups did not reveal any difference in exploration time.

In the learning phase (second phase), we did not find any significant strain difference or any treatment effect in the total exploration time ($F_{4,36}$=0.77, p=0.54) (mean for all groups = 16.07 ± 1.2 sec). All groups also spent approximately the same amount of time exploring each object, with a mean object 1:object 2 exploration ratio of 1.08 ± 0.09 ($F_{4,36}$=1.16, p=0.35).

In the recognition phase (third phase), we also did not observe a significant strain difference or any treatment effects on the total exploration time ($F_{4,33}$=1.03, p=0.41) (10.41 ± 1.22 seconds for all groups). Assessing novel object recognition time relative to the first 5 sec, did not reveal any difference between the strain nor an effect of treatment ($F_{4,23}$=0.96, p= 0.45, see Figure 4-10). The novel object:known object exploration time ratio was also not significantly different between the strains or between the different treatment conditions. ($F_{4,28}$=1.87, p=0.15).

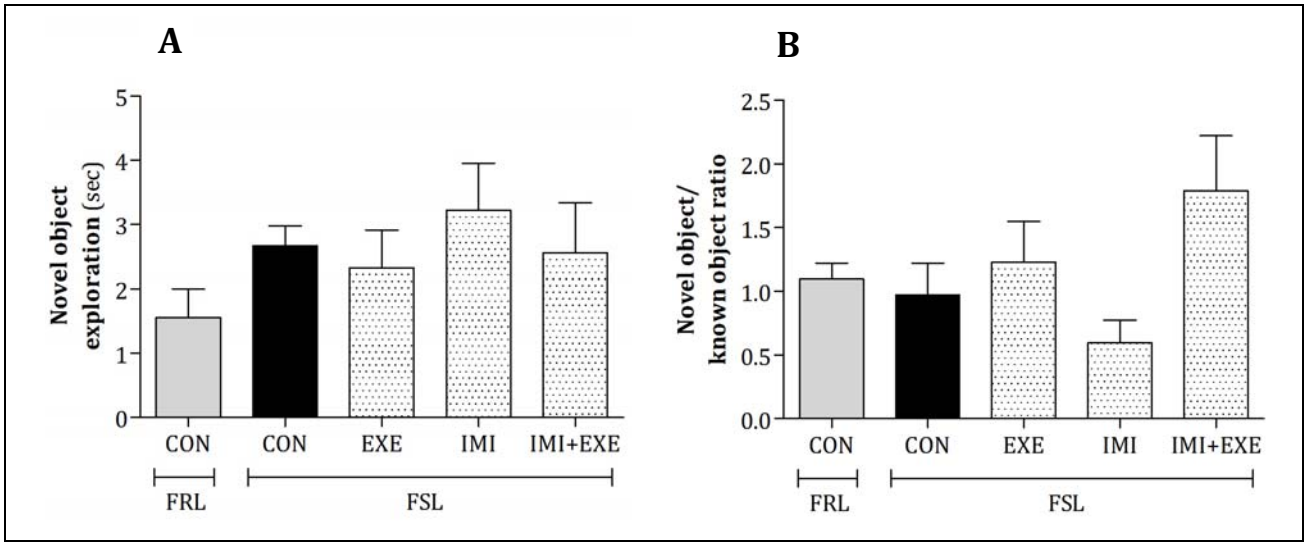


**Figure 4-10: The effects of strain, imipramine and/or exercise treatment on parameters of declarative memory in FSL and FRL rats in the novel object recognition task on T29-T32, namely (A) novel object exploration relative to the first five seconds, and (B) the novel object:known object exploration time ratio.** The number of rats in each group are as follows: FRL-CON(A:4, B:7), FSL-CON(A:6, B:7), FSL-EXE(A:6, B:7), FSL-IMI(4), FSL-IMI+EXE(4). Values are shown as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line), CON (control), EXE (exercise), IMI (imipramine).

### 4.1.5 RT-QPCR

There were no significant differences between the groups in the mRNA expression levels of BDNF ($F_{4,33}$=0.59, p=0.67) VEGFA ($F_{4,36}$=0.28, p=0.89), VEGFR2 ($F_{4,36}$=1.07, p=0.39), NRP1 ($F_{4,36}$=0.58, p=0.68), NRP2 ($F_{4,36}$=0.64, p=0.64), CREB ($F_{4,35}$=0.29, p=0.88) and MR ($F_{4,36}$=0.69, p=0.61), (see Figure 4-11A-G).

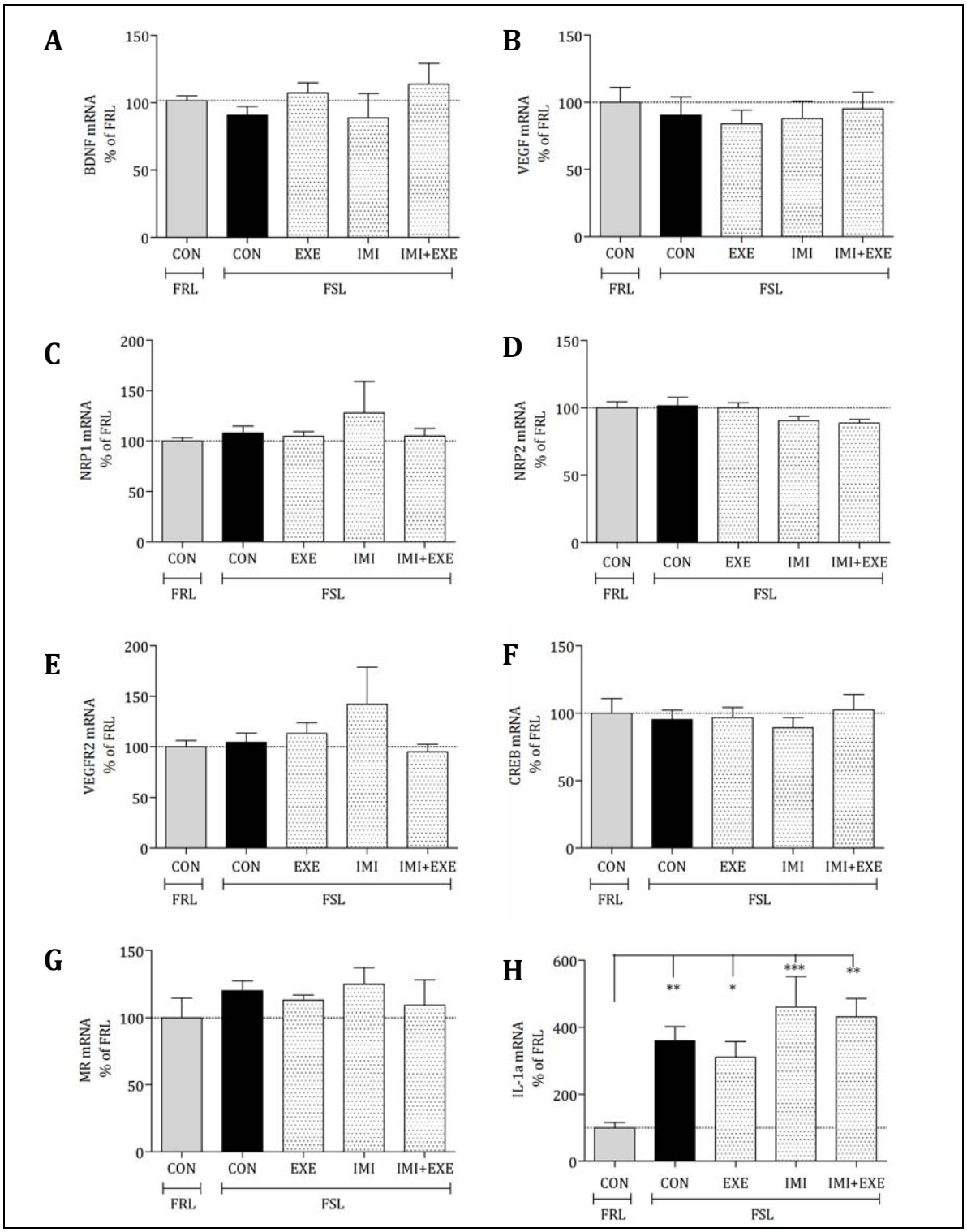


Figure 4-11: **The effects of strain, imipramine and/or exercise treatment on mRNA levels of (A) BDNF, (B) VEGFA, (C) NRP1, (D) NRP2 (E) VEGFR2 (F) CREB, (G) MR,(H) IL-1 in hippocampus of FSL and FRL rats.** The number of rats in each group are as follows: FRL-CON(7), FSL-CON(7), FSL-EXE(8), FSL-IMI(7), FSL-IMI+EXE(6)**.** *p<0.05, **p<0.01, ***p<0.001.Values are shown as the percentage of FRL control and as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line), CON (control), EXE (exercise), IMI (imipramine).

However, when analysing the hippocampal mRNA level of IL-1α, a significant increase was observed in FSL compared to FRL rats ($F_{4,32}$=7.29, p=0.0003) (see Figure 4-11H). Due to technical difficulties, GR could not be determined.

Furthermore, a significantly lower BDNF mRNA level was observed in FSL compared to FRL control rats when they were housed in pairs ($F_{1,28}$=6.72, p=0.015), although no significant effect for housing condition was found ($F_{1,28}$=0.002, p=0.97) (see Figure 4-12).

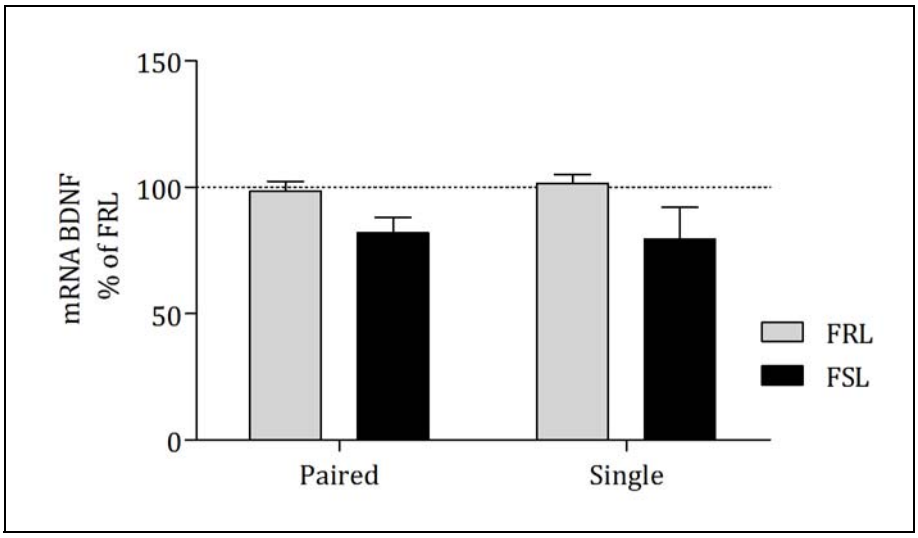


**Figure 4-12: The effects of housing conditions on BDNF mRNA levels in hippocampus of FSL and FRL rats**. The number of rats in each group are as follows: FRL-CON(7), FSL-CON(7), FSL-EXE(8), FSL-IMI(7), FSL-IMI+EXE(6)**.** Values are shown as the percentage of FRL control and as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line), CON (control), EXE (exercise), IMI (imipramine).

### 4.1.6 SERUM BDNF LEVELS

A significant strain difference was found ($F_{4,32}$=7.99, p= 0.0001), with FSL control rats displaying significantly higher plasma BDNF levels relative to FRL rats (p<0.01) (see Figure 4-13).

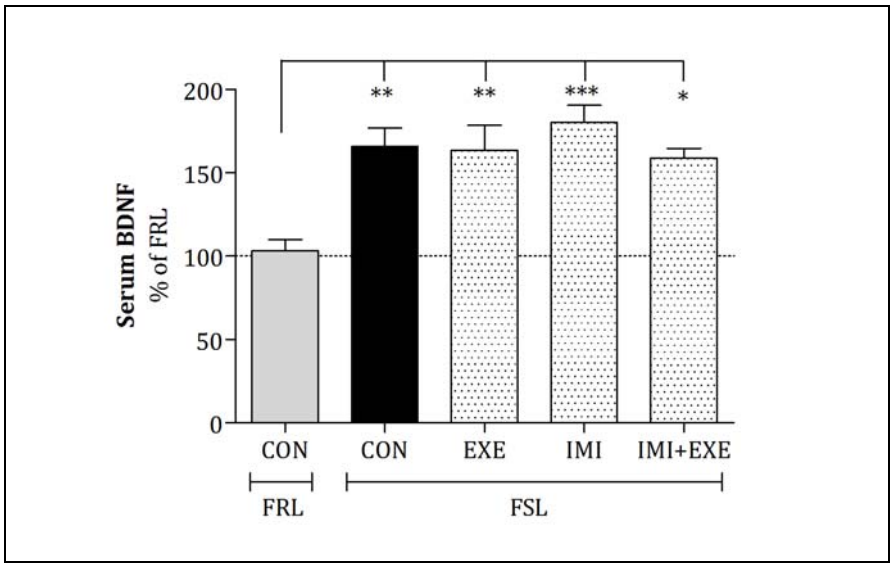


**Figure 4-13: The effect of strain, imipramine and/or exercise treatment on serum BDNF levels in FSL and FRL rats measured after decapitation on T39.** The number of rats in each group are as follows: FRL-CON(8), FSL-CON(8), FSL-EXE(8), FSL-IMI(7), FSL-IMI+EXE(6). *p<0.05, **p<0.01, ***p<0.001. Values are shown as the percentage of FRL control and as the mean ± SEM. Abbreviations used: FRL (Flinders Resistant Line), FSL (Flinders Sensitive Line), CON (control), EXE (exercise), IMI (imipramine).

## 4.2 EXPERIMENT 2: LPS-INDUCED MODEL OF DEPRESSION

In this section, the results from the low-grade inflammation model will be presented.

### 4.2.1 METABOLIC MARKERS

#### 4.2.1.1 WEIGHT GAIN

There were no significant differences in body weight between the groups at the start of the experiment ($F_{1,54}$=0.27, p=0.61). An analysis of the weight gain during the 7 weeks of the experiment revealed a significant lower overall weight gain in the groups treated with LPS ($F_{1,53}$=27.22, p<0.0001) (see Figure 4-14). Furthermore, there were also several significant effects ($F_{2,53}$= 63.20, p<0.0001). Specifically, LPS-treated rats that received exercise (p<0.05) or imipramine (p<0.001) treatment showed a further reduced weight gain compared to rats that received LPS only. This pattern was also evident in the control rats, with a significant reduction in weight gain induced by exercise (p<0.001) and imipramine (p<0.001). Lastly, there was a trend toward an interaction between group and treatment ($F_{2,53}$=2.72, p=0.075).

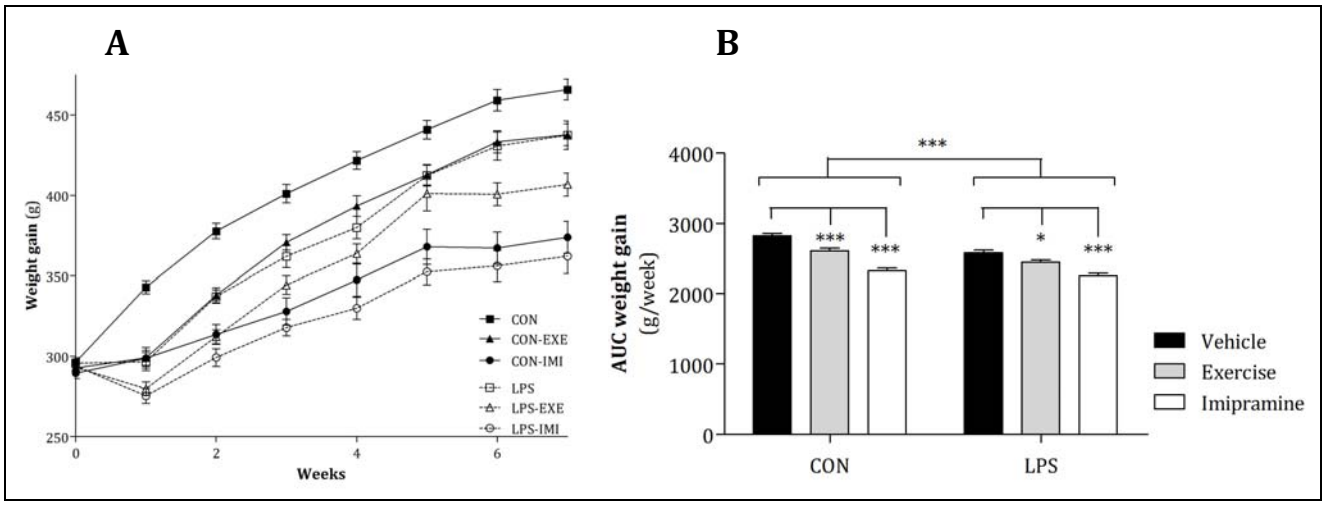


**Figure 4-14: The effect of LPS, imipramine and/or exercise treatment on weight gain in the Sprague-Dawley rats, plotted as (A) the weekly mean of daily weight gain from T0 to T42 (week 0 to 7), and as (B) the area under the curve (AUC) as calculated from (A) from T0 to T42 (week 0 to 7).** The numbers of rats in each group are as follows: CON (10), CON-EXE (10), CON-IMI (9), LPS (10), LPS-EXE (10), LPS-IMI (10). *p<0.05, ***p<0.001. Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide), EXE (exercise), IMI (imipramine).

### 4.2.1.2 FOOD CONSUMPTION

An analysis of overall food consumption revealed that animals treated with LPS had a lower food intake compared to control rats ($F_{1,54}$=15.17, p=0.0003) (see Figure 4-15). In addition, treatment significant affected food consumption ($F_{2,54}$=106.7, p<0.0001). There was no interaction between group and treatment ($F_{2,54}$=2.00, p=0.15).

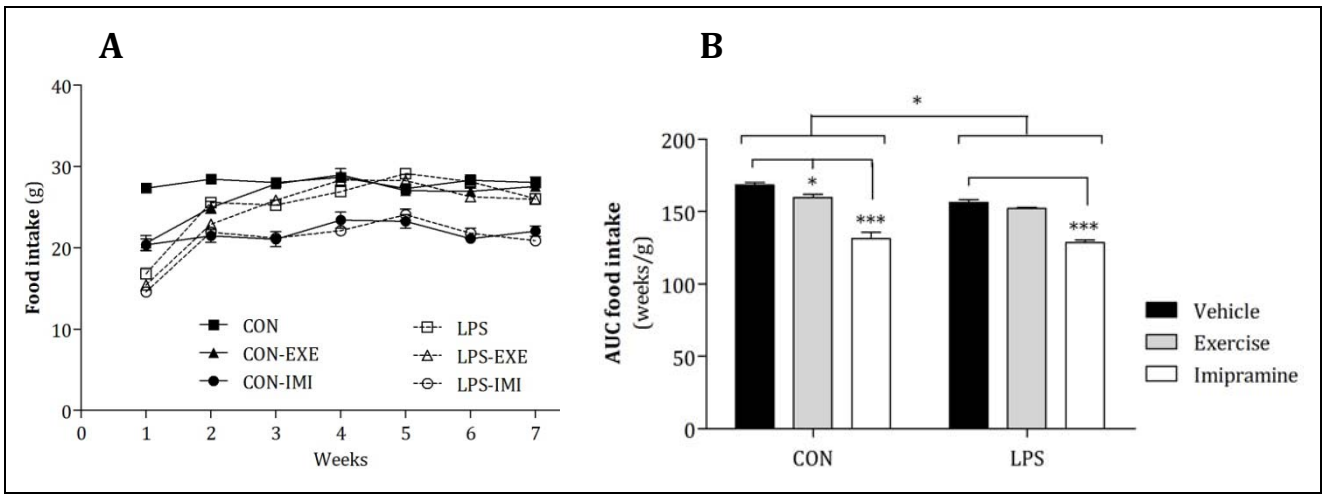


**Figure 4-15**: **The effect of LPS, imipramine and/or exercise treatment on food consumption in Sprague-Dawley rats, plotted as (A) the weekly mean of daily food intake from T0 to T42 (week 1 to 7) and (B) the area under the curve (AUC) as calculated from the (A) from T0 to T42 (week 1 to 7).** The numbers of rats in each group are as follows: CON (10), CON-EXE (10), CON-IMI (9), LPS (10), LPS-EXE (10), LPS-IMI (9). *p<0.05, ***p<0.001. Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide), EXE (exercise), IMI (imipramine).

#### 4.2.1.3 FASTING BLOOD GLUCOSE LEVELS AND THE OGTT

We found a significant increase in fasting blood glucose levels in LPS-treated animals ($F_{1,52}$=20.32, p=0.0001), as well as a combined significant effect of treatment ($F_{2,52}$=7.23, p=0.001) but not for either treatment alone. No interaction between group and treatment was found ($F_{2,52}$=1.03, p=0.37).

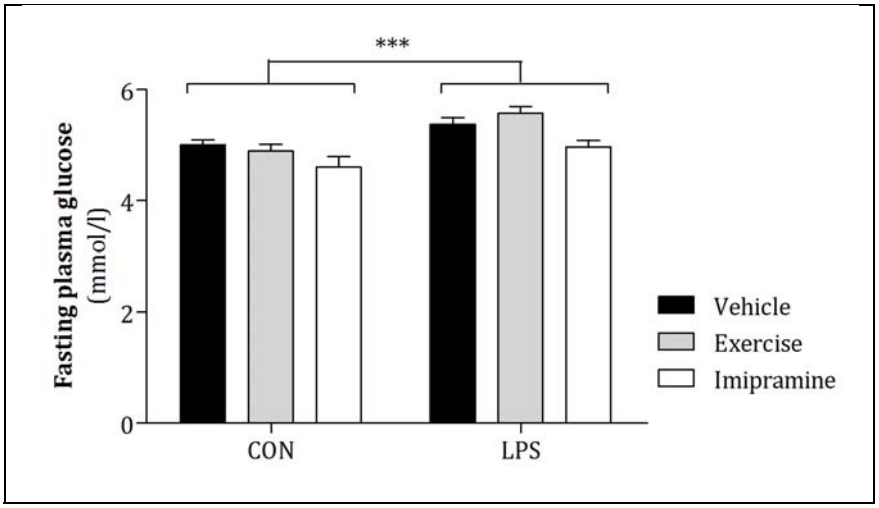


**Figure 4-16: The effect of LPS, imipramine and/or exercise treatment on fasting plasma glucose levels in Sprague-Dawley rats as measured on T56-57**. The numbers of rats in each group are as follows: CON (10), CON-EXE (10), CON-IMI (9), LPS (10), LPS-EXE (10), LPS-IMI (9). ***p<0.001.Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide).

Results from the OGTT showed that blood glucose levels peaked 15 min after glucose administration, after which it slowly decreased (see Figure 4-17A). There was a strong tendency for LPS-treated animals to have an increased glucose response as indicated in the AUC, relative to control animals ($F_{1,53}$=3.69, p=0.060) (see Figure 4-17B). Furthermore, we found a significant effect of treatment ($F_{2,53}$=4.742, p=0.013), in which exercise increased AUC in LPS-treated rats (p<0.001). An interaction between group and treatment was also observed for this parameter ($F_{2,53}$=3.42, p=0.040).

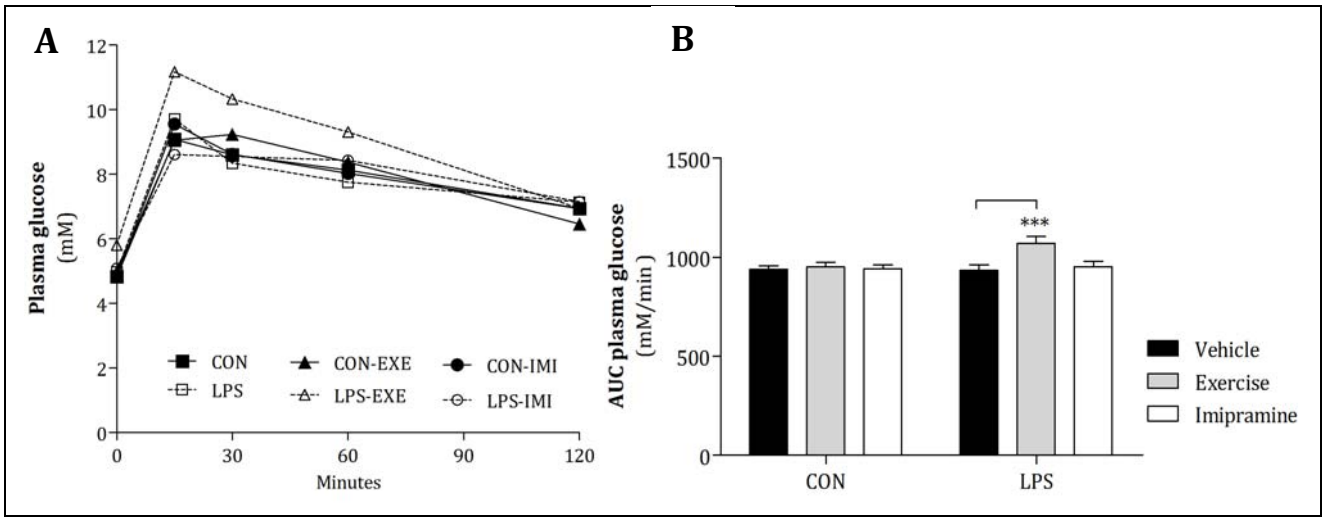


**Figure 4-17: The effect of LPS, imipramine and/or exercise treatment on plasma glucose levels in Sprague-Dawley rats during the oral glucose tolerance test on T49-51, and shown as (A) the time-dependent glucose level changes up to 120 min following glucose administration, and (B) the AUC as calculated from (A)**. The numbers of rats in each group are as follows: CON (10), CON-EXE (10), CON-IMI (9), LPS (10), LPS-EXE (10), LPS-IMI (9). Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide), EXE (exercise), IMI (imipramine).

### 4.2.2 RUNNING BEHAVIOUR

Running behaviour was assessed in both LPS-treated and control rats, see Figure 4-18. Control rats ran on average 730.2 meters per day, whereas LPS-injected rats ran on average 593.3 meters daily. No difference was found in overall running distance between the groups (p=0.47).

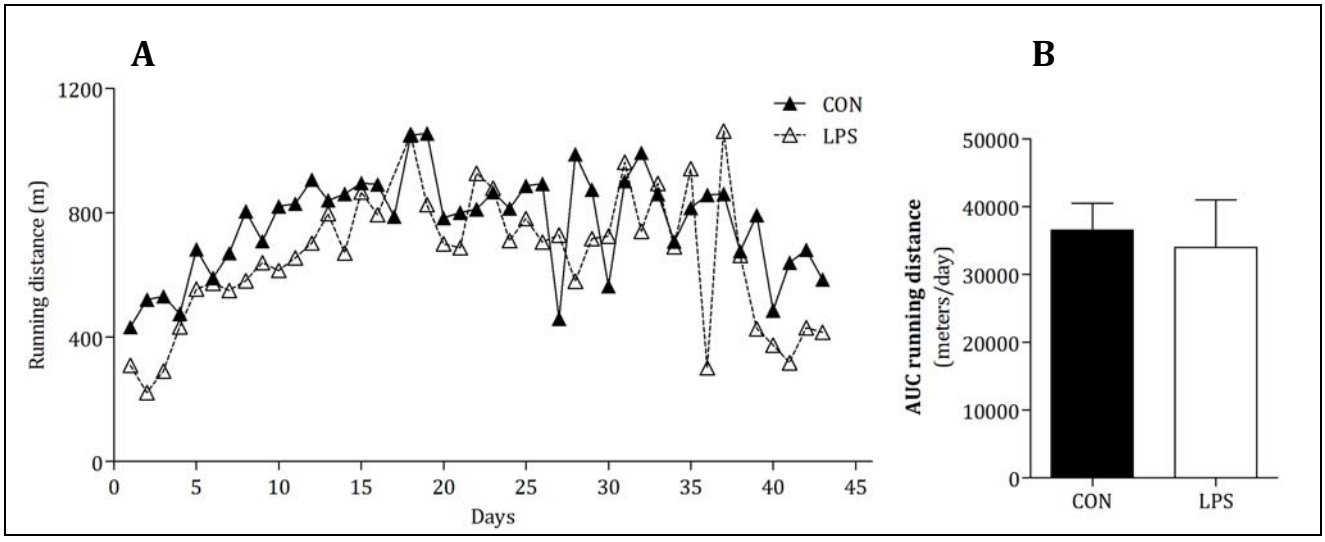


**Figure 4-18: The running distance for control versus LPS-treated rats, shown as (A) the average distance run from T0-43 and (B) the AUC calculated from (A).** The numbers of animals in each group were 10 in both groups from T0-T12, while 2 rats were excluded from T12-T43 due to a defective running detector in one cage. Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide).

By doing an hour-by-hour analysis of the running behaviour in weeks 1 and 4 (see Figure 4-19) we found that the control and LPS-treated rats displayed similar running patterns, with most of their activity taking place in the dark phase. Light vs. dark phase: CON-EXE (week 1: p=0.019, week 4, p<0.0001), LPS-EXE (week 1: p=0.012, week 4: p<0.0001).

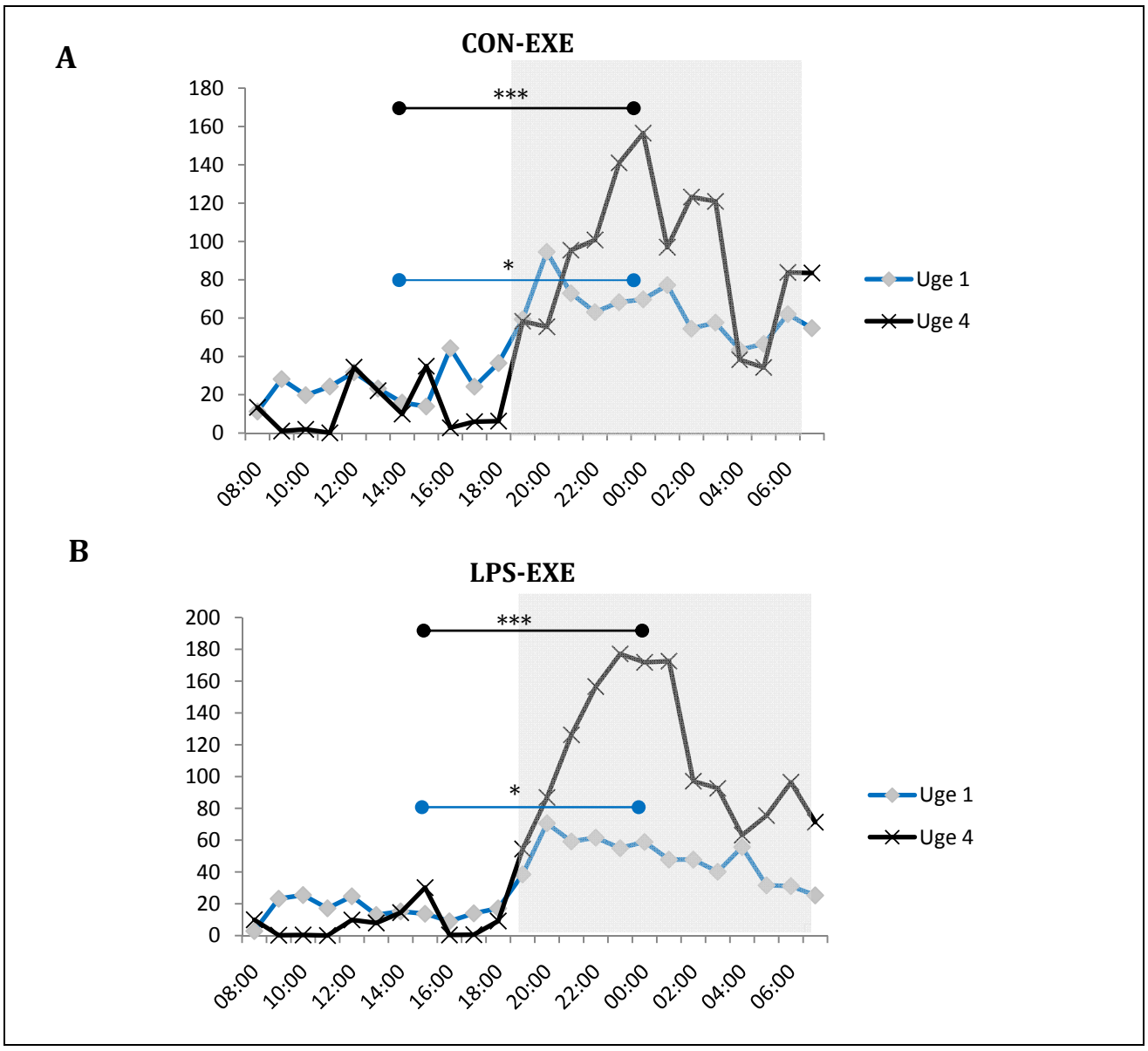


**Figure 4-19: Hour-by-hour evaluations of the mean running distances in weeks 1 and 4 for the two groups that were subjected to exercise, namely (A) CON-EXE and (B) LPS-EXE.** The numbers of rats in each group are as follows: CON-EXE (10), LPS-EXE (10). *p<0.05, ***p<0.001.Values are shown as the weekly mean average of the distance run per hour in each group.

### 4.2.3 BEHAVIOURAL EVALUATION

#### 4.2.3.1 LOCOMOTOR ACTIVITY

LPS significantly reduced locomotor activity ($F_{1,53}$=4.16, p=0.046). No effect was found for treatment ($F_{2,53}$=0.93, p=0.40). and we also did not detect an interaction between group and treatment ($F_{2,53}$=0.75, p=0.48).

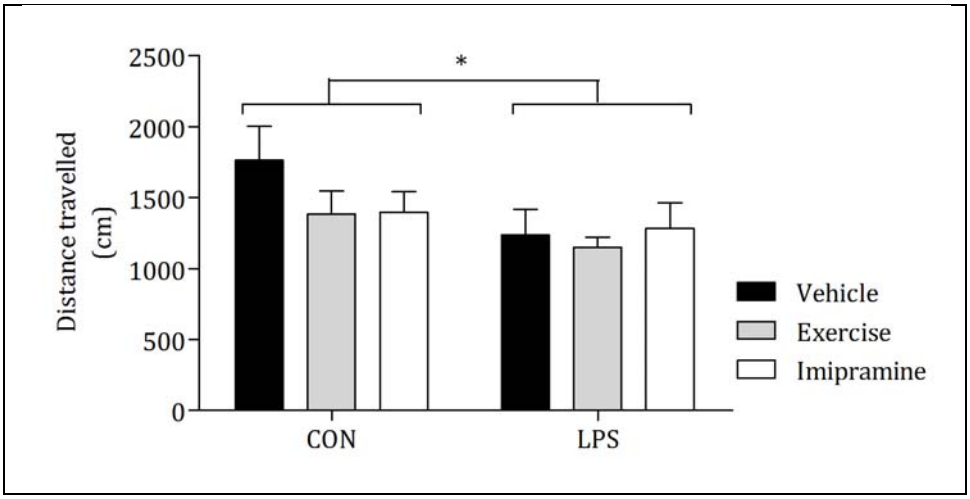


**Figure 4-20: The effect of LPS, imipramine and/or exercise treatment on the locomotor activity of Sprague-Dawley in the open field on T46**. The numbers of rats in each group are as follows: CON (10), CON-EXE (10), CON-IMI (9), LPS (10), LPS-EXE (10), LPS-IMI (10). *p<0.05,Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide).

#### 4.2.3.2 FORCED SWIM TEST

There was a strong trend for LPS-treated animals to display increased immobility in the FST compared to control rats ($F_{1,53}$=3.12, p=0.083), whereas a significant overall treatment effect was found ($F_{2,53}$=9.55, p=0.0003). Both exercise (p<0.01) and imipramine (p<0.01) significantly reduced immobility in LPS-injected rats, and exercise decreased immobility in the control group (p<0.05). No interaction between group and treatment was found ($F_{2,53}$=0.11, p=0.89).

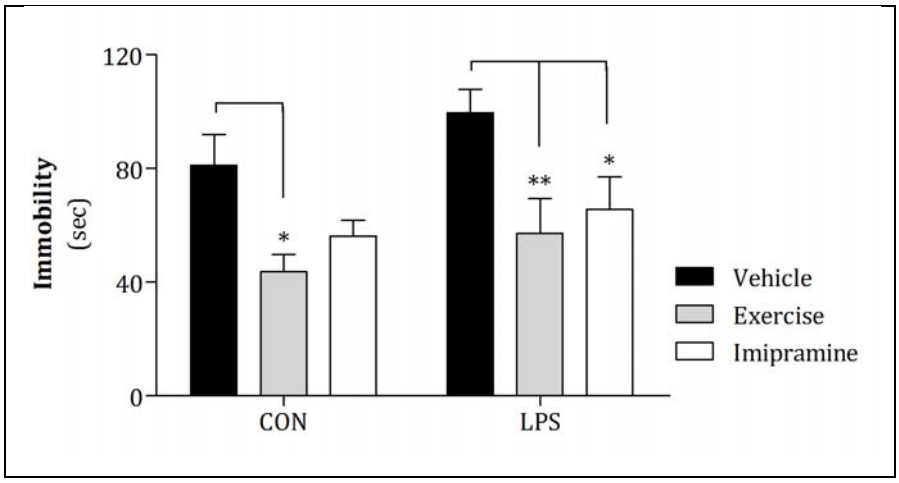


**Figure 4-21: The effect of LPS, imipramine and/or exercise treatment on immobility in Sprague-Dawley rats as measured in an FST on T46**. The numbers of rats in each group are as follows: CON (9), CON-EXE (10), CON-IMI (10), LPS (10), LPS-EXE (10), LPS-IMI (10). One outlier excluded SD>3.*p<0.05, **p<0.01. Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide).

Since both LPS and treatment had affected weight gain, we also performed a Pearson correlation analysis between immobility and body weight, although no association was found.

#### 4.2.3.3 ELEVATED PLUS MAZE

Treatment with LPS did not affect the percentage of time spent in the open arms ($F_{1,44}$=0.21, p=0.64). There was an effect of treatment in the control rats ($F_{2,44}$=4.88, p=0.012), with exercise decreasing the percentage of the time spend on the open arms (p<0.01) (see Figure 4-22A). No interaction between group and treatment was found ($F_{1,44}$=0.48, p=0.62).

LPS also did not affect the total number of entries ($F_{1,51}$=0.46, p=0.50) (see Figure 4-22B). However, there were a significant effect of treatment ($F_{2,51}$=5.82, p=0.005). Imipramine (p<0.05) and exercise (p<0.05) reduced the total number of entries in the LPS- and control rats, respectively. No interaction between group and treatment was found for total entries ($F_{2,51}$=1.46, p=0.24).

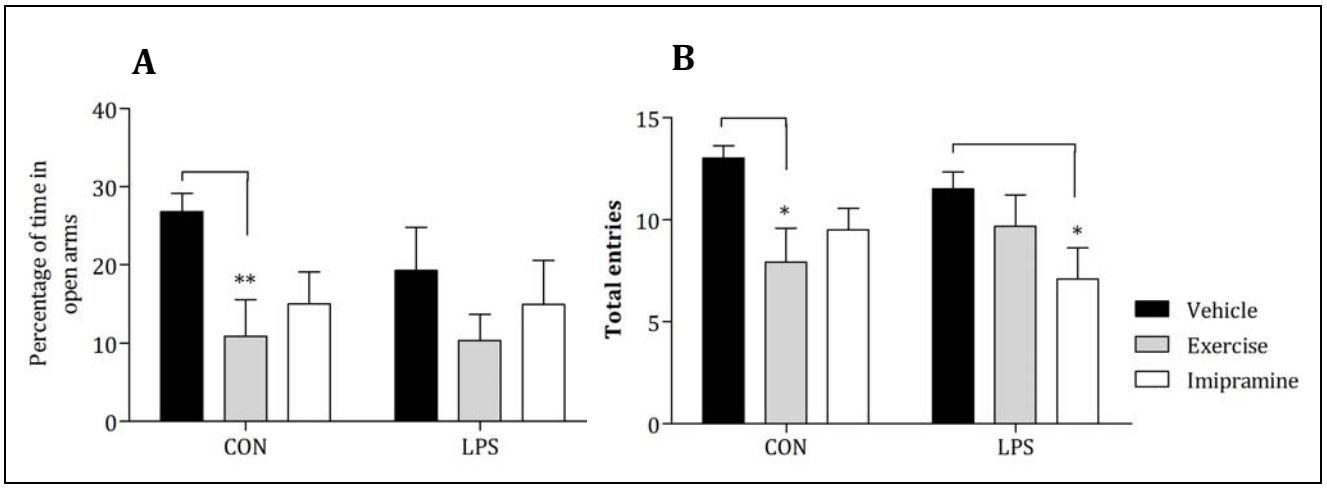


**Figure 4-22: The effects of LPS, imipramine and/or exercise treatment on anxiolytic parameters in Sprague-Dawley rats in the EPM on T45, namely (A) percentage of time spent in open arms, and (B) total number of entries into all arms.**.*p<0.05. **p<0.01. The numbers of rats in each group are as follows: CON (10), CON-EXE (7-10), CON-IMI (8-10), LPS (10), LPS-EXE (7-10), LPS-IMI (8-10). Values are shown as the mean + SEM. Abbreviations used: CON (control), LPS (lipopolysaccharide).

## 4.2.4 ORGAN WEIGHTS

### 4.2.4.1 EPIDIDYMAL FAT MEASUREMENT

LPS treatment significantly reduced the weight of epididymal fat pads, in terms of the percentage of body weight, compared to control rats ($F_{1,52}$=20.10, p<0.0001) (see Table 4-1). There were also significant treatment effects ($F_{2,52}$=10.47, p=0.0002) in which imipramine significantly decreased the weight of epididymal fat pads in both groups (p<0.01). No interaction between group and treatment was found ($F_{2,52}$=0.008, p=0.99).

### 4.2.4.2 LIVER MASS MEASUREMENT

LPS treatment significantly increased the weight of the liver, in term of the percentage of body weight, compared to control rats ($F_{1,52}$=77.26, p<0.0001). There was a significant treatment effect ($F_{2,52}$=3.79, p=0.029), although the ANOVA did not detect an effect for any specific treatment. No interaction between group and treatment was found ($F_{2,52}$=0.16, p=0.85).

**Table 4-1: Epididymal fat and liver weight measurements following LPS, imipramine and/or exercise treatment**

| | CON | | | LPS | | |
|---|---|---|---|---|---|---|
| Group | Vehicle | EXE | IMI | Vehicle | EXE | IMI |
| *Epididymal fat* | | | | | | |
| Weight (g)[a] | 3.22 ± 0.24 | 2.65 ± 0.13[b] | 1.96 ± 1.95[b] | 2.40 ± 0.16 | 1.89 ± 0.15 | 1.41 ± 1.41[c] |
| Weight (% of BW)[a] | 0.72 ± 0.05 | 0.62 ± 0.03 | 0.55 ± 0.05[b] | 0.58 ± 0.03 | 0.48 ± 0.48 | 0.40 ± 0.03[c] |
| *Liver* | | | | | | |
| Weight (g)[a] | 11.01 ± 0.19 | 11.79 ± 0.29 | 9.13 ± 0.30[b] | 13.30 ± 0.61 | 13.85 ± 0.57 | 11.56 ± 0.35[c] |
| Weight (% of BW)[a,d] | 2.48 ± 0.03 | 2.73 ± 0.04 | 2.58 ± 2.58 | 3.23 ± 0.13 | 3.49 ± 0.17 | 3.24 ± 3.23 |

Values are shown as the mean ± SEM. Abbreviation used: BW: body weight.
[a] Significant effect of LPS
[b] Significantly different from CON
[c] Significantly different from LPS
[d] Significant effect of treatment

# 5 DISCUSSION

In the present study, we examined the effect of exercise on two putative animal models of depression, the FSL/FRL and an LPS-induced model. The two models will be discussed individually.

## 5.1 EXPERIMENT 1: THE FSL/FRL RAT MODEL

Individual housing negatively affects the normal phenotype of FSL and FRL rats

Even though the main aim of this experiment was to assess the effect of exercise as an alternative antidepressant treatment, an additional and unexpected factor became of significant importance. A main finding in the present work is that housing the FRL and FSL rats individually had several behavioural and physiological consequences. Singly housed FRL and FSL rats did not, as expected, display the phenotypic characteristic difference in immobile behaviour, which is a well-described distinctive feature of the model, with FSL rats exhibiting exaggerated immobility in the forced swim test compared to FRL rats (Overstreet, 1993). We believe that the housing conditions contributed to this change in behaviour, as we one week prior to initiation of exercise (when all animals were still housed in pairs), confirmed this characteristic in a preliminary FST (see Figure 4-7A). However, at the time of the experimental FST (T32-34), this difference was no longer present (see Figure 4-7B). Additional groups of untreated FSL and FRL rats that were housed in pairs and evaluated in parallel, further strengthen this theory since a clear difference in immobility was found between these two groups (see Figure 4-8).

Social isolation has been widely described in rodents as a stressful condition, and housing rodents individually is widely used as a model for schizophrenia (Van den Buuse et al., 2003). Models for schizophrenia typically involve a social deprivation period that starts at weaning (post-natal day 21) and continues for a period of 6-8 weeks, which then leads to long-term changes by altering brain development and subsequently adult behaviour (Lapiz et al., 2003). Several physiological and behavioural changes have been described and some of the core findings underlying this model include an enhanced functioning of pre-synaptic DA neurons and defects in sensory motor gating mechanisms (measured using pre-pulse inhibition), effects that are similar to observations in human studies (Breier, 1999). However, the social isolation protocols used to model schizophrenia, as well as most other studies that have investigated social isolation, differs significantly from the current study in which the rats were isolated after approximately 70 days after birth. Other social isolation studies in adult rats, that is, animals in which postnatal brain development and social interaction skills have been firmly established, is limited. Although a few papers describe the effects of social isolation in FSL rats, the isolation procedures followed in these studies are diverse. One such study explored the endocrinological consequences on the HPA-axis following social isolation of young male FSL rats (Malkesman et al., 2006). Results showed that seven days of social isolation induced an increase in corticosterone levels, and it was suggested that the negative feedback mechanism of HPA-axis may be impaired, leading to a chronic activation of the HPA-axis, a phenomenon also observed in human depression. Under

basal conditions, the FSL rats exhibit lower levels and reduced serum ACTH levels, while no difference in corticosterone levels between the FSL and FRL rats has been found (Owens et al., 1991). These findings indicate that FSL rats may better represent depressed humans when environmental manipulations are introduced. Another study investigated the dopaminergic effects of social isolation in female adult FSL rats (Bjornebekk et al., 2007). After 7 weeks of isolation, a lower density of dopaminergic $D_2$ receptors was found in the striatal areas of socially isolated FSL rats compared to socially isolated Sprague-Dawley rats. Indeed, it has been reported that dopaminergic neurotransmission is influenced by social stressors, for example, socially dominant primates have an increased dopaminergic $D_2$ receptor density in the basal ganglia compared to subjects in subordinate positions (Grant et al., 1998). This provides a link between the rodent and primate studies, given that prolonged increased dopamine release is known to cause down-regulation of dopaminergic $D_2$ receptors in the brain (Carlsson and Carlsson, 2006). These results support the fact that social stressors can influence the density of dopaminergic $D_2$ receptor, and this may have also occurred in the socially isolated FSL rats in our study.

Although Malkesman did not report the depression-like behavioural consequences of social isolation, in another study by Bjørnebekk published in 2005, an FST was performed following 30 days of social isolation (Bjornebekk et al., 2005), a period similar to the one used in this study. However, in contrast to our results, these authors reported that the FSL rats were more immobile in the FST compared to the FRL strain, although an unconventional FST protocol was used, i.e. behaviour was scored during a 15 min swim session (compared to a conventional 5-7 min test), and it is unclear whether the authors performed a pre-swim session on the previous day, as is sometimes done to induce a higher level of immobility on the day of the test. The study by Malkesman as well as the study by Bjørnebekk published in 2007 used Sprague-Dawley rats as controls rather than the conventional FRL control strain. Given our findings that FRL rats where at least equally affected by the single housing as the FSL rats, in terms of an increased immobility (see Figure 4-8) (we also found an increase in food consumption for isolated FRL rats, see Table 8-1), it could be interesting to establish whether this change in phenotype is consistent in the FRL strain. Unfortunately, in the study mentioned above (Bjornebekk et al., 2005) in which FRL rats were housed individually for 30 days before the FST the authors do not mention the age of the rats at the start of social isolation, and a comparison between our results and those of others therefore remains difficult at present. To date, there has been no specific evaluation of depressive-like behaviour following social isolation of adult FSL and FRL rats. Since FSL/FRL rats are a genetic model of depression, these findings also support the involvement of a gene-environment interaction, although it would have been expected that the FSL strain be most prone to social isolation stress. No clear explanation can currently be given, and this observation may be an interesting topic for future investigation.

Social isolation confounds the antidepressant-like action of imipramine

Another surprisingly finding from this study was that we did not observe any antidepressant-like effects in the FST after chronic treatment with imipramine (see Figure 4-7), an effect that has been previously demonstrated in FSL rats (Overstreet et al., 1995). Although imipramine was administered in the food and the exact administered dose is therefore less predictable, an

insufficient dose is unlikely to explain the inefficacy of the drug in the current study since the dose calculated from the daily food intake did not fall below 10 mg/kg (see Figure 4-3), a dose that have been shown to be effective in decreasing immobility in rats (Kitamura et al., 2002). Social isolation has been shown to affect responses to a wide variety of drugs (Baer, 1971). Interestingly, another study showed that the immobility-reducing effect of imipramine was blocked by ACTH (Kitamura et al., 2002). Therefore, when taking into account the previously described alterations in HPA-axis function in FSL rats following social isolation (Malkesman et al., 2006), this finding suggests that an increased HPA-axis activity may also underlie the absence of antidepressant-like activity by imipramine in our study.

Exercise treatment alone does not have an antidepressant-like action in FSL rats in the FST

In this study, we found that subjecting FSL rats to exercise alone did not have an antidepressant effect in the FST (see Figure 4-7). The effect of exercise as an antidepressant strategy in FSL rats has been sparsely described in the literature. The study by Bjørnebekk et al. in which they studied the effect of running in FSL and FRL rats, it was found that running reduced immobility in the FST in FSL rats compared to non-exercising FSL rats (Bjornebekk et al., 2005). In this study, an experimental set-up similar to ours was used, in that the rats were housed individually and free access to the running wheels were provided for 30 days (Bjornebekk et al., 2005). The different results found in our study may on one hand be explained by differences in running activity, since the FSL rats in the other study ran on average far more than running distance observed in our study. However, as mentioned earlier, there are some uncertainties regarding the FST protocol used in this previous study, i.e. an unconventional 15 minute scoring period was used and it is not clear whether a pre-FST session was performed. In addition, the age of the animals at the time of individual housing is not noted, and, given that social isolation in rodents has different effects at different ages, the results from that study cannot be directly compared to ours. Bjørnebekk et al. also studied the effect of running in FRL rats, and they showed an unexpected behavioural response in this strain, in that there was a trend toward an increased immobility in the FST following access to a running wheel. As mentioned in Section 1.5.2, exercise has been shown to induce physiological alterations that are similar to the stress response, which may be exaggerated by the additional stress of the individual housing conditions. This may therefore explain why the FRL rats from the study by Bjørnebekk et al. did not gain any antidepressant effects from running, and further suggests that FRL may be more prone to the effects of social isolation, and is in line with the results from our study. Similarly, the beneficial effect of exercise on the FSL rats in our study may also have been counteracted by the stressful condition of being housed alone. However, the mechanisms underlying these changes remain unclear, and there is a need for novel studies to explore the effects of exercise in FSL/FRL rats, but in paradigms where there are no confounding social isolation factors.

Exercise and imipramine treatments have additive antidepressant-like effects in the FST

Interestingly, the combination of exercise and imipramine treatment showed an additive antidepressant-like action in FSL rats by potently decreasing immobility in the FST, an effect that was not evident with either treatment alone (see Figure 4-7). Therefore, the results from this study indicate that the isolated housing conditions may decrease the motivation of FSL rats to

exercise, and that this demotivation can be prevented or reversed by concurrent and chronic imipramine treatment. Thus, it appears that only when running behaviour is increased above a certain level can it have significant antidepressant effects. Indeed, our results show that FSL rats that received imipramine had a significant higher running wheel activity level compared to the FSL rats that did not receive antidepressant drug treatment (see Figure 4-4).

The combination of exercise with antidepressant drug treatment has been shown to have an antidepressant effect in FSL rats. Bjørnebekk et al investigated the antidepressant-like action of combined voluntary wheel running plus the selective serotonin inhibitor, escitalopram, in the FST (Bjornebekk et al., 2010). In line with our findings these authors also found a combined treatment effect, as was illustrated by decreased immobility in the FST relative to vehicle treatment. Although these authors did include separate groups that received either exercise or escitalopram, they did not compare the combined treatment group to these, and it is therefore unclear whether the combined exercise and escitalopram treatment was more efficient than either of the treatments alone. In summary, the results from our study indicate that the resulting phenotype that is produced by exposing a genetically susceptible rat strain (FSL rats) to social isolation, only respond to a combined antidepressant drug plus exercise treatment, in terms of a decrease in immobility in the FST.

FSL and FSL rats do not display strain differences in anxiety-like behaviour in the EPM

We did not find any strain differences in basal anxiety-like behaviour measured in the EPM between FSL and FRL rats (see Figure 4-9). Similar findings have been previously described for FRL and FSL rats (Wegener et al., 2011), whereas SD rats displayed increased anxiety-related behaviour relative to both FSL and FRL rats (Wegener et al., 2011). In addition, FSL and FRL rats respond equally to the administration of the anxiolytic diazepam, by showing a similar increase in the time spent in the open arms of the EPM (Overstreet et al., 1995). These findings are important since they separate the depressive-like and anxiety-like phenotypes of FSL/FRL rats, and therefore support the validity of this model as a model for depression only. Some studies have even reported that FSL rats display less anxiety. For example, young (post-natal day 40) FSL rats showed less anxiety-like behaviour in both the open field and EPM compared to SD (Braw et al., 2006). Moreover, when adult (post-natal day 92) FSL rats are subjected to a high fat diet, this increased time on open arms indicating a trend toward an anxiolytic effect of the diet, whereas immobility in the FST was increased in these rats (Abildgaard et al., 2010). This further separates the depression- and anxiety-like characteristics of FSL rats, and may support the idea that different pathophysiologies are involved in anxiety and depressive disorder.

Exercise or imipramine treatments do not alter anxiety-like behaviour of FSL rats in the EPM

Chronic treatment with either exercise or imipramine, or the combination of these, did not alter the anxiety-like behaviour of FSL rats in the EPM (Figure 4-9). That imipramine did not affect behaviour in test could be explained by this drug being an antidepressant with no anxiolytic activity (File and Johnston, 1987). To our knowledge, it has not been evaluated whether exercise has any anxiety-like effects in FSL rats. However, studies using other rat strains have examined the anxiolytic effects of exercise, although the results of these are somewhat contradicting. One

study evaluated the effects of 10 weeks of treadmill exercise in SD rats in the open field and EPM (Fulk et al., 2004). These authors found that exercise reduces anxiety-like behaviour in both tests. In contrast, another study that investigated the effect of voluntary running in the open field and EPM in an animal model of maternal separation, found that SD rats with free access to the a running wheel (maternally separated rats and controls), displayed higher levels of anxiety in both tests (Grace et al., 2009). Therefore, there remain significant inconsistencies in the literature, and more studies that explore the anxiolytic effect of exercise on FSL/FRL rats are needed.

FSL and FRL rats did not display strain differences in declarative memory in the ORT

We evaluated the effects of exercise and imipramine treatment on declarative memory in FSL rats by subjecting them to an object recognition task (ORT. We did not find any differences between the two strains (Figure 4-10). Only a few studies have been published on cognitive abilities and memory function in the FSL/FRL model. Bushnell et al. investigated working and reference memory in the FSL/FRL rats, by using a test based on choice accuracy in appetitive tasks (Bushnell et al., 1995). This study indicated that memory function is not impaired in FSL rats. On the other hand, Abildgaard et al. reported that FSL rats may have a reduced memory function compared to FRL rats, based on results from the ORT (Abildgaard et al., 2010). Because the last-mentioned study had experimental setup similar to ours, it is difficult to find an explanation for the different results. Of note, in our study, half of the animals were subjected to the ORT twice due to technical difficulties in setting up the experiment. Although it did not seem to have an effect on total exploration time, it did appear to increase the variance within the groups, thereby making it difficult to find differences between the two strains. In addition, it may be argued that the individual housing conditions in this study may have contributed to disturbances in memory function. Therefore, additional studies are needed to investigate memory function in FRL and FSL rats.

Exercise or imipramine treatment did not alter declarative memory in FSL rats in the ORT

We did not find effects for exercise or imipramine treatment on declarative memory in FSL rats in the ORT (see Figure 4-10), which may also be attributed to the factors mentioned above. To our knowledge, there are currently no publications describing exercise-induced changes in memory function in the FSL/FRL model. However, increasing evidence suggest that neurogenesis may have an important role in learning and memory (Deng et al., 2010) and experiments on FSL rats have shown that exercise increases cell proliferation in the hippocampus compared to non-exercising FSL rats (Bjornebekk et al., 2005). This supports the suggestion that learning and memory in the FSL strain may be improved by exercise, but was not observed in this study due to the difficulties mentioned above. Indeed, another preclinical study using Long Evans rats that were subjected to 4 weeks of voluntary exercise also showed an exercise-induced improvement in object recognition memory (Hopkins and Bucci, 2010). Therefore, because of the procedural shortcomings of this part of the experiment, further studies are needed to establish whether exercise can improve memory function in FSL rats.

FSL rats present with increased hippocampal IL-1α levels relative to FRL rats

An interesting finding from this study is that the mRNA level of the pro-inflammatory cytokine, IL-1α, was found to be increased in the hippocampus of FSL rats, but not in FRL rats (see Figure 4-11H). To our knowledge, this has not been investigated in the FSL/FRL model of depression prior to this study. Increasing evidence suggests that major depression is accompanied by the activation of the inflammatory response system, and that pro-inflammatory cytokines may play a role in the aetiology of depression (Maes, 1999). The immune response has only been sparsely described in the FSL and FRL rats. One study reported that FSL rats exhibit an altered immune response, by showing that FSL rats has a decreased activation of natural killer (NK)-cells compared to FRL rats (Friedman et al., 1996). NK-cells are essential components in the host against viral and bacterial infections and cell activation is necessary for host defence. A decreased NK-cell response found in the FSL rats corresponds to observations from depressed humans (Blume et al., 2011). A more recent study investigated the serum levels of several pro-inflammatory cytokines in FSL and FRL rats (Carboni et al., 2010), and showed that IL-1α was increased in FSL rats compared to FRL rats. This cytokine is a constitutively expressed cytokine that activates a cascade of immune system responses following an acute infection, and has been suggested to inhibit GR gen-translocation and function (Wang et al., 2004). In line with this, GR function has been suggested to be reduced in patients with major depression (Webster et al., 2002), and increased IL-1α levels could therefore be involved in the impairment of the negative feedback mechanism in HPA-axis. Unfortunately, the mRNA expression levels for the GR were not successfully measured in this study due to technical difficulties. This interaction could be an interesting focus for future investigation in FSL rats, together with the gene expression measurements of other pro-inflammatory cytokines. Nevertheless, this finding supports the involvement of pro-inflammatory cytokines in the FSL/FRL rat model of depression, as well as the construct validity of this model.

FSL rats presented with decreased hippocampal BDNF mRNA expression relative to FRL rats

Another interesting result from this experiment came from the analysis of the gene expression levels of BDNF in the hippocampus. Although no difference was found when analysing BDNF levels from single-housed rats, a collective analysis of both pair- and individually housed rats revealed a difference in mRNA expression between the two strains (see Figure 4-12). Specifically, FSL rats presented with a significantly lower level of hippocampal BDNF compared to FRL rats. This observation is also supported by the findings of a previous study (Elfving et al., 2010a), although another report did not show any difference in hippocampal BDNF between FSL and FRL rats (Angelucci et al., 2000, Biagini et al., 2001).

Exercise or imipramine treatment did not alter BDNF mRNA expression in FSL rats

Treatment with either exercise or imipramine, or the combination of these treatment did not affect the level of BDNF in hippocampus (see Figure 4-11A). Although there have not been any previous studies that have evaluated the effects of exercise or imipramine on BDNF expression in FSL/FRL rats, an up-regulation of BDNF expression in the hippocampus has been shown previously in Wistar rats following chronic imipramine treatment (5 and 10 mg/kg, p.o. for 14

days) (Rogoz and Legutko, 2005). However, contrasting results have also been reported in a study were the down-stream effects on intracellular signalling following chronic administration of imipramine were investigated (Reierson et al., 2009). It is widely believed that an initial increase in cAMP underlies the antidepressant activity of most antidepressant drugs by leading to an activation of CREB and a subsequent increase in BDNF expression (see Section 1.3.3) (Coyle and Duman, 2003). However, Rogoz et al. found a decreased hippocampal cAMP level following imipramine treatment and no change in BDNF level, suggesting that imipramine might have a different antidepressant signalling mechanism. Taken together, these results suggest that the exact antidepressant mechanism(s) of imipramine are still not fully understood.

It has been suggested that the level of hippocampal BDNF is correlated to the running distance of FSL rats. (Bjornebekk et al., 2005). This was hypothesized from results showing that FRL rats had an increased running activity compared to FSL rats, and that these rats also showed an increase in hippocampal BDNF expression. In our study, FSL rats that were subjected to a combined treatment of exercise plus imipramine ran an average distance comparable to the FRL rats in the study of Bjørnebekk. However, the BDNF expression level was not altered by this treatment condition in the FSL rats in our study, and it therefore appears that BDNF expression may not only be affected by running, but is also related to the genetic background of the animal.

Basal serum BDNF levels are increased in FSL rats relative to FRL rats, but are not affected by imipramine or exercise treatment

We found that FSL rats displayed a significant higher serum concentration of BDNF compared to FRL rats (see Figure 4-13). This result corresponds to a previous study where an inverse correlation between the blood and brain levels of BDNF was also shown in FSL and FRL rats (Elfving et al., 2010a), although it is in contrast with data from humans that suggest that serum levels of BDNF is decreased during depression (Karege et al., 2005).

Treatment with exercise or imipramine, or the combination of these treatments, did not affect the serum level of BDNF. To our knowledge, there have been no other studies that have assessed serum or plasma BDNF levels in FSL and FRL rats following these treatments. From human studies, there is increasing evidence that acute aerobic exercise increases plasma BDNF levels in both healthy and chronically ill persons (Knaepen et al., 2010). However, given the contrasting results obtained from humans and rat studies, it is currently unclear what to expect regarding exercise-induced alterations in BDNF serum levels in FSL rats, whereas, our results suggest that no such changes occur. Other recent evidence also suggests that BDNF is produced in muscle cells following exercise, where it act locally without being released into the circulation (Matthews et al., 2009). Thus, the examination of muscle tissue might be an alternative approach to evaluate peripheral BDNF levels in FSL rats following exercise.

Food consumption and weight gain are lower in FSL rats relative to FRL rats

We found that FSL rats presented with a slower weight gain (see Figure 4-1) and a reduced food consumption (see Figure 4-2) compared to FRL rats. In agreement with this, previous studies have reported similar differences in appetite and body weight in FSL rats (Overstreet et al., 2005).

This further strengthens the face validity of the FSL rat as a model of depression, since weight loss and reduced appetite is sometimes also observed in human depression.

A trend for reduced food-intake and weight gain is evident in FSL rats relative to FRL rats

Although there were found no significant effects of exercise or imipramine treatment on weight gain, a trend for a decreased food consumption was observed in animals treated with imipramine (see Figure 4-2). While this has not been observed in FSL rats before, other studies have found similar results in healthy rats. One study reported decreases in food consumption and weight gain in rats following chronic treatment with imipramine at a similar dose as was used in this study (Mogensen et al., 1994). The effect of imipramine could be due to the sedative effect of the antidepressant, as suggested by File and Tucker (File and Tucker, 1986). Another explanation for the altered feeding behaviour in these animals could be that the groups that were treated with imipramine had their food changed to hand-formed pellets following the mixing of the drug with the food, which may have resulted in an altered texture and possibly also an altered taste. However, this does not rule out that imipramine may indeed induce these effects by a pharmacological means.

Fasting blood glucose levels are higher in single- compared to pair-housed FSL rats

Interestingly, fasting blood glucose levels were significantly higher in FSL rats that were housed individually compared to pair-housed FSL rats (see Figure 4-6B). This is also a novel observation in FSL and FRL rats. Fasting blood glucose is often used as a marker for insulin resistance in humans to determine the risk for developing type 2 diabetes. Moreover, an increased fasting blood glucose level is also a component of the metabolic syndrome, which is a collection of risk factors that predisposes an individual to chronic illnesses such as cardiovascular disease and diabetes. Interestingly, a study in humans observed that social isolation was also associated with the metabolic syndrome (Horsten et al., 1999) and, together with the results from this study, suggests that social isolation can alter important metabolic functions that may predispose an individual to a pre-diabetic state. There is also increasing evidence that suggests a co-morbidity between depression and the metabolic syndrome, which may be explained by overlapping pathophysiologies. In line with this, a previous study has shown an increased fasting blood glucose in FSL rats when they are metabolically challenged with a high-fat diet (Abildgaard et al., 2010). This suggests that, in addition to serving as a model for a vulnerable phenotype for depression, the FSL/FRL rat model may also in some aspects represent a vulnerable phenotype for metabolic syndrome.

Synopsis

In summary, our findings on the FSL/FRL model of depression in this study suggest that individual housing of these rats can alter important behavioural characteristics, including the well-documented difference in depressive-like behaviour, as well as important metabolic functions. Therefore, this factor may have interfered with the "normal" physiological and behavioural responses that have been reported in FSL and FRL rats, such as antidepressant-like effects for exercise and imipramine treatment. Though the FSL rats did not respond to exercise or

imipramine separately, when the treatment strategies were combined, an antidepressant effect was observed as a decrease in immobile behaviour in the FST. The exposure of this genetically vulnerable phenotype to social isolation could therefore create a different phenotype that could be interesting to investigate in more detail.

## 5.2 EXPERIMENT 2: THE LPS-INDUCED MODEL OF DEPRESSION

Chronically LPS-treated rats presents with sickness behaviour rather than depression-like behaviour

In the second part of this project our main finding was, surprisingly, that long-term administration of a low dose of LPS to Sprague-Dawley rats induced sickness behaviour that persisted for the duration of the experiment. Some of the symptoms of sickness behaviour include a decreased food intake, weight loss, and decreased activity level (see Section 1.3.4), all of which were manifestations in our study (see Figures 4-14, 4-15, 4-20). Sickness behaviour could very easily be mistaken for a depression-like state, since many of the symptoms of these two states overlap (Loftis et al., 2010). This finding was somewhat unexpected, as previous unpublished results from our group have suggested that the initial sickness behaviour that is observed following chronic LPS administration would subside after 1 week of treatment. The eventual model obtained in this experiment may therefore not by definition represent a valid model of depression, but rather illustrate prolonged sickness behaviour with symptoms that overlap with some of the characteristics of depression.

We initially hypothesized that the rats would recover from the sickness behaviour, and following long-term LPS treatment would develop a tolerance against this pathogen. This would have enabled us to assess whether a low-grade inflammation state could be a valid model for depression. This notion was based on findings from clinical and pre-clinical studies where it has been suggested that depression can be a late development following earlier appearing sickness, such as chronic infections (Dantzer et al., 2011) (Gibb et al., 2009). A distinction between sickness behaviour and depressed symptoms in humans has been shown in a longitudinal clinical study in patients treated with cytokine immunotherapy (Capuron et al., 2000). Whereas all patients responded to the first few injections of cytokine therapy by presenting with symptoms of sickness, only one third developed a major depressive episode after a few days to several weeks of treatment. Interestingly, it appears that sickness behaviour and major depression share at least some biological substrates in their pathologies, which include an overproduction of endogenous pro-inflammatory cytokines and a dysregulation of the HPA axis (Loftis et al., 2010). Importantly, however, sickness behaviour presents with pyrexia (Hart, 1988), a symptom that is not typically associated with depression. Therefore, it would have been very interesting to measure the body temperature of the rats in our study, but due to our initial hypothesis, this was not carried out. A measure of the rectal temperature would have provided us with a more detailed indication of whether the rats were “sick” or “depressed”. This measurement should certainly be included in future work.

Although we observed a trend for LPS-treated animals to present with depression-like behaviour in the FST (see Figure 4-21), the interpretation of these results are somewhat complicated to interpret since a decreased locomotor activity was evident in these rats (see Figure 4-20). In other words, a decreased swimming behaviour in the FST may not be caused by a reduced motivation to escape the cylinder, but rather a general reduced locomotor activity in the animals. In hindsight, an additional evaluation of other depression-like behavioural parameters may have been assessed, such as the saccharine preference test, which does not depend on locomotor activity, but a decreased preference for a glucose solution added to the drinking water, which is regarded as a measure of anhedonia, i.e. the inability to experience pleasure (Katz, 1982). However, further analysis of the harvested tissue samples may assist in differentiating whether the animals were affected by a depression-like state or suffered from sickness behaviour. Such analysis could involve determination of the level of IDO, an enzyme that converts the serotonin precursor, TRP, to kynurenine, and is activated by pro-inflammatory cytokines (see Section 1.3.4). Indeed, a human study have shown that patients treated with cytokine immunotherapy have a reduced plasma level of TRP, and this appears to be correlated with the severity of depressive symptoms (Capuron et al., 2002). This suggests that IDO activation might play a role in the cytokine-induced depressive symptoms observed in these patients. Interestingly, IDO may also represent a point of divergence between sickness behaviour and depression, since it has been demonstrated that the inhibition of IDO in rats prevents the LPS-induced development of depression-like behaviour but not sickness behaviour (O'Connor et al., 2009).

Exercise treatment reversed the LPS-induced increase in immobility in the FST

We found that the increased immobility induced by LPS treatment in the FST was reversed by exercise treatment (see Figure 4-21). Given that our model appears to represent sickness behaviour rather than a model of depression as such, this treatment strategy may therefore have acted as an anti-inflammatory mediator in addition to its hypothesized antidepressant action. In line with this, it has often been reported that regular exercise has protecting effects against all-cause mortality (Klavestrand and Vingard, 2009), and it can be suggested that some of the reported antidepressants effects of exercise may be related to an anti-inflammatory action. For example, exercise stimulates the release of IL-6 that induces anti-inflammatory effects by stimulating anti-inflammatory cytokines, as well as producing inhibitory effects on pro-inflammatory cytokines (Petersen and Pedersen, 2006). In addition, exercise shifts the balance of T lymphocytes in favour of an anti-inflammatory response (see Section 1.5.4), and reduces the expression of toll-like receptors, the suggested target via which LPS exerts its actions (Gleeson et al., 2006, Gleeson, 2007).

Imipramine treatment reversed the LPS-induced increase in immobility in the FST

We further observed that imipramine reduced immobile behaviour in the FST in rats that were chronically treated with LPS, suggesting that this antidepressant drug may have counteracted the inflammatory effects of LPS. This result is in line with previous findings from

a study were imipramine was administered to sepsis survivors rats (Tuon et al., 2007). Animals had undergone a cecal perforation which resulted in a severe immune response and were 10 days later administered imipramine or saline. Depressive-like behaviour was reversed by imipramine, indicating that LPS-induced depressive symptoms were sensitive to the action of imipramine. In another study, different classes of antidepressants were tested for their anti-inflammatory effects on carrageenan-stimulated (a polysaccharide expressed on the cell walls of certain red algae, administered to induce inflammation) paw oedema (Abdel-Salam et al., 2003). This study found that imipramine (at doses of 3.75, 7.5 and 15 mg/kg) reduced paw inflammation, and hereby may demonstrated some anti-inflammatory properties. However not all antidepressants show anti-inflammatory effects, since the same study found that the SSRI, sertraline, caused a dose-dependent enhancement of paw oedema. Therefore, the relationship between antidepressants and inflammation appear to be more complex as previously thought, and this is proving to be a controversial field in modern research. Taken together, although both exercise and imipramine reduced the hypothetical depression-like behaviour in the FST, it remains very difficult to distinguish between the antidepressant- and putative anti-inflammatory effects of these treatment strategies, given the concurrent decrease in locomotor activity observed in LPS-treated rats in this study.

Running behaviour in the rats was unaffected by chronic treatment with LPS

Some of our main reasons for believing that the LPS-treated rats suffered from a sickness behaviour was based on the observations that this pathogen induced a decrease in locomotor activity (see Figure 4-20), food-intake (Figure 4-15) and body weight (Figure 4-14). However, another interesting observation in this experiment was that LPS did not reduce the running activity of the rats (Figure 4-18). A previous study that investigated wheel-running activity in rats that were injected with the bacteria Bacillus Calmette-Guerin (BCG), observed a decrease in running behaviour for 9 days following the injection (Moreau et al., 2008). In addition the febrile response to BCG was measured as an indicator of sickness behaviour, and they reported an increase in body temperature that lasted for 5 days post-injection. These findings suggest that the decrease in running activity lasts longer than the sickness behaviour, and is somewhat in contrast to our findings. It can be speculated that the unchanged running behaviour following LPS treatment in this experiment was attributed a "rewarding feeling" experienced by the rats during or after running (also described by others (Smith et al., 2008)), or an instinctive drive to "self-medicate".

Anxiety-like behaviour of the rats in the EPM was unaffected by chronic treatment with LPS

We did not observe any LPS-induced changes in anxiety-like behaviour in the EPM (see Figure 4-22). However, since the rats had a lower locomotor activity in the open field test, one might suspect that a decreased activity level may also have influenced behaviour in the EPM. Findings from another study, however, is in contrast to our data, by reporting an anxiogenic effect for LPS in the EPM, as measured by a decrease time spent in the open arms (Sah et al., 2011). However, the experimental design in their study consisted of a single injection of LPS,

and at a higher dose (1 mg/kg versus 600 µg/kg in our study, i.p.), and any or both of these factors may explain the different responses observed in the EPM between the two experiments. Another study investigated the anxiety-modulating effect of treatment with the pro-inflammatory cytokine, IL-1β, at a sub-pyrogenic dose (50 ng/kg) in rats, has also reported increased anxiety-like behaviour in the EPM (Sokolova et al., 2007). Once again, the time-course used in this study differed from our treatment schedule, in that the rats were treated for only 14 days in that study. One clinical investigation, however, did not show a correlation between circulating cytokine levels and the severity of anxious-depressive symptomatology (Haack et al., 1999), and is in line with our results that suggest that chronic inflammation does not precipitate anxiety-related changes.

Fasting blood glucose levels in the rats were increased by chronic treatment with LPS

We found that chronic treatment with LPS induced an increase in fasting blood glucose levels in the rats (see Figure 4-16). This finding is in agreement with previous unpublished data from pilot studies performed within our group, where we observed a similar effect (Buhl, 2010, unpublished data). Hyperglycemia may be a response to insulin resistance, and defective glucose sensing at the β-cell which could indicate pre-diabetes (Ferrannini et al., 2011). In fact, an increased fasting glucose and insulin resistance are also indicators of the metabolic syndrome, and hereby risk factors for type 2 diabetes and cardiovascular diseases. Thus, there is growing evidence that increased levels of cytokines may be a common denominator in a variety of chronic diseases (Bagry et al., 2008). Both overnutrition and inactivity has been associated with a hypersecretion of cytokines which can eventually lead to insulin resistance and diabetes in genetically or metabolically predisposed individuals (Esposito and Giugliano, 2004), whereas increased circulatory levels of IL-6 and TNF-α have been found to interfere with glucose uptake by impairing the insulin receptor, and hereby leading to insulin resistance (Krogh-Madsen et al., 2006). Since LPS is known to increase the levels of both IL-6 and TNF-α (see Section 1.6.2), this pathogen may have also impaired glucose transport in the rats in our study, thus resulting in the observed increased fasting glucose levels.

The weight of the liver of the rats were increased by chronic treatment with LPS

In this study we found that LPS induced an increase in the average weight of the liver in the rats (see Table 4-1). Similarly, administration of the pro-inflammatory mediator TNF-α has previously been shown to increase the weight of the liver in rats (Endo et al., 2007). In this study, a single injection of TNF- α was found to increase the levels of triglycerides in the liver, whereas further histological analysis also revealed an accumulation of hepatic fat deposits, and thus indicates that acute inflammation may induce hepatic steatosis. These inflammation-induced hepatic changes were suggested to be caused by an increased enzymatic activity in the liver, hereby increasing fatty acid synthesis (Endo et al., 2007), or could also be a consequence of increased transport of fatty acids from adipose tissue into the liver (Feingold

et al., 1990). Such an inflammation-induced hepatic steatosis may also explain the increased liver weight observed in this study. Furthermore, an accumulation of fat in the liver has also been linked with impaired insulin signalling (Hotamisligil et al., 1994) and hepatic insulin resistance (den Boer et al., 2004). Given that insulin is a crucial mediator of glucose uptake, the increased fasting blood glucose levels observed in this study after LPS treatment may also be connected to the increase in liver weight. However, further studies that explore the inter-relationship between cytokines, triglycerides and insulin in the liver are required to affirm this hypothesis. Lastly, another explanation for the increase in liver mass observed in this study may involve an enhanced production of cytokines and acute-phase immune proteins in the liver in response to LPS treatment.

We also found that epididymal fat pads were reduced in LPS-injected rats. In a previous study it was shown that a chemically induced colitis also increased the weight of the liver and decreased mesenteric fat deposits in mice, (Li et al., 2008). Another study has reported increased fat pads in rats following a high-fat diet (Abildgaard et al., 2010), and, in addition to the obvious dietary consequences, it may be speculated that this result may also involve an inflammatory component. However, given the contrasting effects on the size of fat pads between the last-mentioned study and our experiment, is appears that low-grade inflammation induced by LPS exerts a different effect in adipose tissue compared to a high-fat diet.

# 6 SUMMARY AND CONCLUSIONS

In this study we evaluated the antidepressant potential of exercise, a promising alternative antidepressant strategy, as a treatment on its own, as well as a possible adjunct to current antidepressant therapies. For this reason, we used two different models of depression to investigate the effects of exercise on two distinct facets of the underlying aetiology and/or pathology of depression, namely genetic (FSL/FRL rats), and environmental (LPS-induced) aspects. Although we encountered significant difficulties in both experiments, several noteworthy findings were made in this project.

In the **FSL/FRL rat model of depression**, a significant setback was that the well-documented depression-like phenotype of the FSL rats relative to the FRL rats, as evident in the FST, disappeared when evaluated after 5 weeks. We also did not detect antidepressant activity for the positive control, imipramine. This questioned the face and predictive validity of the model, since the genetic make-up of the FSL/FRL rats should provide the abovementioned phenotypical difference, and respond to clinically effective antidepressants. We attributed these findings to the single-housed condition that was required for the accurate measurement of individual running distances. Despite this factor, however, our results did suggest that exercise might be an effective adjunct to antidepressant drug therapy, by showing that the combination of exercise and imipramine in FSL rats had antidepressant activity in the FST. Especially since none of these treatments alone displayed this effect. Interestingly, this finding may represent a likely scenario in humans, where depressed individuals have a reduced motivation to exercise, but which can be improved by initial antidepressant drug therapy. In addition to the behavioural effect observed in this experiment, exercise in combination with imipramine also appeared to have a beneficial action on the metabolism of FSL rats.

The **LPS-induced model of depression** is not as well-validated as the FSL/FRL rats as a model for depression. As previously mentioned, this model is still in an experimental stage, and it is based mostly on unpublished observations from a collaborating laboratory. The results from our study have, however, highlighted a problem that may prevent the successful use of the LPS-induced model as a valid model for depression. After 8 weeks of LPS treatment, we were unable to differentiate between sickness behaviour and depression-like behaviour per se. This meant that we were unable to distinguish whether the observed treatment effects were mediated by anti-inflammatory-, or by "true" antidepressant mechanisms. Nevertheless, we found that both exercise and imipramine treatment reduced immobility in the FST, which suggests that exercise exerts at least some beneficial anti-inflammatory and/or antidepressant action(s). Furthermore, this project also shed some light on the metabolic state of rats following chronic treatment with LPS, by showing that LPS-treated rats have an increased fasting blood glucose level compared to untreated animals. Interestingly, an elevated fasting blood glucose level is associated with the metabolic syndrome in humans, which displays a high co-morbidity with major depression. In this sense, this finding provides some support for the construct validity of an LPS-induced model of depression.

Taken together, this project illustrates the complexities that are involved when modelling a multifaceted human mental disorder such as depression, in rats. In addition to the technical

difficulties surrounding the experimental design of animal studies investigating treatment effects, the multi-factorial nature of depression makes the studying of the efficacy of exercise in depression even more difficult. For this same reason, it is not all that surprising that currently available antidepressant drugs, all with relatively specific pharmacological mechanisms, are widely believed to be sub-effective in the treatment of depression, and this also reflects our limited understanding of the underlying neurobiology of depression. The results from this study support the efficacy of a promising, albeit primitive putative antidepressant therapy, namely physical exercise, that has much more general and wide-spread effects throughout the body in different physiological systems, and therefore could theoretically be a useful option in the effective treatment of major depression.

# 7 FUTURE PERSPECTIVES

Although the results obtained from this study helped us to gain important insight into several aspects regarding the underlying pathophysiology of depression as well as the putative antidepressant benefit of exercise, many questions remain unanswered, while our findings also created a number of new prospects for future investigation.

Firstly, future studies that investigate the effect of exercise in rats should take into consideration the effect of housing, and preferably design the experiment with at least two rats per cage. An obvious problem with this set-up is the inability to measure the running activity for each rat individually. One solution to this problem is to simply calculate the average running distance for two rats, as we did in the LPS-induced model, although this does not take into account inter-individual differences in running behaviour. Another option is to use a system where a microchip is implanted under the skin of the rat, which registers each time the rat passes through a gate. This system not only gathers accurate running data of each rat, but also prevents rats from entering the running wheel at the same time. This technology has, however, been slow to develop, and it remains exceedingly expensive.

As mentioned above, the LPS-induced model of depression is still in its developmental phase, and the results from this project provided useful insight into ways that this model may be improved for future use. Firstly, an easy and reliable method to distinguish between sickness behaviour and depression-like behaviour could be to measure the rectal temperature of the rats, and thereby establishing whether the animals present with fever. In addition, an improved approach to measure locomotor activity may be to monitor the activity of the animals in their home cages by using infrared sensors. This would avoid the influence of the novel environment in the open field test. LPS can also be administered via a surgically implanted minipump. This method will reduce the stress of daily injections, as well as the fluctuating circulation levels that is an unavoidable consequence of this daily administration method, while a lower dose can also be considered to decrease the risk of sickness behaviour. Furthermore, an investigation into the serum and brain levels of a number of inflammatory mediators, such as TNF-α, IL-6 and IL-1 may be interesting to investigate in this LPS-induced model. Additionally, the enzyme, IDO, may be an interesting focus for future investigation, given its possible role in a depression-like state and not in sickness behaviour.

Lastly, it could be very interesting to combine the two models that were used in this project. By doing this a new model could be created, with a possibly enhanced validity and a better representation of human depression, by incorporating a gene-environment interaction aspect into the model.

# 8 ADDENDUM

In this Addendum several additional data that was not shown in the Results section will be presented.

## 8.1 EXPERIMENT 1: THE FRL/FSL MODEL OF DEPRESSION

In Tables 8-1 and 8-2, the results from the pair-housed rats are shown, together with the results of the single-housed rats for comparison.

**Table 8-1: Additional metabolic results (in bold) for the pair-housed FSL and FRL rats**

| | Pair-housed | | Single-housed | | | | |
|---|---|---|---|---|---|---|---|
| **Group** | **FRL** | **FSL** | **FRL** | **FSL** | **FSL-EXE** | **FSL-IMI** | **FSL-IMI+EXE** |
| **Size (n)** | **8** | **8** | 8 | 8 | 8 | 7 | 6 |
| **Age (days)** | **75.00 ± 1.17** | **70.63 ± 1.83** | 73.50 ± 1.02 | 70.63 ± 1.57 | 70.88 ± 1.09 | 71.13 ± 1.27 | 73.33 ± 1.20 |
| **Weight (g)** | | | | | | | |
| **T0** | **254.5 ± 7.9** | **243.625 ± 13.7** | 265.75 ± 8.8 | 241.5 ± 18.3 | 302,6 ± 15,9 | 288,3 ± 10,1 | 309,2 ± 14,2 |
| **T39** | **123.38 ± 6.95** | **107.75 ± 8.99** | 148.5 ± 5.61 | 107.38 ± 10.30 | 113,3 ± 6,9 | 86.14 ± 11.18 | 76.83 ± 10.13 |
| **Total food intake** | **1059 ± 16.67** | **990.1 ± 21.93** | 1174 ± 29.59 | 1011 ± 29.54 | 1072 ± 34.4 | 1596 ± 35.7[b] | 1846 ± 72.7 |
| **Fasting plasma glucose** (mM) | **3.15 ± 0.12** | **3.52 ± 0.14** | 3.56 ±0.11 | 3.73 ± 0.19 | 3.57 ± 0.13 | 3.99 ± 0.23 | 2.98 ± 0.23 |
| **OGTT AUC (min/mM)** | **13.76 ± 0.27** | **14.16 ± 0.32** | **12.94 ± 0.19** | **13.99 ± 0.37** | **14.03 ± 0.23** | **14.53 ± 0.17** | **15.22 ± 0.83** |

Values are shown as the mean ± SEM.

**Table 8-2: Additional behavioural results (in bold) for the pair-housed FSL and FRL rats**

| | Pair-housed | | Single-housed | | | | |
|---|---|---|---|---|---|---|---|
| **Group** | **FRL** | **FSL** | **FRL** | **FSL** | **FSL-EXE** | **FSL-IMI** | **FSL-IMI+EXE** |
| **FORCED SWIM TEST** Immobility (sec) | | | | | | | |
| T(-7) | **75.63 ± 7.70** | **201.3 ± 12.02** | 73.75 ± 6.99 | 185.6 ± 15.94 | 179.4 ± 17.71 | 180.0 ± 14.70 | 180.0 ± 10.88 |
| T32-34 | **188.1 ± 14.14** | **271.3 ± 13.62** | 220.6 ± 12.55 | 255.7 ± 12.93 | 220.6 ± 12.97 | 240.7 ± 17.47 | 164.2 ± 19.38 |
| Swimming (sec) | **86.88 ± 10.52** | **67.50 ± 7.31** | **85.00 ± 6.41** | **65.00 ± 8.93** | **100.6 ± 6.08** | **61.43 ± 4.46** | **70.83 ± 11.36** |
| Climbing (sec) | **145.0 ± 14.70** | **81.25 ± 8.06** | **114.4 ± 10.92** | **99.29 ± 10.43** | **98.75 ± 13.62** | **117.9 ± 14.51** | **185.0 ± 26.61** |
| **OPEN FIELD** | | | | | | | |
| Distance traveled (cm) | **1922 ± 273.0** | **1819 ± 212.9** | **1641 ± 181.0** | **1898 ± 224.4** | **2439 ± 321.3** | **1257 ± 323.0** | **1739 ± 337.4** |

Values are shown as the mean ± SEM.

In **Figure 8-1**, the results from a pilot study are shown where we evaluated the baseline differences in running behaviour between FSL and FRL rats. Although the number of animals in the FRL group is small (n=4), it was clear that FRL rats had a substantially higher running activity than the FSL rats, and was found to be statistically significant following a Student's t-test ($p<0.01$).

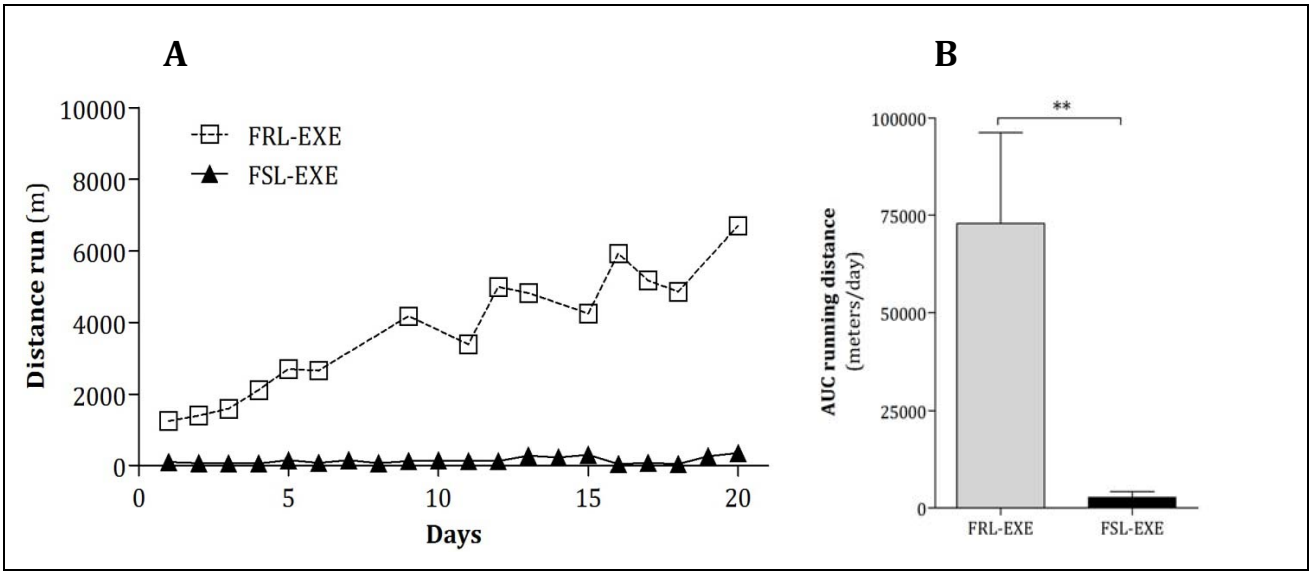


**Figure 8-1: The baseline running distances (m) of FSL and FRL rats as measured in a pilot study. (A) shows the daily distance run by each group for 20 days (some points missing due to a software failure), (B) shows the AUC calculated from (A)**. The numbers of rats in each group are as follows: FRL-EXE (4), FSL-EXE (8). **p=0.0025. Abbreviations used: FRL (Flinders resistant line), FSL (Flinders sensitive line), EXE (exercise).

## 8.2 EXPERIMENT 2: THE LPS-INDUCED MODEL OF DEPRESSION

In Table 8-3, additional behavioural parameters (i.e. swimming and climbing) that were measured in the FST in this experiment are shown, together with the immobility values for comparison.

**Table 8-3: Additional results from the FST (in bold) in LPS-treated animals**

| **FORCED SWIM TEST** (sec) | | | | | | |
|---|---|---|---|---|---|---|
| **Immobility** | 72.22 ±7.13 | 43.50 ± 6.15 | 56.11 ± 5.51 | 99.50 ± 8.25 | 57.00 ± 12.25 | 65.50 ± 11.44 |
| **Swimming** | **69.00 ± 7.37** | **77.00 ± 7.68** | **63.89 ± 5.51** | **51.00 ± 5.90** | **78.50 ± 8.13** | **47.00 ± 47.00** |
| **Climbing** | **150.0 ± 13.48** | **179.5 ± 11.63** | **180.0 ± 9.20** | **149.5 ± 11.24** | **164.5 ± 12.03** | **187.5 ± 16.65** |

Values are shown as the mean ± SEM.

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