An investigation into the antidepressant-like profile of pioglitazone in a genetic rat model of depression

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Abstract

Major depression is a highly prevalent mood disorder with chronic debilitating effects. Additional to a rising rate in incidence, depression is highly co-morbid with other psychiatric disorders, but also chronic cardiometabolic illnesses that present with an inflammatory component.

The exact aetiology of depression is still unknown, being multifactorial in its possible aetiology. Various hypotheses have attempted to shed light on both endogenous and exogenous risk factors as well as the underlying pathology that may lead to the development of the disease. This has led to a wide range of mediators being implicated, including biogenic amines, the HPA-axis, neurotrophic factors, inflammatory agents, the cholinergic system and circadian rhythm, to name a few.

The mechanisms of action of current treatment strategies, except for a few atypical and novel treatment approaches, are limited to interactions with monoamines and are at best only 65% effective. Many of these are also plagued by troubling side-effects, relapse and recurrence. It has therefore become imperative to explore novel targets for the treatment of depression that may produce more rapid, robust and lasting antidepressant effects with a less daunting side-effect profile.

The strong co-morbidity between depression and various cardiometabolic disorders, including cardiovascular disease, atherosclerosis, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) has led to the proposal that a metabolic disturbance may be a vital component that drives inflammatory and immunological dysfunction in depression. Supporting of this is evidence for a role of inflammatory cytokines and neurotrophic factors in the pathogenesis of depression.

It has also been demonstrated that a link exists between insulin- and nitric oxide (NO)-mediated pathways in the brain, which further highlights the role of oxidative stress and cell damage. Furthermore, evidence supports a role for oxidative stress and NO in T2DM and/or insulin resistance. Insulin has also been implicated in various physiological processes in the central nervous system (CNS) and may also influence the release and reuptake of neurotransmitters.
Preclinical and clinical evidence has provided support for the antidepressant-like effects of insulin-sensitizing peroxisome proliferator activated receptor (PPAR)-γ agonists, such as rosiglitazone and pioglitazone. In preclinical studies, however, these effects are limited to acute treatment with pioglitazone or sub-chronic (5 days) treatment with rosiglitazone. It is well-recognized that such findings need to be confirmed by chronic treatment paradigms. The aim of the current study was therefore to further investigate the proposed antidepressant-like effects of pioglitazone in a genetic animal model of depression, the Flinders sensitive line (FSL) rat, using a chronic treatment protocol.

The FSL rat model was reaffirmed as presenting with inherent depressive-like behaviour compared to its more resilient counterpart, the Flinders resistant line (FRL) rat. Moreover, imipramine demonstrated a robust and reliable antidepressant-like effect in these animals using the forced swim test (FST), thus confirming the face and predictive validity of the FSL rat model for depression.

In contrast to previous preclinical studies, acute dose-ranging studies with pioglitazone in Sprague Dawley rats delivered no significant anti-immobility effects in the FST, whereas results similar to that seen in the dose-ranging studies were observed following chronic treatment using FSL rats. Since altered pharmacokinetics could possibly influence the drug’s performance, another route of administration, viz. the subcutaneous route, was utilized as an additional measure to exclude this possibility. The results of the subcutaneous study, however, were congruent with that observed after oral treatment.

In order to confirm an association between altered insulin sensitivity and antidepressant action and demonstration by recent studies that thiazolidinediones may augment the efficacy of existing antidepressants, we therefore investigated whether concomitant treatment with gliclazide (an insulin releaser and insulin desensitizer) or pioglitazone (an insulin sensitizer) may alter the antidepressant-like effects evoked by chronic treatment with imipramine. Pioglitazone did not positively or negatively affect the antidepressant effect of imipramine, although gliclazide tended to decrease the anti-immobility effects induced by this antidepressant. Taken together and considering the current available literature, this finding supports evidence linking the insulin-PPARγ pathway to depression. However, further
explorative studies are required to delineate the role of insulin sensitivity and glucose homeostasis in depression and antidepressant response.

**Keywords:** peroxisome proliferator activated receptor (PPAR)-γ, metabolic syndrome (MetS), major depression, pioglitazone, gliclazide, Flinders sensitive line (FSL)
Major depressie is ’n baie algemene gemoedsteurnis met ’n kroniese afdalingseffek op pasiënte. Bykomend tot ’n verhoogde insidensie, kom depressie dikwels voor in kombinasie met ander psigiatriese afwykings, asook kroniese kardiometaboliëse siektetoestande wat met ’n inflammatoriese komponent presenteer.

Die presiese etiologie van depressie is steeds onbekend, syne die multifaktoriële aard daarvan. Verskeie hipoteses het al gepoog om lig te werp op beide die endogene en eksogene risikofakteore, sowel as die onderliggende patologie wat tot ontwikkeling van die siektetoestand mag aanleiding gee. Dit het daartoe geleë dat ’n wye verskeidenheid mediatore, waaronder biogene amiene, die HPA-as, neurotrofiese faktore, inflammatoriese agente, die cholinergiese stelsel en die sirkadiëse ritme, hierby betrek is.

Die werkingsmeganismes van huidige behandelingstrategieë, buiten vir enkele atipiese en nuwe benaderings, is beperk tot interaksies met monoamiene en is ten beste slegs 65% effektief. Baie van hierdie benaderings het egter ook steurende newe-effekte, terugslae en herhalende episodes tot gevolg. Dit het daarom noodsaaklik geword dat nuwe teikens vir die behandeling van depressie, wat ’n vinniger aanvang van werking het, sowel as kragtige en blywende antidepressiewe effekte met ’n minder ontmoedigende newe-effek profiel, verken moet word.

Die hoë ko-morbiditeit tussen depressie en verskeie kardiometaboliëse toestande, insluitend kardiovaskulêre siektes, arteriosklerose, tipe 2 diabetes mellitus (T2DM) en metaboliese sindroom (MetS), het aanleiding gegee tot die voorstel dat ’n metaboliese afwyking moontlik ’n baie belangrike komponent kan wees wat die inflammatoriese en immunologiëse wanfunksionering in depressie dryf. Hierdie voorstel word verder ondersteun deur bewyse vir ’n rol vir inflammatoriese sitokiene en neurotrofiese faktore in die patogenese van depressie.

Daar is bykomend aangetoon dat daar ’n verband tussen insulien- en stikstofoksied (NO)-bemiddelde weë in die brein bestaan – ’n verdere beklemtioning van die rol van oksidatiewe stres en selskade. Voorts is daar ook bewyse dat oksidatiewe stres en NO ’n belangrike rol speel in T2DM en/of insulienweerstandigheid. Insulien is ook betrokke by verskeie fisiologiëse
prosesse in die sentrale senuweestelsel (SSS) en kan moontlik ook die vrystelling en heropname van neurotransmittors beïnvloed.

Beide prekliniese en kliniese studies het ondersteunende bewyse gelewer vir die antidepressiewe effekte van die insuliensensitisierende peroksisoomproliferator-geactiveerde resceptor (PPGR)-γ-agoniste soos rosiglitasoon en pioglitasoon. Hierdie resultate in prekliniese studies is egter tot dusver slegs aangetoon tydens akute behandelings met pioglitasoon of subkroniese behandeling (5 dae) met rosiglitasoon en dit is noodsaaklik dat sulke bevindings bevestig moet word in 'n kroniese behandelingsraamwerk. Die doelwit van die huidige studie was dus om die voorgestelde antidepressiewe effekte van pioglitasoon verder te ondersoek in 'n genetiese dieremodel van depressie, die Flinders sensitiewe lyn (FSL)-rot, deur gebruik te maak van 'n kroniese behandelingsprotokol.

Die huidige studie het herbevestig dat die FSL-dieremodel met inherente depressiewe gedrag presenteer in vergelyking met die meer geharde en lewenskragtige Flinders weerstandige lyn (FWL) rot. Bowendien het imipramien 'n krachtige en betroubare antidepressiewe effek tydens die geforseerde swemtoets in hierdie dieremodel vertoon en daardeur die sig-en voorspelbaarheidsgeldigheid van die FSL-depressiemodel herbevestig.

In teenstelling met vorige prekliniese studies het akute dosis-responsstudies met pioglitasoon in Sprague Dawley-rotte geen beduidende anti-immobiliteitseffekte in die geforseerde swemtoets veroorsaak nie, terwyl resultate soortgelyk aan dié wat in die dosis-responsstudies gesien is, waargeneem is na kroniese behandeling van FSL-rotte. Aangesien farmakokinetiese aspekte die effektiwiteit van die middel kan beïnvloed, is daar van 'n addisionele toedieningsroete, nl. die subkutaneuse roete, gebruik gemaak as bykomende maatreël om hierdie moontlikheid uit te skakel. Die resultate van die subkutaneuse toedieningstudie was egter ooreenstemmend met dié van die orale behandelingstudie.

Om die verband tussen gewysigde insuliensensitiwiteit en antidepressiewe werking, asook die aanduiding uit onlangse studies dat tiasolidiendione moontlik die doeltreffendheid van bestaande antidepressiewe middels kan verhoog, te bevestig het ons dit ondersoek of die gelykydige toediening van imipramien met óf gliklasied ('n insulienvrysteller en
insuliendesensitiseerder) óf pioglitasoon (‘n insuliensensitiseerder) die antidepressiewe werking wat deur kroniese behandeling met imipramien ontlok word, kan wysig. Pioglitasoon het nie die effek van imipramien op ‘n positiewe of negatiewe wyse beïnvloed nie, alhoewel gliklasied ‘n geneigheid getoon het om die anti-immobiliteitseffeekte van hierdie antidepressant te onderdruk. Samevattend en inaggenome die beskikbare literatuur, ondersteun hierdie bevinding bewyse wat die insulien-PPGRγ-weg met depressie verbind. Verdere studies is egter nodig om die rol wat insuliensensitiwiteit en glukose-homeostase in depressie en antidepressantrespons, speel, te ondersoek.

**Sleutelwoorde:** peroksismoproliferator-geakteerde reseptor (PPGR)-γ, metaboliese sindroom (MetS), major depressie, pioglitasoon, gliklasied, Flinders sensitiewe lyn (FSL)
I wish to express my sincere appreciation to the following people:

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Excerpts from the current study have been presented as follows:

Role of the Peroxisome Proliferator Activated Receptor (PPAR)-γ Pathway in Mood Regulation and Antidepressant Action.


(Presented as podium presentation at the 6th International Conference on Pharmaceutical and Pharmacological Sciences (6th ICPPS) in Durban, South Africa, 25-27 September 2011.)
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<td>A</td>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
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<tr>
<td></td>
<td>ALS</td>
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<td>B</td>
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<td>brain-derived neurotrophic factor</td>
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<td></td>
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<td>C</td>
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<td></td>
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<td>cAMP response-element binding protein</td>
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<td>E</td>
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<tr>
<td>F</td>
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<td>Flinders resistant line</td>
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<tr>
<td></td>
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<tr>
<td>G</td>
<td>GABA</td>
<td>gamma-amino butyric acid</td>
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<tr>
<td>H</td>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>I</td>
<td>ICPE</td>
<td>International Consortium of Psychiatric Epidemiology</td>
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<td>P</td>
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<td>peroxisome proliferator-activated receptor</td>
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<td>pedunculopontine nucleus</td>
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<tr>
<th>Abbreviation</th>
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<td>5-HT</td>
<td>serotonin</td>
</tr>
<tr>
<td>SASH</td>
<td>South African Stress and Health</td>
</tr>
<tr>
<td>SCN</td>
<td>suprachiasmatic nucleus</td>
</tr>
<tr>
<td>SHR</td>
<td>spontaneously hypertensive rats</td>
</tr>
<tr>
<td>SNRI</td>
<td>serotonin and noradrenalin reuptake inhibitor</td>
</tr>
<tr>
<td>SRI</td>
<td>serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
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<tr>
<td>TCA</td>
<td>tricyclic antidepressant</td>
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<tr>
<td>TNF</td>
<td>tumour necrosis factor</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WKY</td>
<td>Wistar Kyoto</td>
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<td>WMH</td>
<td>World Mental Health</td>
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CHAPTER 1

Introduction

1.1. Problem statement

Depression is a heterogeneous syndrome, consisting of a range of underlying pathophysiologies (Nestler et al. 2002). The World Health Organization (WHO)’s World Mental Health (WMH) surveys have suggested that about half of the population of six countries (among which the United States, France and South Africa) will eventually suffer from a mental disorder (Kessler et al. 2007), while current statistics indicate that about ten percent of individuals in South Africa will suffer from a major depressive episode during their lifetime (Tomlinson et al. 2009).

Another factor complicating this already bleak scenario is the frequent occurrence of depression together with other co-morbid conditions that include, but is not limited to, other psychiatric disorders, especially anxiety disorders (Blazer et al. 1994, Gorman 1996, Kessler et al. 1997, Schoevers et al. 2003), cardiovascular diseases (Halaris 2009), metabolic syndrome (MetS) (Capuron et al. 2008, Mendelson 2008, Skilton et al. 2007) and inflammatory and autoimmune diseases, e.g. cancer, rheumatoid arthritis (Brown et al. 1982, Danner et al. 2003, Dantzer et al. 2008, Ford et al. 2004, Pop et al. 1998). Indeed, the prevalence of depression is doubled in individuals with type 2 diabetes mellitus (T2DM) (Anderson et al. 2001, Egede et al. 2002), while patients with MetS are more likely to have depression than those without MetS (Capuron et al. 2004).

Since the first seminal discoveries in the 1950’s and 1960’s that led to the development of our current armamentarium of antidepressant drugs, much progress has been made with regard to our understanding of the neurobiology of depression and antidepressant action. However, even though a range of drugs are now available for the treatment of depression, the vast majority of these drugs interact with the monoaminergic system by interfering with the reuptake and/or metabolism of biogenic amines (Nestler et al. 2002, Krishnan et al. 2008, Vetulani et al. 2000). Furthermore the use of these antidepressants is associated with a delayed onset of action, poor treatment outcomes, and a high prevalence of relapse and
recurring depressive episodes that may lead to an increased occurrence of suicide (Nestler et al. 2002, Krishnan et al. 2008, Paykel 1998, Trivedi et al. 2006). In addition, these drugs, especially the tricyclic antidepressants (TCAs), are associated with troubling side-effects that adversely affect patient compliance (Berken et al. 1984, Furukawa et al. 2002).

Although several breakthroughs have been made regarding the development of novel treatment strategies, few of these have successfully been implemented in practice. The exploration and use of novel treatment strategies to target underlying causes of both depression and its co-morbid diseases are essential to improve efficacy, treatment outcomes and compliance. In this regard it may be beneficial to explore the use of drugs targeting possible overlapping neurobiological causes of the afore-mentioned co-morbid illnesses.

There is an abundance of evidence linking inflammation and inflammatory markers to depression (Capuron et al. 2008, Dantzer et al. 2008, Krishnan et al. 2008, Loftis et al. 2004). The peroxisome proliferator activated receptor (PPAR)-γ is associated with suppression of the immune response through its ability to inhibit the synthesis and release of inflammatory cytokines (Guri et al. 2010, Lee et al. 2005, Martin 2009, Ramakers et al. 2007). It is also central in the regulation of carbohydrate metabolism and is a vital biological mediator of the intracellular actions of insulin (Guo et al. 2006, Kamon et al. 2003, Rangwala et al. 2003). In fact, T2DM and insulin resistance present with many characteristic symptoms and biochemical abnormalities consistent with an inflammatory state (Capuron et al. 2008, Skilton et al. 2007, Raison et al. 2006). Furthermore, activation of PPARγ has been found to improve various central nervous system (CNS) dysfunctions with an inflammatory component, including spinal cord injury, brain injury, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), Alzheimer’s disease and depression (Eissa Ahmed et al. 2009, Heneka et al. 2007, Kemp et al. 2009, Kummer et al. 2008, McTigue 2008, Morgenweck et al. 2010, Ramanan et al. 2010). This knowledge has been extended in recent years so that PPARγ is now proposed to have a central role in a number of psychiatric diseases (Eissa Ahmed et al. 2009, Bright et al. 2008, García-Bueno et al. 2010, Kemp et al. 2011).
Evidence has suggested that the insulin-sensitizing thiazolidinedione group of PPARγ-agonists may exert antidepressant-like effects in a clinical setting, an observation that was first made in type 2 diabetics (Kemp et al. 2009, Kemp 2010). Indeed, pre-clinical studies in rodents have confirmed the antidepressant-like effects of rosiglitazone (Eissa Ahmed et al. 2009), while, during the conducting of the current study, a similar response has been described for pioglitazone by Sadaghiani and colleagues (2011), incidentally also the subject of this investigation. However, the latter study only documented an antidepressant response following acute administration of pioglitazone. Since depression only responds to chronic, but not acute treatment with antidepressants, this requires extension to chronic treatment to confirm its possible antidepressant-like actions. Moreover, even though a number of recent clinical studies have found that PPARγ-agonists may augment the action of concurrently administered antidepressants (Kemp et al. 2011, Rasgon et al. 2010), neither of the above two studies investigated the effect of co-administered thiazolidinediones on the antidepressant-like actions of a known antidepressant.

The above findings potentially implicate the PPARγ receptor in the pathogenesis of depression and suggest that thiazolidinediones and other PPARγ-agonists may represent a novel therapeutic avenue in the treatment of depression. However, further research is necessary to more broadly validate the pharmacological effects of PPARγ-agonists such as pioglitazone as putative antidepressants. Moreover, it is now imperative that the antidepressant effects of the thiazolidinediones in general, and in particular pioglitazone, be studied following chronic treatment and also to explore the possibility of using PPARγ-agonists to augment the actions of existing antidepressants. This study has set about to address these issues. Importantly, this work makes a further valuable contribution by exploring these questions in a genetic rodent model of depression, the Flinders sensitive line (FSL) and Flinders resistant line (FRL) rat.
1.2. Project hypothesis, aims and objectives

1.2.1. Hypothesis

- Using the FSL rat model of depression, chronic administration of the PPARγ-agonist and insulin sensitizer, pioglitazone, will present with antidepressant-like actions comparable to the known antidepressant, imipramine, the latter following chronic administration.

- Chronic administration of the insulin releaser and a drug that essentially compromises insulin sensitivity, gliclazide, will worsen depressive-like behaviour in the FSL rat.

- Chronic administration of the PPARγ-agonist, pioglitazone, but not the insulin releaser, gliclazide, will enhance the antidepressant-like actions of the known antidepressant, imipramine, in the FSL rat, the latter following chronic administration.

- Chronic administration of the insulin releaser, gliclazide, will attenuate the antidepressant-like effects of imipramine in the FSL rat, the latter following chronic administration.

1.2.2. Study aims and objectives

- Primary aims and objectives:

  - Demonstrate the ability of acute pioglitazone treatment to improve depressive-like behaviour in the standard Sprague Dawley rat

  - Reconfirm that the FSL rat presents with inherent depressive-like behaviours relative to its healthy FRL-counterpart when subjected to the FST
Demonstrate that this depressive-like behaviour can be reversed by chronic treatment with a known antidepressant compound, such as the serotonin reuptake inhibitor (SRI), fluoxetine, or the TCA, imipramine.

Investigate the antidepressant-like effects of the PPARγ-agonist, pioglitazone, following a chronic treatment protocol in the FSL rat.

Secondary aims and objectives:

To compare the pioglitazone response to either that of fluoxetine or imipramine using the forced swim test (FST), a primary behavioural screening tool for depressive-like and antidepressant-like behaviours. This will be undertaken in order to confirm earlier findings describing the antidepressant-like effects of thiazolidinediones in rodents using the FST following acute and sub-chronic treatment (Eissa Ahmed et al. 2009, Sadaghiani et al. 2011). Pending the outcome of this study, this work will then be extended to prove the same hypothesis using the FSL rat, a genetic rodent model of depression. However, before the latter study can be undertaken it will first be necessary to demonstrate that the FSL rat presents with inherent depressive-like behaviours (refer to primary aims and objectives).

The study will then investigate the antidepressant-like effects of the insulin-releasing drug, gliclazide, following chronic treatment in the FSL rat and conclude with an investigation into whether pioglitazone or gliclazide can modify the antidepressant-like actions of a known antidepressant following chronic treatment in the FSL rat.

The FST, an established animal screening model for antidepressant efficacy, was used as primary measure of antidepressant-like effects in all instances.

1.3. Project design

This project will comprise two behavioural studies viz. an acute dose response analysis and a chronic treatment behavioural study. Since no treatment-related data on the use of pioglitazone in rats were available at the time of designing the study, a dose response analysis
will initially be performed in Sprague Dawley rats (n=8 per group) (see Chapter 2, Methods) in order to confirm earlier findings and to establish a proper dose for pioglitazone that can be applied in a later chronic treatment regimen. These data will then be compared to that of acute treatment with a known antidepressant, either fluoxetine or imipramine. In the chronic behavioural study the antidepressant-like effect of pioglitazone will be compared to that of fluoxetine and/or imipramine, as well as gliclazide, an insulin releasing agent. In order to improve the sensitivity and validity of the findings, the latter behavioural tests will be performed in FSL rats (n=12 per group) and the results compared to that of a vehicle-treated control group. A group of Flinders resistant line (FRL) rats will also be treated with the vehicle to establish the depressive phenotype of the FSL rat as quantified in the FST. The FST will be preceded by an assessment of general locomotor activity.

1.4. Expected results

Building on the hypothesis presented earlier, and considering what is known regarding the current thinking on the neurobiology and pathophysiology of depression, as well as the established behavioural effects of PPARγ-agonists, the following results may be expected:

- It is proposed that fluoxetine and/or imipramine will present with antidepressant-like effects using the FST following both acute and chronic treatment regimes in Sprague Dawley and FSL rats, respectively.

- It is proposed that pioglitazone will present with antidepressant-like effects in the FST following both acute and chronic treatment regimes in Sprague Dawley and FSL rats, respectively. Considering that pioglitazone is approximately 10 times less potent than rosiglitazone (Junichi et al. 2000), it is predicted that pioglitazone will present with antidepressant-like effects at a dose of between 70-120 mg.kg⁻¹/day.

- With the strong positive correlation in the literature between depression and T2DM, it is postulated that the insulin releaser, gliclazide, will worsen depressive behaviour
in the FSL rat and will attenuate the antidepressant-like response of a known antidepressant, i.e. either fluoxetine or imipramine

- Considering earlier clinical and preclinical data confirming the role of PPARγ-agonists in depression (Eissa Ahmed et al. 2009, Heneka et al. 2007, Kemp et al. 2009, Kummer et al. 2008, McTigue 2008, Morgenweck et al. 2010, Ramanan et al. 2010), it is predicted that chronic treatment with pioglitazone will attenuate the depressive-like behaviour typical of the FSL rat and will bolster the antidepressant effects of a known antidepressant (either fluoxetine or imipramine) in this model

1.5. General points

This dissertation will be written and submitted in the standard format for thesis/dissertation submission, as approved by the North-West University. This format includes an introductory chapter, a chapter covering the relevant literature overview, chapters containing experimental results and a discussion thereof and finally a chapter containing concluding remarks and suggestions for future study.
2.1 Depression

It has been more than half a century since several classes of medication were discovered by chance for use in the treatment of depression, viz. the tricyclic antidepressants (TCAs) and the monoamine oxidase inhibitors (MAOIs). These drugs soon became established as an essential part of the treatment strategy of depression and forever changed the way in which mood disorders are managed. However, despite their initial promise, these drugs are at best only 65% effective, while troublesome side-effects and a slow onset of action have compromised their success as effective antidepressants (Nestler et al. 2002, Fava 2003, Holtzheimer et al. 2006, Machado-Vieira et al. 2009). Moreover, although many new classes of antidepressant drugs have been developed since then, little advances have been made in developing novel drugs with improved efficacy, while there is still debate as to the exact mechanisms by which these drugs mediate their mood elevating effects (Holtzheimer et al. 2006) as well as a lack of clarity about the genetic and neurobiological foundations of depression (Nestler et al. 2002).

Depression is a multifactorial illness, comprising not only of genetic and environmental determinants, but also consisting of a host of mood, cognitive, endocrine and neuronal abnormalities (Nestler et al. 2002, Krishnan et al. 2008). Indeed, before improved pharmacotherapies can be expected, we need to understand the various pathways in the brain that are responsible for the regulation of mood.

2.1.1 Incidence and demographics of depression

With a lifetime prevalence of 8-12% and a median age of onset in the early to mid-twenties in most countries (Andrade et al. 2003), depression is not only one of the most common neuropsychiatric disorders, but also one of the most disabling (Kessler et al. 2005). A survey by the International Consortium of Psychiatric Epidemiology (ICPE) indicates that up to half of people with a lifetime history of major depressive disorder also have a history of at least one anxiety disorder. Major depressive episodes have been found to be strongly co-morbid with,
and temporally secondary to, anxiety disorders, with primary panic and generalized anxiety disorders being the most powerful predictors of the first onset of secondary major depressive disorder (Andrade et al. 2003).

Population studies have also shown that depression is almost twice as common in females as in males, the higher incidence in females being attributed to factors such as intra-psychological and psychosocial gender roles and other gender-related aspects such as endocrine stress reactions and neuropsychological processes (Kuehner 2003). The risk of developing depression also increases with neurological disorders like stroke, Parkinson’s disease and multiple sclerosis and also in the first year after giving birth (Rickards 2005).

In South Africa, the South African Stress and Health (SASH) study found that mood and anxiety disorders had the highest incidence among common mental health disorders (Herman et al. 2009), with depression having a lifetime prevalence of 9.7% and occurring more frequently in individuals with a low level of education (Tomlinson et al. 2009).

### 2.1.2 Aetiology of depression

There is no consensus about the cause of depression. Rather its aetiology is believed to be multifactorial, being most strongly correlated with prior stressful life events, genetic risk (heritability ≈40%), and various unknown disease genes (Fava et al. 2000, Kendler et al. 2001). It may also be idiopathic, a side-effect of drugs (e.g. interferon-α or isotretinoin) or secondary to systemic illness (Nestler et al. 2002, Drevets 2001). Pathogenesis may also be attributed to abnormal activity of the hypothalamic-pituitary-adrenal (HPA) axis, alterations in neurotrophic signalling or abnormal hippocampal neurogenesis (Krishnan et al. 2008).

Despite increasing prosperity, improved health care and a thriving antidepressant industry, the prevalence of depression and other anxiety disorders continues to rise (Lambert 2006). This increase may be attributed to several factors, including industrialization and the younger age at which individuals are being exposed to stressful conditions (Robinder 1999) as well as the progressive occurrence of less active lifestyles, unhealthy diets (Beydoun et al. 2010,
Thomson et al. 2010) and chronic medical illnesses such as T2DM, cardiovascular disorders, hypertension and obesity (Patten 2005).

Taking this into account, it is unlikely that depression could be associated with any specific cause, considering that many biological and non-biological processes, environmental aspects and various risk factors may contribute to the development of depression in any given individual (Hankin 2006, Maja et al. 2010).

2.1.3 Pathophysiology

Various hypotheses regarding the pathophysiology of depression have been proposed. Although the following hypotheses are not meant to be an exhaustive list of possible theories and hypotheses, they nevertheless represent the most popular approaches toward understanding the illness:

2.1.3.1 The biogenic amine hypothesis

Since the discovery of the monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), the pathophysiology of depression has been dominated by the amine hypothesis. It postulates that depression is caused by a deficit in monoamine function in the brain, specifically noradrenalin and serotonin (Berton et al. 2006). These two monoamines demonstrate a wide distribution throughout the brain, but are especially evident in the reward and cortico-limbic regions of the brain, such as the ventral striatum, hippocampus, frontal cortex, hypothalamus, amygdala, olfactory bulb and others (see Fig. 2-1 and Fig. 2-2). These deficits may be restored by antidepressant drugs that interfere with monoaminergic signalling, e.g. MOAIs, TCAs, and in later years the serotonin reuptake inhibitors (SRIs).

The greatest shortcoming of this hypothesis, however, is the rapid action of these drugs on endogenous monoamine pathways (Krishnan et al. 2008), but that does not translate into prompt behavioural enhancements. Indeed, the mood-elevating effects of currently prescribed
antidepressant drugs can take several weeks (and in some cases up to months) to take effect (Machado-Vieira et al. 2009).

Figure 2-1: Serotonergic projection from the raphe nuclei in the human brain. (Adapted from Nutt et al. 1999)
2.1.3.2 The dysregulation hypothesis

Dysregulation of the HPA-axis has been reported in depressed individuals (Holsboer 2000) which may lead to altered neuroendocrine activity (Nemeroff 1996, Owens et al. 1993), especially corticotrophin-releasing factor (CRF) that mediates the synthesis of adrenocorticotropic hormone (ACTH) by the anterior pituitary (Fig. 2-3). Impaired HPA negative feedback leads to elevated circulating corticosteroid levels (Pariante et al. 2001) which may damage hippocampal neurons (Nestler et al. 2002) (also see Fig. 2-4) leading to loss of hippocampal volume (Holsboer 2000) and further dysregulation of the HPA-axis (Nestler et al. 2002). The dysregulation of the HPA-axis then leads to secondary changes in monoaminergic transmission.
For an antidepressant drug to induce a proper antidepressant response, it has been suggested that it is imperative that it demonstrates the ability to readjust HPA-axis abnormalities (Nemeroff 1996, Holsboer et al. 1996, Nemeroff 1988) – it has also been proposed that the mechanism by which antidepressants mediate their clinical effects is related to normalization of the dysregulated HPA-axis (Holsboer 2000, Nemeroff et al. 2002).

Figure 2-3: Regulation of the Hypothalamic-Pituitary-Adrenal Axis. Prominent neural inputs to the paraventricular nucleus (PVN) of the hypothalamus include excitatory afferents from the amygdala and inhibitory afferents from the hippocampus. Ascending monoamine pathways (not shown) also serve as important inputs. CRF is released by these neurons and acts on the corticotrophs of the anterior pituitary to release ACTH that reaches the adrenal cortex via the bloodstream where it stimulates the release of glucocorticoids. Glucocorticoids suppress CRF and ACTH synthesis and release. At higher levels, glucocorticoids impair and may damage the hippocampus which may initiate and maintain a hypercortisolemic state related to depression. (Adapted from Nestler et al. 2002)
2.1.3.3 Neuroplasticity

The basis of this hypothesis is based on the ability of the brain to undergo structural alterations in reaction to various stimuli (Duman 2002). Failure of this process to function normally may lead to various neuroplastic changes, e.g. loss of synaptic interactions, increased atrophy and cell death, suppressed neural cell proliferation and changes in receptor density (Duman et al. 2000). A role for various molecular determinants have also been described – these include cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) – that are also altered by stress (see Fig. 2-4) and antidepressant treatment (Duman 2002). Both serum BDNF levels and CREB phosphorylation and protein levels are reduced in depressed individuals (Karege et al. 2002, Nibuya et al. 1996, Shimizu et al. 2003), while an inverse relationship exists between serum levels of BDNF and the severity of depression (Shimizu et al. 2003). It has however been shown that antidepressant treatment is able to reverse the aforementioned deficit in BDNF (Aydemir et al. 2005) and to increase phosphorylation and binding of CREB (Frechilla et al. 1998, Laifenfeld et al. 2005).
Figure 2-4: Schematic representation depicting potential routes by which stressors and cytokines could influence depressive state. A stressor could potentially influence major depression through two major routes that feed into several interconnected loops. Stressors and cytokines both increase hypothalamic CRH release. In addition to activating HPA functioning, CRH may influence serotonin (5-HT) processes, and GABA activity may act as a mediator in this regard. This, in turn may influence depression directly, or may do so by impairing neuroplastic processes. An alternative, although not necessarily mutually exclusive pathway, involves cytokine/stress activation of various signalling pathways. These would influence oxidative or apoptotic mechanisms, leading to altered growth factor expression (e.g., BDNF), hence again favouring impaired neuroplastic processes, culminating in depression. (Adapted from Anisman et al. 2008)

Seemingly, the activation of CRH in response to stressors has an effect on serotonin regulation, which in turn affects gamma amino butyric acid (GABA) transmission. This has led to the suggestion that SSRIs may illicit their effects indirectly through influencing GABA’ergic transmission (Zhong et al. 2004). The possibility also exists that interactions between CRH and GABA may have depressive effects since CRH may influence the activity of noradrenalin and serotonin (Ruggiero et al. 1999) or may even induce serotonin receptor changes, thereby affecting GABA in the frontal cortex (Tan et al. 2004).
2.1.3.4 The cholinergic-adrenergic hypothesis

This hypothesis revolves around imbalances between central cholinergic and adrenergic neurotransmitter activity in those areas of the brain that regulate affect, with depression being a disease of cholinergic dominance (Janowsky et al. 1972). On the other hand, mania may be attributed to an over-activity of noradrenergic neurotransmission compared to cholinergic transmission (Fritze et al. 1995).

Cholinergic receptors are widely expressed in the brain (see cholinergic projection in Fig. 2-5). Cholinergic neurotransmission innervates both the hippocampus and frontal cortex (Mash et al. 1986, Spencer Jr. et al. 1986) where it is involved in attention, learning and memory (Sarter et al. 1999, Everitt et al. 1997) – functions that are severely affected in mood disorders.

In support of this hypothesis, it has been shown that cholinomimetics may induce depressive symptoms, such as anhedonia, in healthy volunteers (Risch et al. 1981). As already pointed out, depression is also associated with deficits in cognitive processes, which are largely influenced by cholinergic function (Deutsch 1971, Jerusalinsky et al. 1997). It has been suggested that citalopram’s ability to improve memory deficits may be attributed to enhancing acetylcholine release (Egashira et al. 2006). Furthermore, lithium up-regulates cholinergic receptors in the hippocampus (Marinho et al. 1998), while many of its neurobiological effects can be related to cholinergic influence (Ghasemi et al. 2011, Harvey et al. 1990a, Harvey et al. 1990b, Liebenberg et al. 2010). The cholinergic system also seems to play an important role in the antidepressant effects of phosphodiesterase-5 inhibitors (Liebenberg et al. 2010).

Despite the evidence of cholinergic involvement in depression and antidepressant action, there have also been a great many inconsistencies in the literature involving cholinergic-based drug therapies for the treatment of depression (Dagyte et al. 2011, Ferguson et al. 2000, Gatto et al. 2004, Goldman et al. 1983, Howland 2009b, Shytle et al. 2002), foremost among these being that if depression is a hypercholinergic state, why are anticholinergic agents ineffective as antidepressants (Fritze et al. 1995, Goldman et al. 1983, Gillin et al. 1995)? The complexity of the relationship between the cholinergic system and mood regulation is evident in preclinical...
studies on sildenafil, where both atropine and sildenafil alone are ineffective antidepressants, yet a combination of sildenafil plus atropine is comparable in efficacy to known reference antidepressants in a known animal model of depression (Liebenberg et al. 2010, Brink et al. 2008). Nevertheless, centrally acting anticholinergics may be rapidly effective in treatment resistant depression (Drevets et al. 2010, Furey et al. 2006, Furey et al. 2010), thus emphasizing that the cholinergic system plays a definite, yet poorly understood, role in the aetiology of depression. Moreover, the widely used genetic animal model of depression, the Flinders sensitive line (FSL) rat, was initially observed to present with increased activity of the cholinergic system in a number of limbic brain regions (Overstreet et al. 2003).

![Figure 2-5: Major neural cholinergic projections. The pedunculopontine nucleus (PPN) projects to the hypothalamus, while the nucleus basalis projects to the neocortex, hippocampus and amygdala. (Adapted from (Bohnen et al. 2011))](image-url)
2.1.3.5 The circadian rhythm hypothesis

Disrupted sleep cycles are one of the notable symptoms observed in depressed individuals (Berger et al. 1993) (see Table 2-1). One could therefore anticipate that depression would be associated with a disruption in circadian rhythm (Wirz-Justice et al. 1993) – a physiological feature that in mammals is controlled by the suprachiasmatic nucleus (SCN) (see Fig 2-6) (Hastings 1997). The SCN is regarded as the “master clock” responsible for regulating a number of physiological (e.g. body temperature, heart rate, blood pressure), behavioural (mood, cognition, sleep-wake cycle, locomotor activity) and biological (e.g. cortisol, thyroid stimulating hormone, parathyroid hormone) processes in the body (Hickie et al. 2011). It is not surprising then that depression is associated with the dysregulation of many of these physiological processes, such as hypercortisolemia, deficits in cognition and mood, and cardiovascular abnormalities (Hickie et al. 2011). Circadian rhythms are ultimately determined by the mutual interplay between the SCN and the pineal gland, the source of the circadian rhythm regulator, melatonin (Altun et al. 2007).

The phase and amplitude of SCN rhythm is determined by the balance between melatonin (at M₁ and M₂ receptors) and serotonin (at 5HT₂c receptors), with depression being characterised by a relative loss of the melatonin surge in the dark cycle, resulting in loss of SCN regulation via the pineal gland (Hickie et al. 2011, Racagni et al. 2011). Many patients who suffer from depression also present with chronic insomnia and a decrease in REM latency after night-time sleep has commenced (Riemann et al. 2001) as well as increased day-time body temperature (Rausch et al. 2003). This may be the result of deviations in pathways responsible for regulating the sleep/wake cycle (Armitage 2007), for instance decreases in (Parry et al. 2001) and earlier offset times and shorter durations of release (Parry et al. 1990) of melatonin. These patients also present with increased cortisol levels compared to healthy individuals, with the morning spike in cortisol levels tending to occur earlier (Yehuda et al. 1996). The overall increase in cortisol is associated with neurotoxic effects on a number of brain regions, especially the hippocampus that is responsible for controlling the stress response (Fig 2-3).
Consequent atrophy of the hippocampus results in failed regulation of the stress response, leading to ongoing hypercortisolemia and maladaptive behaviour (Harvey et al., 2003).

The presence of chronic insomnia increases the risk of developing an affective disorder and may probably increase the odds of developing co-morbid cardiovascular and metabolic disturbances (Riemann et al. 2011). Most notable, and in strong support of this hypothesis, is the fact that compounds which enhance melatonergic function, for instance the novel antidepressant, agomelatine (see §2.1.6), have noteworthy antidepressant effects.

2.1.3.6 Inflammatory and neurodegenerative hypotheses

As mentioned in section 2.1.4 below, various brain structures undergo both structural and cellular changes in patients suffering from depression which may be attributed to enhanced neurodegeneration and decreased neurogenesis (Maes et al. 2009). Neurogenesis may be compromised by stressful conditions (Gould et al. 1997) (Fig. 2-2) and stressors may also be responsible for developmental abnormalities in brain regions involved in stress responses (Bremner et al. 1998), whereas environmental enrichment (Kempermann et al. 1997) and most antidepressant treatments (Malberg et al. 2000) have the ability to stimulate neurogenesis.

A strong relationship has been demonstrated between depression and the presence of inflammation and its associated inflammatory mediators (Capuron et al. 2008, Anisman et al. 2003) (also see Fig. 2-4). These mediators include the proinflammatory cytokines, interleukin (IL) -1, -2, -6 and 8, interferon (IFN)-γ and tumour necrosis factor (TNF)-α (Schiepers et al. 2005) that, when administered to a healthy individual, may induce a syndrome described as sickness behaviour (Capuron et al. 2004, Yirmiya 1997) (a state in which many of the symptoms coincide with those seen in depression (Dantzer et al. 2008)).

An occurrence that lends further support for the involvement of inflammation in affective disorders is the high incidence of co-morbidity between depression and diseases that present with chronic inflammation, i.e. coronary heart disease (Vieweg et al. 2010), metabolic
syndrome (MetS) (Skilton et al. 2007), multiple sclerosis (Gold et al. 2009) and rheumatoid arthritis (Creed et al. 1992).

2.1.4 Neuroanatomy of depression

When considering all the brain structures involved in mood and cognitive function as well as in the stress response, it can be expected that several brain regions will be affected by mood disorders. However, functional abnormalities in specific brain regions have been proposed to be central to the pathophysiology of depression, these include the prefrontal cortex and the limbic region, most notably the hippocampus, amygdala and ventral striatum (Nestler et al. 2002) (see Fig. 2-6).

Neuroimaging studies have been central in identifying the key structures involved in the pathophysiology of depression, showing decreases in hippocampal volume of up to 15% in depressed patients (Campbell et al. 2004), as well as reductions in grey-matter volume and glial density in the prefrontal cortex and the hippocampus (Sheline 2003). These regions are thought to mediate the cognitive aspects of depression, such as feelings of worthlessness and guilt (Krishnan et al. 2008).

Even though most research into the neuroanatomy of mood disorders has focused mainly on the hippocampus and prefrontal cortex, there is an increasing realisation that several subcortical structures are also implicated, especially regions involved in reward, fear and motivation (Yadid et al. 2001), such as the nucleus accumbens, amygdala, and hypothalamus. The hypothalamus is especially important for its role in the stress response and intermediary metabolism, both of which are strongly affected in depression, leading to, for example, altered biological rhythms, hypercortisolemia, sleep disturbances, and altered immune function, as well as altered metabolic profile – the latter leading to altered glucose metabolism and obesity (Gardner et al. 2011, Harvey 2008).

Other brain imaging studies have used changes in blood flow and glucose metabolism as measures to implicate certain brain regions affected in depression by comparing resting brain
state activity of depressed patients to that of healthy patients (Koenigs et al. 2009). These studies have implicated putative roles for the ventromedial and dorsolateral prefrontal cortex in depression, as well as the basal ganglia (Soares et al. 1997).

![Anatomy of the human brain](image)

**Figure 2-6: Anatomy of the human brain.** Regions most affected in depression are indicated. (Adapted from Thatcher et al. 2008)

### 2.1.5 Symptomatology and diagnosis of depression

Core symptoms of depression include depressed mood, anhedonia (reduced ability to experience pleasure from natural rewards such as food, sex and social interaction), fatigue, irritability, difficulties in concentrating, and abnormalities in appetite and sleep (‘neurovegetative symptoms’) (Nestler et al. 2002, Krishnan et al. 2008, Knol et al. 2006). Apart from an associated increased risk of death due to suicide, an increased risk of developing coronary artery disease and type 2 diabetes mellitus (T2DM) also contributes to increased mortality in patients suffering from depression (Krishnan et al. 2008).
Diagnosing depression is subjective and is based on the presence of a combination of at least five of the symptoms described in Table 2-1, as stipulated by criteria specified in the Diagnostic and Statistic Manual (DSM-IV). The presenting symptoms must be evident for a period longer than two weeks (Nestler et al. 2002) with at least one of the symptoms being either depressed mood or loss of interest of pleasure (American Psychiatric Association 2000).

Table 2-1: Diagnostic criteria for major depression (adapted from Nestler et al. (2002) and Akechi et al. (2009))

- Depressed mood
- Irritability
- Low self esteem
- Feelings of self-reproach, worthlessness and guilt
- Decreased ability to concentrate and think
- Decreased or increased appetite
- Weight loss or weight gain
- Insomnia or hypersomnia
- Low energy, fatigue or increased agitation
- Decreased interest in pleasurable stimuli
- Recurrent thoughts of death and suicide

Diagnosing depression is complicated by the co-occurrence of depression with other psychiatric disorders, e.g. anxiety disorders (Brunello et al. 2000) and Parkinson’s disease (Friedman et al. 2004) – diseases that share many of the symptoms of depression.

2.1.6 Treatment options

Although pharmacotherapy is the most widely used treatment of depression, other modalities include psychotherapy, electroconvulsive therapy (ECT), magnetic stimulation, vagal nerve stimulation, deep brain stimulation, and exercise (Nestler et al. 2002, Berton et al. 2006) – all these approaches being effective in treating depression to some extent. From a pharmacotherapy view-point, treatment has almost exclusively focused on an interaction with
monoaminergic systems, particularly increasing serotonin and/or noradrenalin mediated systems in the brain (Lenox et al. 2002).

Total sleep deprivation has also been shown to rapidly improve depressive symptoms in ≈50% of patients. The effect, however, is short-lived and lasts only a few days (Giedke et al. 2002). Exercise and physical activity have also proved to be effective in alleviating depressive symptoms (Blumenthal et al. 2007, Dunn et al. 2005). Although the exact means by which these somatic approaches to treatment elicit their effects are not totally understood, they provide a valuable aid in treating depressed individuals by providing rapid effects.

Most currently available antidepressants mediate their mood elevating effects through, for example, the inhibition of serotonin or noradrenalin reuptake, e.g. TCAs, SRIs, or by the inhibition of monoamine oxidase, the major catabolic enzyme for monoamine neurotransmitters (Nestler et al. 2002, Nemeroff 2008). Even though it may be argued that SSRIs are potentially less or just as effective antidepressants as the TCAs, their use is still favoured due to their safer and improved side-effect profile (Vetulani et al. 2000, Anderson 2000, Gallo 1999).

Various atypical antidepressants have also been introduced that target monoaminergic systems, or other systems, in a way that is different to the traditional approaches described above. Such agents include for example mirtazapine, a multitarget antidepressant with antagonist activity on the α2-adrenergic receptor, trazodone, that mainly blocks 5HT2 and α1-adrenergic receptors and also bupropion that inhibits noradrenalin and dopamine reuptake (Holtzheimer et al. 2006, Berton et al. 2006, Brunton et al. 2011, Papakostas et al. 2008), and more recently the melatonergic antidepressant, agomelatine (Hickie et al. 2011, Howland 2009a, Howland 2009a, De Bodinat et al. 2010).
Figure 2-7: Sites of action of antidepressants. Schematics representing noradrenergic (top) and serotonergic (bottom) nerve terminals. SSRIs, SNRIs, and TCAs increase noradrenergic or serotonergic neurotransmission by blocking the noradrenalin or serotonin transporter at presynaptic terminals (NET, SERT). MAOIs inhibit the catabolism of noradrenalin and serotonin. Some antidepressants such as trazodone and related drugs have direct effects on serotonergic receptors that contribute to their clinical effects. Chronic treatment with a number of antidepressants desensitizes presynaptic autoreceptors and heteroreceptors, producing long-lasting changes in monoaminergic neurotransmission. Post-receptor effects of antidepressant treatment, including modulation of GPCR signalling and activation of protein kinases and ion channels, are involved in the mediation of the long-term effects of antidepressant drugs. Note that NE and 5-HT also affect each other's neurons. (Brunton et al. 2011).
Considering that available antidepressant treatments have limited efficacy, a delay in clinical efficacy as well as cause various adverse effects (Nestler et al. 2002, Fava 2003, Holtzheimer et al. 2006, Machado-Vieira et al. 2009), there is an increasing need to explore novel targets for the treatment of depression that may produce more rapid, robust and lasting antidepressant effects yet with a less daunting side-effect profile.

2.1.7 Treatment problems/challenges

Ideally, an antidepressant drug should have a fast onset of action, a favourable side-effect profile and induce 100% remission rates, as well as an absence of relapse (Rosenzweig-Lipson et al. 2007). However, currently available antidepressants still have several shortcomings, as are illustrated in Table 2-2.

Table 2-2: Unmet clinical needs for marketed antidepressants. (Rosenzweig-Lipson et al. 2007)

<table>
<thead>
<tr>
<th>Efficacy in refractory patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy in treatment resistant depression</td>
</tr>
<tr>
<td>▪ Recovery</td>
</tr>
<tr>
<td>▪ Relapse</td>
</tr>
<tr>
<td>▪ Recurrence</td>
</tr>
<tr>
<td>Faster onset of antidepressant action</td>
</tr>
<tr>
<td>Reduction of cognitive deficits</td>
</tr>
<tr>
<td>Treatment of symptomatic pain accompanying depression</td>
</tr>
<tr>
<td>Decreased side-effect profile</td>
</tr>
<tr>
<td>▪ Sexual dysfunction</td>
</tr>
<tr>
<td>▪ Gastrointestinal events</td>
</tr>
<tr>
<td>▪ Weight gain</td>
</tr>
<tr>
<td>▪ Cardiovascular</td>
</tr>
</tbody>
</table>

Although pharmacotherapy and psychotherapy may both be effective in treating depression, many patients don’t react sufficiently to the available forms of treatment. Today’s treatments remain sub-optimal, with only ≈50% of all patients demonstrating complete remission (Nestler et al. 2002), while up to 20% show minimal or no reaction to even the most aggressive
interventions (Fava 2003). In addition, intolerable side-effects, as well as a slow onset of action, prompts many patients to prematurely discontinue their medication leading to a further range of problems and associated complications, foremost among these being an increased risk of relapse and recurrence (Holtzheimer et al. 2006, Harvey et al. 2003). It is also now an accepted fact that long-term treatment is necessary to limit the number and intensity of subsequent depressive episodes (Holtzheimer et al. 2006).

As has been highlighted earlier (see §2.1.3.1), the cause of depression is far from being a simple deficiency in central monoamines. MAOIs and monoamine reuptake inhibitors produce immediate increases in monoamine transmission (Krishnan et al. 2008), whereas their mood-enhancing properties require a number of weeks to reach effect. In fact, many patients do not show adequate improvement after even months of treatment (Machado-Vieira et al. 2009). This indicates that enhanced serotonergic or noradrenergic neurotransmission per se is not the only requirement for clinical efficacy (Nestler et al. 2002). Indeed, neurotrophins, neurogenesis and the concept of neuroplasticity have now taken centre stage in our understanding of depression and the mechanisms of action of antidepressants (Krishnan et al. 2008, Manji et al. 2003). There is also the realization that neuroendocrine and metabolic dysfunction contribute to the eventual development of depression, and together with the above, has now provided a new framework for understanding the neurobiology and treatment of depression.

2.2 Metabolic dysfunction and inflammation in depression

Despite the elaborate hypotheses described earlier, current theories on serotonergic dysfunctions, cortisol hypersecretion, etc. do not adequately explain the neurobiology of depression. In fact, that currently available antidepressants are effective in less than two thirds of depressed patients highlights the desperate state of this situation. New evidence, however, strongly supports the claim that inflammatory and neurodegenerative processes play an important role in depression and that depression may, at least partly, be caused by inflammatory processes involving inflammatory cytokines, oxygen radical damage, altered tryptophan metabolism and other excitatory messengers such as glutamate (Maes et al. 2009).
The strong correlation between depression and various cardiometabolic disorders (see Section 2.2.2 and below) suggests that a metabolic disturbance may be a vital component that drives inflammatory and immunological dysfunction in depression. In fact, some investigators have suggested that depression may encompass neuropathological components reminiscent of a metabolic encephalopathy (Harvey 2008). Importantly, efforts to develop new antidepressant drugs are becoming more cognoscente of neurometabolic pathways as novel avenues for drug development (Bright et al. 2008, Kemp et al. 2011), and is the focus of this dissertation.

2.2.1 Incidence of MetS and T2DM

In 1997, an estimated 124 million people worldwide had diabetes mellitus, 97% of these having T2DM (Amos et al. 1997) whilst in the US, it is estimated that 12.9% of the adult population currently suffer from diabetes mellitus (Cowie et al. 2009). According to Nichols & Moler (2010), 36.5% of this population suffer from MetS, of which 13.3% developed T2DM within five years. This is consistent with findings by the National Health and Nutritional Examination Survey that reported a 34% incidence of MetS. Moreover, the prevalence of MetS increases with age and body mass index (BMI) (Ervin 2009).

2.2.2 Evidence for a relationship between MetS and major depressive disorder (MDD)

2.2.2.1 Pre-clinical evidence

Evidence from pre-clinical studies have found that Flinders sensitive line (FSL) rats, a genetic animal model of depression, are more prone to developing severe depressive-like behaviour after long-term feeding of a high fat diet than are their normal controls, while these animals are also more prone to developing various metabolic disturbances (Abildgaard et al. 2011).

Metabolic status is greatly influenced by mood and motivation (Krishnan et al. 2008). Insulin and the satiety hormone, leptin, influence metabolism by decreasing feeding behaviour, thus
acting as satiety signals (Davis et al. 2010). Interestingly, leptin produces a dose-dependent antidepressant-like response in rodents (Lu et al. 2006), while similar pre-clinical studies have emphasized the antidepressant-like effect of insulin-sensitizers, particularly the PPARγ-agonists, rosiglitazone and pioglitazone. Indeed, rosiglitazone has been demonstrated to evoke an antidepressant-like effect in rodents (Eissa Ahmed et al. 2009), while similar studies have demonstrated this response with pioglitazone (Sadaghiani et al. 2011) as well as a novel thiazolidinone, NP031115, of which the effects have also been shown to be PPARγ dependant (Rosa et al. 2008).

2.2.2.2 Clinical evidence

MetS is characterized by various co-existing abnormalities such as insulin resistance and hyperglycaemia (Ford et al. 2002), and shares certain characteristics with depression (Skilton et al. 2007). Furthermore, the prevalence of depression is doubled in individuals with T2DM (Anderson et al. 2001) while patients with MetS are much more likely to have depression than those without MetS (Capuron et al. 2004).

Many antidepressant drugs lead to alterations in glucose metabolism and weight-gain (Brown et al. 2008, Derijks et al. 2008, Harvey et al. 2000, Rubin et al. 2008) and it has been demonstrated that the SSRIs, for example, have the ability to improve at least some pathological factors associated with cardiovascular disease (Halaris 2009) – a disease that is often associated with obesity and MetS (Abate 2000, Ezquerra et al. 2008). Indeed, fluoxetine is preferred in T2DM patients considering its anorectic actions and beneficial effects on glycaemic control (Connolly et al. 1995, Daubresse 1996, Gray et al. 1992). Some antidiabetic drugs, viz. the PPARγ-agonists, also improve symptoms of depression in patients with abdominal obesity and/or MetS (Kemp et al. 2009, Kemp et al. 2011).

These co-morbidities observed between mood disorders, obesity and other cardiometabolic disorders (Skilton et al. 2007, McElroy et al. 2004) lead to the assumption that depressive symptoms in these patients may be associated with increased visceral fat mass (Capuron et al. 2004, Vogelzangs et al. 2010). Inflammation is a key component of MetS (Yudkin et al. 1999),
while central obesity, another element of this syndrome, is a major determinant of low-grade chronic inflammation (Santos et al. 2005). Adipose tissue has the ability to produce and secrete a number of inflammatory molecules, including C-reactive protein and proinflammatory cytokines (Guzik et al. 2006, Wellen et al. 2003). Taking into consideration that inflammatory responses play an important role in the pathophysiology of depression (as discussed in Section 2.1.3.6) (Wellen et al. 2005), inflammation represents a major mediator in the development of both mood disorders as well as MetS (Capuron et al. 2008). This also suggests that inflammatory processes, chronically activated in MetS, could participate in mood alterations (Danner et al. 2003, Ford et al. 2004, Alesci et al. 2005, Tiemeier et al. 2003). Importantly, it has been demonstrated that treatment of both T2DM and co-morbid depression with antidepressants results in significant improvement of both diseases (Abrahamian et al. 2009).

T2DM has been associated with moderate cognitive deficits, as well as neurophysiological and structural changes in the brain (Biessels et al. 2002). These brain changes bear a strong resemblance to that observed in depression, while diabetes-associated depression may also be driven by similar biochemical changes accompanying the disease (Haeser et al. 2007), as well as vascular causes and/or changes in the blood-brain barrier (Mooradian 1997). Several meta-analyses have concluded that depression and T2DM are highly co-existent (Knol et al. 2006). Indeed, the pathophysiology of these diseases overlap and may be independent risk factors for each other (Anderson et al. 2001, Knol et al. 2006).

T2DM doubles the odds of depression and as many as one in every three individuals with T2DM has depression at a level that impairs normal functioning and quality of life (Anderson et al. 2001, Knol et al. 2006). Several neuroendocrine and neurotransmitter abnormalities associated with both depression and T2DM have been identified (Lustman et al. 1992), further pointing to the possibility of shared aetiology. In agreement with studies in animals, the PPARγ-agonist and insulin sensitizer, pioglitazone, has been found to display antidepressant efficacy in patients with depression (Kemp et al. 2009, Kemp 2010, Kemp et al. 2011), thus lending further support for an overlapping pathophysiology between major depression and MetS.
2.2.3  The role of inflammation in MetS and MDD

In addition to their role in the immune system, cytokines are important modulators of mood and may activate receptors within the CNS. In this way proinflammatory cytokines, such as IL-1, IL-6 and TNF-α, produced either peripherally or centrally, may lead to sickness behaviour (refer to §2.1.3.6) and the development of symptoms of depression in vulnerable individuals (Dantzer et al. 2008). Furthermore, chronic cytokine elevations have been proposed to evoke neurotransmitter changes that are interpreted by the brain as stressors and may contribute to the development of depression (Anisman et al. 2003).

Depressed patients have been found to have higher levels of proinflammatory cytokines (Anisman et al. 2003), which in turn strongly contribute towards various pathophysiological domains that characterize depression, including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity and behaviour (Danner et al. 2003, Ford et al. 2004, Raison et al. 2006, Tiemeier et al. 2003, Miller et al. 1999, Musselman et al. 2001). Proinflammatory cytokines also stimulate HPA-axis hormones as well as CRH in both the hypothalamus and the amygdala, which, in the latter, plays an important role in fear responses and anxiety-related behaviour (Raison et al. 2006). Administration of cytokines to humans results in altered functioning in brain regions relevant to the development of depressive symptoms and many patients treated with interferon (IFN)-α develop a behavioural syndrome closely related to major depression (Capuron et al. 2004). Incidentally, IFN-α induced depression is also responsive to standard antidepressant therapy (Musselman et al. 2001). Cytokine-induced depression is associated with alterations in serotonin metabolism through the enzyme indoleamine-2,3-dioxygenase (IDO) (Capuron et al. 2002), as well as alterations in CRH function (Schiepers et al. 2005, Capuron et al. 2003). Importantly, depression induced by IFN-α also involves the nitric oxide (NO) cascade (Hashioka et al. 2007, Suzuki et al. 2003), the latter known to play an important role in the neurobiology and treatment of depression (Dhir et al. 2011, Harvey 1996, Harvey 2008). In a recent study in Black Africans, it was found that depressive symptoms were associated with an excess burden of sub-clinical vascular disease (Hamer et al. 2011). More specifically, MetS was found to be a significant mediator of the
association between depressive symptoms and mean carotid intima media thickness, accounting for approximately 21% of the effect. Together, the above data highlight the importance of inflammation in chronic diseases like cancer, T2DM, coronary artery disease and MetS, and that these may contribute to an increased risk of developing depression.

In recent years depression has been associated with changes in redox status and increased oxidative stress or diminished oxidative defence systems (Harvey 2008). The brain has relatively low levels of antioxidant defences, as well as high lipid content that are highly susceptible to attack by reactive oxygen species (Halliwell 1994). Many of the changes in oxidative status in depression may be directly related to increased inflammatory response due to the presence of other systemic illnesses, such as endocrine and metabolic disorders and cardiovascular disorders. Furthermore, changes in certain neurotransmitter systems in the brain, especially glutamate and GABA, increase the risk of oxidative stress in the brain and subsequent neuronal oxidation and cell death (Harvey 2008). One of the more prominent redox active molecules released by changes in glutamate activity in the brain is NO which, as alluded to earlier, is well described as being a contributing factor towards the development of depression.

2.2.4 Insulin

Insulin is involved in the regulation of glucose homeostasis. Insulin receptors are widely expressed in the CNS (Unger et al. 1991, Woods et al. 1985) and are unevenly distributed throughout the brain, whilst being densely expressed in the cerebral cortex, olfactory bulbs, hippocampus, cerebellum, and hypothalamus. Insulin is transported into the brain from peripheral tissues via the cerebrospinal fluid (CSF) (Woods et al. 1985), while the hormone may also be synthesized by neurons in the brain (Craft et al. 1996). Recognized physiological effects of insulin in the CNS include regulation of food intake, control of glucose uptake and trophic actions on neuronal and glial cells (Wozniak et al. 1993). Importantly, brain insulin may influence the release and reuptake of neurotransmitters (Sauter et al. 1983). Indeed, subjects with peripheral insulin resistance also present with insulin resistance in the brain, particularly in
the prefrontal cortex and other areas of the brain involved in motivation and reward (Mendelson 2008). T2DM is also associated with increased oxidative stress (Rösen et al. 2001) probably as a result of glucose auto-oxidation through which glucose is oxidized in the presence of free metal ions, leading to superoxide and hydroxyl radical release and finally protein oxidation (Wolff et al. 1987).

Substantial evidence exists linking the insulin and NO mediated pathways in the brain. Insulin up-regulates the expression of neuronal nitric oxide synthase (nNOS) in various brain cell lineages at both protein and mRNA levels (Yuan et al. 2004), confirming that insulin regulates the expression of nNOS in the brain. Furthermore, Serino et al. (1998) have demonstrated that the expression of the nNOS gene in the paraventricular and supraoptic nuclei increases in diabetic rats, suggesting that hyperglycaemia and plasma hyperosmolality may cause up-regulation of the nNOS gene in the hypothalamus. There is also mounting evidence in support of a combined role for oxidative stress and NO in T2DM and/or insulin resistance (Brillante et al. 2009) and a role for increased NO and insulin/PPARγ signalling following stress, possibly presenting as a susceptibility factor in the subsequent development of depression (García-Bueno et al. 2010, Sadaghiani et al. 2011). In fact, increased stress-related activation of the NMDA-NOS cascade has been proposed to be a vulnerability factor in stress-sensitive individuals (Wegener et al., 2010).

2.2.5 Peroxisome proliferator activated receptor (PPAR)-γ

2.2.5.1 Physiology

PPARγ forms part of the super family of nuclear hormone receptors (Houseknecht et al. 2002) and, when activated, may illicit effects on various physiological systems, including muscle tissue, adipose tissue and β-cells to regulate metabolism and mediate the actions of insulin (Guo et al. 2006, Kamon et al. 2003, Rangwala et al. 2003). PPARγ also regulates the expression of genes related to lipid and glucose metabolism, inflammatory processes and cellular differentiation (Kapadia et al. 2008).
Agonist-induced activation of PPARγ by the thiazolidinedione class of oral antidiabetic drugs results in adipocyte differentiation and increases insulin sensitivity in adipocytes, skeletal muscle and the liver. These drugs are used in the treatment of T2DM, with rosiglitazone being the most potent and selective agent in this class (Houseknecht et al. 2002).

### 2.2.5.2 Role of PPARγ in the CNS and MDD

Antidiabetic treatments, e.g. thiazolidinediones, may have the capacity to treat pathological processes in mood disorders via neuroprotective, neurotrophic and anti-inflammatory mechanisms. Considering the hypothesized role of insulin signalling pathways in neuroplasticity and neuroprotection, modulation of these pathways have also been proposed as an approach to treating depression (Eissa Ahmed et al. 2009, McIntyre et al. 2008, Rasgon et al. 2004). Cognitive function in particular, has the potential to be effectively preserved or even augmented by the neurotrophic actions of antidiabetic treatments. Given the prominence of cognitive deficits in depression, euglycaemic agents may have significant clinical potential (McIntyre et al. 2006).

PPARγ receptors are also present in various brain regions, including the hippocampus, striatum, frontal cortex and hypothalamus (Cimini et al. 2005, Drew et al. 2006, Moreno et al. 2004) and are also expressed in immune cells (Heneka et al. 2007). It is therefore not surprising that PPARγ has been associated with suppression in immune response through its ability to inhibit the expression of inflammatory cytokines (Guri et al. 2010, Lee et al. 2005, Martin 2009, Ramakers et al. 2007) and to have actions on pathways involved in apoptosis, cellular proliferation and cellular resilience (Sertznig et al. 2007). Moreover, it has been demonstrated that metabolites of serotonin act as PPARγ-agonists in the periphery (Waku et al. 2010, Watanabe et al. 2011), which further indicates the possibility of an underlying link between the biochemical pathways of MDD and MetS.

Pioglitazone crosses the blood-brain barrier (Maeshiba et al. 1997), implying that antidepressant actions observed in the clinical and preclinical environment may be attributed
to a central effect. However, such psychotropic actions may also be ascribed to their effects in the periphery – including decreased abdominal obesity, decreased inflammation or improved insulin sensitivity (Kemp et al. 2011).

The ability of these drugs to reduce microglial activation (Heneka et al. 2005), decrease neuronal damage (Zhao et al. 2006), as well as enhance brain glucose utilization through increased neuronal mitochondrial biogenesis (Strum et al. 2007) further supports the evidence that thiazolidinediones may be clinically effective in treating neuropsychiatric disorders.

A gene association between PPARγ and depression has been observed (Ji-Rong et al. 2010). Indeed, the anti-inflammatory pathway, 15d-PGJ2/PPARγ, 15d-PGJ2 being the most recently discovered prostaglandin and serving as an endogenous ligand for PPARγ, also presenting with anti-inflammatory properties (Scher et al. 2005), has been suggested as a possible biological marker in psychiatric diseases (García-Bueno et al. 2010). Moreover, activation of PPARγ has been demonstrated to improve various CNS-dysfunctions with an inflammatory component, including spinal cord injury, brain injury, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), Alzheimer’s diseases and depression (Eissa Ahmed et al. 2009, Heneka et al. 2007, Kemp et al. 2009, Kummer et al. 2008, McTigue 2008, Morgenweck et al. 2010, Ramanan et al. 2010).

Preclinically it has been demonstrated that ligands of PPARγ inhibit inflammation and oxidative stress following stress exposure in rats (García-Bueno et al. 2005) by inhibiting increases in iNOS (inducible NOS) activity as well as TNF-α (Munhoz et al. 2008). Moreover, both rosiglitazone and 15d-PGJ2 increase the neuronal metabolism of glucose and prevent stress-induced suppression of glutamate uptake (García-Bueno et al. 2007).

In light of the literature discussed here, it is evident that thiazolidinediones may represent a valuable new treatment for depression as well as cognitive and somatic deficits associated with this disorder.
2.3 Animal models of depression

The main purpose of animal models is to study the neurobiology of disease and to predict the effects of drugs in vivo (Borsini et al. 1988). Ideally, an animal model must accurately represent the clinical features of a given human disorder. As has been described earlier, depression is a heterogeneous disease with unknown causes as well as a number of neurobiological discrepancies. Hence, it is a major challenge to develop an animal model that accurately reflects the symptomatology, neurobiology and treatment response evident in depression when there is still so much debate as to the exact underlying biology of the disorder. In order to do this, a putative animal model is required to be validated with respect to three principle criteria (Overstreet et al. 1995, Willner 1984, Willner 1991, Willner et al. 2002), which are:

- Face validity: the extent to which the animal model mirrors the behaviour and specific symptoms observed in the human condition
- Construct validity: the degree to which the theoretical rationale behind the animal model correlates with that of the human disorder
- Predictive validity: the ability of the animal model to predict the theorized outcome, e.g. identify drugs with potential therapeutic value in humans

Broadly speaking, animal models may be either pathological or induced. Essentially the former is a “sick” animal that is representative of the actual human illness, while an induced model is a healthy animal that is exposed to a series of stressors that induce certain depressive-like behaviours in the animal.

A number of putative animal models of depression have been developed, termed pathological models, and include for example chronic mild stress (CMS) (Willner et al. 1992), the Flinders sensitive line (FSL) rat, Wistar Kyoto (WKY) rats (Pare 1989, Pare et al. 1993) as well as Lewis (LEW) and spontaneously hypertensive (SH) rats (Hinojosa et al. 2006). Important to note is that none of these models fully recreate the human disorder, but if their purpose and limitations are carefully considered, they all have the potential to provide useful information on
depression and its treatment. For the purpose of the current study, only the FSL model will be discussed.

2.3.1 The Flinders sensitive line (FSL) rat as a relevant animal model of depression

The FSL rat was originally developed in an attempt to develop a line of rats bred to be genetically resistant to the anticholinesterase agent, diisopropyl fluorophosphates (DFP). Paradoxically, this in-bred line of Sprague Dawley rats was found to be more sensitive to DFP, and was named the Flinders sensitive line (FSL). The Flinders resistant line (FRL) rat, on the other hand, was initially developed to be more resistant to DFP (Overstreet et al. 2005), although in the end this strain was found to be no more resistant to DFP than normal Sprague Dawley rats but only in comparison with FSL rats (Overstreet et al. 1979, Russell et al. 1982). Although the model may have failed in its original intent, it soon became apparent that the FSL rat presented with very unique bio-behavioural qualities compared to its FRL control and compared to normal Sprague Dawley rats. Foremost among these characteristics is that FSL rats display several behaviours that closely resemble the symptoms of depression in humans (Overstreet et al. 2005; Table 2-3), thus presenting with robust face validity for depression.

Table 2-3: Behavioural characteristics modelled in FSL rats that reflect symptoms of depression (adapted from Yadid et al. 2000))

<table>
<thead>
<tr>
<th>Symptom/characteristic</th>
<th>FSL rats</th>
<th>Major depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>General activity</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>REM-sleep amount</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>REM-sleep latency</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Appetite</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Anhedonia</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Enhanced response to antidepressants</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Apart from the characteristics described in Table 2-1, the FSL rodent model also presents with important construct validity for depression. Thus, FSL rats adhere to the cholinergic supersensitivity hypothesis of depression (see Section 2.1.3.4) that has been described in depressed humans (Drevets 2001, Perlis et al. 2002), presenting with increased sensitivity to muscarinic
receptor agonists (Overstreet et al. 1982) and altered expression of muscarinic receptors in various limbic brain regions (Brand et al. 2011, Overstreet et al. 1984). Moreover, FSL rats also demonstrate important abnormalities with respect to serotonergic (Overstreet et al. 1994, Zangen et al. 1997), glutamatergic (Wegener et al. 2010) and neurotrophic signalling (Elfving et al. 2010), as well as various cardio-metabolic defects (Abildgaard et al. 2011, Solskov et al. 2010), thus incorporating other important theories of depression. Depressive-like symptoms in FSL rats also respond to chronic but not acute treatment with antidepressants (Yadid et al. 2000, Kanemaru et al. 2009) and is capable of detecting both classical TCAs and SSRIs (Pucilowski et al. 1993a, Pucilowski et al. 1993b, Schiller et al. 1992), as well as atypical antidepressants e.g. the melatonergic antidepressant, agomelatine (Overstreet et al. 2005). Thus the FSL rat model represents a genetic rodent model of depression that fulfils the criteria of face, construct, and predictive validity (Yadid et al. 2000, Overstreet 1993).

2.4 Behavioural tests relevant to depression

A number of behavioural tests are used for the general assessment of animal behaviour. Although a diverse range of tests related to depression have been developed in animals, each test usually has a specific purpose, for example to assess inherent anxiety, spatial memory, learned helplessness, etc. Consequently, a single test is seldom used on its own, while a combination of assessments will provide the greatest accuracy and validity. For this reason, only test immediately relevant to this study will be covered.

2.4.1 Open field test (OFT)

The OFT is used to measure inherent levels of aversion or anxiety in rodents, and also to assess the animal’s general locomotor activity (Dunne et al. 2007, Prut et al. 2003). Rats will spend more time close to the protective walls as opposed to the centre of the test arena due to a natural aversion towards illuminated open spaces. The subjects are regarded as being more “anxious” when spending more time in this form of behaviour (Montgomery 1955, Overstreet et al. 2004). In behavioural research, the OFT is usually done in conjunction with other behavioural tests, e.g. the FST and NORT (novel object recognition test), as a control of
locomotor activity. In general, if locomotor activity is influenced negatively by drug treatment, it should be considered whether the reduced locomotor activity may have an unfavourable effect on the results of other behavioural studies. Other methods for assessing locomotor activity are, however, also used – one of the most popular being computerised scoring of horizontal and vertical activity using a locomotor box monitored by a series of infrared light beams. One such example is the DigiScan® Animal Activity Monitor (refer to §3.2.2) used in this study.

2.4.2 Novel object recognition test (NORT)

According to the American Psychiatric Association (2000), cognitive dysfunction is evident in depression and characterized by impaired memory and concentration. Depression may also be associated with diverse memory disturbances (Kasahara et al. 2006) as a result of hippocampal shrinkage that invariably follows the neurotoxic effects of elevated levels of glucocorticoids that is typical of depression (Woolley et al. 1990).

The novel object recognition test (NORT) is a test of declarative memory in rodents, relying on the premise that rats prefer to explore a novel object relative to a familiar one. The NORT has relevance for depression as a measure of cognitive function that is known to be affected in depressed patients (Ennaceur et al. 1988). Thus, increased exploration time of a novel object, relative to a familiar object, would indicate improved cognitive function.

2.4.3 Forced swim test (FST)

The rat forced swim test (FST) was developed by Porsolt and colleagues (1978) for use in rodents (rats and mice). This test is still the most widely used tool for preclinical assessment of antidepressant-like activity due to its ease of use, reliability across laboratories and ability to detect a broad spectrum of antidepressant agents (Borsini et al. 1988). The test is based on the observation that rats, following initial escape-directed movements, develop an immobile posture when placed in an inescapable cylinder of water. The immobility is thought to reflect a failure in persistence in escape-directed behaviour, also referred to as behavioural despair.
(Lucki 1997). When animals are reintroduced to the FST 24 hours later, and following successive doses of an antidepressant, the animals actively persist in escape-directed behaviour for longer periods compared to vehicle-treated animals. However, the major drawback of the traditional FST is that it is unreliable in the detection of SSRI-type antidepressants (Detke et al. 1995). In an effort to enhance the sensitivity of the traditional FST to SSRIs, several procedural modifications have been made (Lucki 1997, Cryan et al. 2002). These developments include increasing water depth from 15-18 cm to 30 cm and/or distinguishing between different behavioural components of active swimming behaviour, namely climbing behaviour, swimming behaviour and immobility (as described in section 3.3.3.3.). The modified FST has since been used with great success, indicating the reliability of this modified version (Reneric et al. 2001, Reneric et al. 2002).

The major advantage of the modified FST is that it distinguishes between agents that enhance central serotonin and noradrenalin levels. For example, agents that enhance noradrenalin levels typically decrease immobility with a corresponding increase in climbing activity, while agents that enhance serotonin levels, such as SSRIs, also decrease immobility, but increase swimming behaviour (Lucki 1997, Cryan et al. 2000). A typical application of these useful qualities of the modified FST and how it may be used to corroborate the behavioural and neurochemical effects of putative antidepressant compounds is described in Harvey et al. (2010).

Another potential drawback of the FST is the discrepancy between the duration of effective drug treatment in the FST and the time to clinical response in humans. Acute antidepressant treatments are sufficient to reverse immobility in rats in the FST, whereas such treatment is ineffective in humans. Indeed, antidepressant treatment may require a number of weeks before an elevation in mood is observed. However, it has also been demonstrated that doses of antidepressant drugs that are found to be inactive following acute administration may in fact elicit antidepressant-like effects when administered chronically (Detke et al. 1997). Moreover, chronic treatment programmes have also been evaluated for use in the FST and found to be
effective (Reneric et al. 2002) and hence is often used to corroborate acute response data in the FST (e.g. Harvey et al. 2010).

When applying the FST to antidepressant response in the FSL model, it has been found that the initial pre-swim is not necessary in order to evoke the typical immobile posture in these animals (Liebenberg 2009). This is very likely due to the increased stress-responsiveness of these animals. Thus, pre-swim conditioning is not necessary and ill-advised when using FSL rats that display spontaneous immobility in the FST.

2.5 Synopsis

The treatment of depression is currently sub-optimal, with few new drugs with improved efficacy reaching the market. A possible exception would probably be agomelatine, a novel melatonergic-serotonergic agent that acts via the re-entrainment of circadian rhythms, which has recently been registered world-wide for the treatment of depression (Hickie et al. 2011, Howland 2009a, De Bodinat et al. 2010). Depression is one of the most common neuropsychiatric disorders and also one of the most significant causes of disability. Furthermore the causes of depression are multifactorial and the exact neurobiological mechanisms unclear. Matters are further complicated by co-morbidity and increased risks of developing depression when already suffering from various other psychiatric and cardio-metabolic disorders. The associated risk of developing depression when suffering from metabolic disorders or other chronic illnesses also leads to an increasing prevalence of depression as less active lifestyles and unhealthy diets become more common.

The pathophysiology and treatment of depression has mainly been based on the biogenic amine hypothesis of depression, while more recently increased emphasis on the role of neurotrophins, neurogenesis and neuroplasticity has also been explored. However, the central role for neuro-immune and metabolic dysfunction in the mutual interaction between metabolic disorders and depression can further assist our understanding of the neurobiology and treatment of depression.
Depression has been found to be co-morbid with various metabolic disorders of which MetS may be the most interesting – with patients presenting with increased levels of proinflammatory cytokines and various other biological markers which may be associated with an increased risk of developing depression. In this regard, the PPARy-agonist group of insulin sensitizing drugs (which are used in the treatment of T2DM and MetS) have been shown to improve central conditions associated with inflammation – most fascinating of these being depression. These drugs may represent a novel approach to the treatment of depression, and thus contribute to continuing attempts to develop antidepressant drugs with improved efficacy and onset of action.

Various animal models have been developed to screen potential antidepressants for therapeutic effects, some of which display excellent validity for the human illness. The FSL genetic rodent model of depression has great value due to it being a pathological animal model of depression (as opposed to an induced model) with robust face, construct and predictive validity for depression. While a number of antidepressant screening tools are available to the researcher, the FST and especially its modified version allow for in-depth analysis of both behaviour and neurochemical factors that may contribute to antidepressant response. Combining the FST with the FSL rodent model of depression thus provides a robust means by which to test new theories of antidepressant drug action.

In the following chapters, the current study will be presented including a detailed description of the methods (Chapter 3), the results (Chapter 4), a discussion of the data and their relevance to depression (Chapter 5), and closing with a conclusion on how these findings extend our current knowledge of depression, as well as providing some suggestion for future studies (Chapter 6).
This chapter presents the experimental methods employed in the current study, including experimental layout, animal models, materials, drugs used and their dosages, and behavioural studies.

The treatment of the animals and behavioural testing were conducted at the Animal Research Centre at the Potchefstroom campus of the North-West University in accordance with the ethics application, NWU-00099-10-S5, as approved by the Research Ethics Committee of the North-West University. Preparation of all drugs for administration to the animals was carried out in the Laboratory of Applied Molecular Biology (LAMB) at the Potchefstroom campus of the North-West University.

3.1 Overview

The project is divided into two components, namely a series of pilot studies and a main experimental study:

3.1.1 Pilot studies

This section consists of a dose-ranging analysis, initially in Sprague Dawley rats in an acute treatment regime and later repeated in Flinders sensitive line (FSL)-rats following a chronic treatment regime. The results from these studies were critical in defining the treatment parameters used for the main experimental study (see below). Since this was a process of decisions based on empirical data, selected results from these pilot studies are presented in the text in order to enlighten the reader as to the reasoning behind these decisions. However, the comprehensive presentation of these results and their discussion are presented in Chapters 4 and 5.

3.1.2 Main experimental study

This chronic treatment study forms the primary focus of the dissertation and was performed exclusively in FSL rats. The animals were treated orally with the relevant test drugs for a period...
of 7 days. In addition, a number of treatment groups were carefully selected in which to repeat this 7-day chronic treatment via the subcutaneous route. This was done in order to rule out the possibility that pharmacokinetic issues associated with the oral route could predetermine the nature of the results obtained.

3.2 Materials used

3.2.1 Drugs

Fluoxetine and pioglitazone were a generous gift from Cipla-Medpro (Bellville, South-Africa). Imipramine and gliclazide were obtained from Sigma-Aldrich (South-Africa).

3.2.2 Instruments

The following instruments were used during this study:

- Perspex® cylinders (diameter 180 mm, height 600 mm) and Perspex® boxes (100 cm x 100 cm x 50 cm) (Porsolt FST apparatus)
- Digiscan® Animal Activity Monitor (DAAM, AccuScan® Instruments, Columbus, OH, USA)
- Novel object recognition apparatus (manufactured by the Instrument Making Department of the Faculty of Engineering, NWU)
- Video cameras were mounted directly above (or opposite to) apparatus used in behavioural assessments
- Sartorius® BP211D balance
- Transsonic® 310 (Faust, D-78224 Singen, Germany) sonication bath
- Laboratory glassware
- Eppendorf® pipettes and tips
3.3 Methods

3.3.1 Animals and animal housing

Male Sprague Dawley, FRL and FSL rats were used in the study. Rats weighing 240 ± 20 g on the day of behavioural testing were randomly allocated to their respective treatment groups. In order to maintain the overall good health of the animal and to reduce the incidence and spread of infectious diseases, the animals are bred and housed in the Animal Research Centre of the North-West University, Potchefstroom campus. This environment ensured aseptic conditions throughout by daily washing of floors with disinfectants and obligatory wearing of boots, masks and head covering. Extraneous sounds and noises were kept to a minimum.

All animals were housed in groups of 4 rats/cage in identical polypropylene cages (380 mm x 380 mm x 230 mm) with free access to Epol® mice cubes with water available *ad libitum*. Animals were housed under controlled environmental conditions, including a 12:12 hour light-dark cycle (lights on 06:00 to 18:00), positive air pressure with an air change rate (ACR) of 18 times per hour, and constantly controlled temperature (21 ± 2°C) and humidity (±55%).

3.3.2 Preparation and administration of drugs

Pioglitazone is insoluble in water (Soltanpour et al. 2009). In various animal studies pioglitazone was administered orally, either by mixing it with food (Li et al. 2005, Ohga et al. 2007) or delivering it via oral gavage in either a methylcellulose or 0.9% saline suspension (Barbiero et al. 2011, Fujita et al. 2003, Pathan et al. 2006). The latter method (suspension in 0.9% saline), however, is impractical since the drug rapidly precipitates from the suspension before being administered to the animal. Therefore, in order to ensure accurate oral dosing, the drug was carefully weighed using a Sartorius® BP211D balance before being added to a 3% methylcellulose solution. This solution was then sonicated in a sonication bath and suspended using a magnetic plate stirrer.
All other drugs (and vehicle control) were prepared in the same way before being administered orally to the animals so as to ensure consistency throughout all the treatment groups.

The animals were weighed each morning, the respective dosages calculated and drug suspensions/solutions prepared. Drugs were administered in volumes of 4 mL.kg\(^{-1}\). In the acute dose-ranging study, rats received drug 24 hours, 6 hours and 1 hour prior to the FST. In the chronic treatment protocol, drugs were administered at the same time every morning for 7 days, with the last dose being the morning before testing.

### 3.3.3 Behavioural assessments

#### 3.3.3.1 Measurement of general locomotor activity

Before subjecting the rats to the FST, the animals were habituated in a dark room for 20 minutes, after which their general locomotor activity was recorded during a 5-minute session in the DigiScan\textsuperscript{®} Animal Activity Monitor (see Fig. 3-1). Locomotor activity was quantified and expressed as number of beam breaks and distance travelled (cm).

![Figure 3-1: Digiscan\textsuperscript{®} Animal Activity Monitor](image)
3.3.3.2 The rat forced swim test (FST)

In the acute dose-ranging analysis, Sprague Dawley rats were exposed to a 15 minute preswim session 24 hours before the final test swim.

On the day of the test session, rats were allowed to habituate to their surroundings for a period of 30 minutes. Thereafter the FST commenced by placing a rat into each of four transparent Perspex® cylinders containing 30cm of clean water (see modifications to Porsolt FST, Chapter 2, §2.2.7.3). While swimming, the animals were digitally recorded in order to analyze the footage at a later stage. Afterwards, the animals were removed from the cylinders, dried with paper towels and returned to their home cages.

When scoring videos recorded during the FST, three specific behavioural components were distinguished and measured:

- Immobility: no additional activity is observed other than that required to keep the rat’s head above water
- Climbing (also known as struggling): upward directed movements of the forepaws along the side of the cylinder
- Swimming: swimming movements (either horizontal or downward) throughout the cylinder
Each rat was scored separately. The most prominent of the behavioural components were identified and measured in five second intervals and expressed in terms of the amount of time (in seconds) spent performing this behaviour over a total period of 5 minutes. Subsequently, the combined time of the three components observed added up to 300 seconds.

3.3.4 Project layout

3.3.4.1 Pilot study: A dose-ranging analysis

Before attempting any chronic treatment studies, a dose-ranging analysis was performed in order to determine appropriate dosages for the various drug treatments. The primary reason for this being firstly to corroborate our findings with that presented by similar studies published in the literature, and secondly to enable accurate dosage extrapolation for application in the chronic studies. Furthermore this was done in order to conform to animal ethic requirements, e.g. to exclude harm/discomfort to the animals.

Dosages used were based on earlier studies in rats (see in text) and all drug-treated groups were compared to methylcellulose vehicle-treated control groups. The dose-ranging analysis was performed in Sprague Dawley rats, whereas subsequent experimental studies were...
performed on FSL and FRL rats in order to minimize strain on the animal centre’s breeding program.

3.3.4.1.1 **Acute treatment: Sprague Dawley rats**

An acute dose-ranging study for pioglitazone, gliclazide and fluoxetine was performed in Sprague Dawley rats, with group assignments and dosing as presented in Table 3-1. In all instances, drugs were administered orally, as described in Section 3.3.2., using a 3% methylcellulose suspension as vehicle (control):

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>n-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>n/a</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Fluoxetine</td>
<td>10 mg.kg(^{-1}) *</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Gliclazide</td>
<td>1 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Gliclazide</td>
<td>5 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Gliclazide</td>
<td>10 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Gliclazide</td>
<td>30 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>Gliclazide</td>
<td>50 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>Pioglitazone</td>
<td>1 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>Pioglitazone</td>
<td>3 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>Pioglitazone</td>
<td>5 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>Pioglitazone</td>
<td>10 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>Pioglitazone</td>
<td>20 mg.kg(^{-1})</td>
<td>8</td>
</tr>
</tbody>
</table>

The dosing range for fluoxetine, gliclazide and pioglitazone was based on earlier studies in rats:

* *(Liebenberg et al. 2010, Reneric et al. 2002)*

*#*(Mikov et al. 2008, Satyanarayana et al. 2006, Stetinová et al. 2007)*

‡ *(Barbiero et al. 2011, Fujita et al. 2003, Pathan et al. 2006)*
• Group 1: The control group received only the vehicle (methylcellulose solution) p.o. via oral gavage.

• Groups 2-12: The various drug-treatment groups received their appropriate drug dosages (as presented in Table 3-1 above and Figure 3-3 below) suspended in the vehicle methylcellulose solution, administered p.o. via oral gavage.

Figure 3-3: Schematic illustration of the treatment timeline for acute dose-ranging study in Sprague Dawley rats

Drug dosages for the chronic treatment study (3.3.4.1.2.) were decided based on results from the above pilot study.

3.3.4.1.2 Chronic treatment: FSL rats

An acute dose-response protocol was initially tested in order to confirm whether acute dosing would indeed be a viable treatment option, especially since the FST is widely recognised to be sensitive to acute treatment. However, due to a lack of significant results in the acute dose-ranging analysis above, particularly for the positive control (see Chapter 4, Figure 4.3 & 4.4), it became apparent that either acute dosing or the oral route of administration, or both, may be undermining a meaningful response in the FST. Alternatively, the Sprague Dawley rat is known to be more resistant to stressful conditions (Bielajew et al. 2002, Ma et al. 2004, Wu et al. 2010), so that using this animal may preclude a meaningful result in the FST. FSL rats have
also been shown to respond more robustly to chronic than acute antidepressant treatment in the FST (Overstreet 1993, Dremencov et al. 2004). It was therefore decided to explore the above drug responses in a more stress-sensitive animal using a chronic oral treatment protocol. The FSL rat, a genetic animal model of depression, is widely regarded as being stress sensitive (see Chapter 2, §2.2.6.1). Since our laboratory has been actively involved with depression studies using this animal (Liebenberg et al. 2010, Van Zyl 2008), it was decided to replace Sprague Dawley rats in the study with FSL rats. The revised study protocol still necessitated the use of a chronic treatment protocol (even for the dose-ranging analysis) since the FSL rat exhibits a decrease in immobility in the FST only after chronic and not acute treatment (Pucilowski et al. 1993a, Raber et al. 1997). In order to confirm the depressive-like phenotype of the FSL rat, a FRL-control group was added to the study. Finally, considering the less than adequate response to fluoxetine in the acute treatment study, and since the FST is known to be less sensitive to SSRI-type antidepressants (Detke et al. 1995), fluoxetine was replaced with imipramine as positive control. Indeed, tricyclic antidepressants are widely accepted to produce a robust and reliable response in the FST (Kornstein et al. 2000, Mason et al. 2009). Nevertheless, imipramine was tested over two doses, namely 20 mg.kg\(^{-1}\) and 30 mg.kg\(^{-1}\) to re-affirm its maximum efficacy as a function of oral dose.

Since no studies in rodents using pioglitazone were available at the time when this protocol was designed, it was decided to consider the dosages used for rosiglitazone. Consequently, the initial dosage range for pioglitazone used in 3.3.4.1.1 was based on an earlier study using rosiglitazone (Eissa Ahmed et al. 2009), namely 1-12 mg.kg\(^{-1}\). Literature however confirms that pioglitazone is approximately 10 times less potent than rosiglitazone in inhibiting PPAR\(\gamma\) \textit{in vivo} (Junichi et al. 2000). The oral doses used for pioglitazone were therefore increased from 1-20 mg.kg\(^{-1}\) to 30-120 mg.kg\(^{-1}\). The doses used, however, did not exceed the acute toxic dose for this compound (LD\(_{50}\) = 1814 mg.kg\(^{-1}\)) (Ito et al. 2004).

The new group assignments for the adjusted treatment protocol are presented in Table 3-2 and Figure 3-4.
Table 3-2: Treatment regime for the chronic dose-ranging study (p.o.) in FSL rats (7 days)

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>n-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle (FSL)</td>
<td>n/a</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle (FRL)</td>
<td>n/a</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Imipramine (FSL)</td>
<td>20 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Imipramine (FSL)</td>
<td>30 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Pioglitazone (FSL)</td>
<td>30 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Pioglitazone (FSL)</td>
<td>70 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>Pioglitazone (FSL)</td>
<td>120 mg.kg(^{-1})</td>
<td>8</td>
</tr>
</tbody>
</table>

- **Group 1**: The FSL control group received only the vehicle (3% methylcellulose solution) p.o. via oral gavage.
- **Group 2**: An FRL group received only the vehicle (3% methylcellulose solution) via oral gavage.
- **Groups 3+4**: These groups received imipramine at the dose indicated, dissolved in the methylcellulose vehicle and administered p.o. via oral gavage.
- **Groups 5-7**: Each of these groups received pioglitazone at the dose indicated, suspended in the methylcellulose vehicle and administered p.o. via oral gavage.
3.3.4.2 Pilot study: chronic treatment via the subcutaneous (s.c.) route

As evinced in the Results (Chapter 4, Figure 4-7), FSL rats presented with significantly increased immobility in the FST compared to their healthy FRL controls, thus confirming the face validity of the model for depression, whereas imipramine was also effective in significantly decreasing this immobility at both doses tested, thus confirming the predictive validity of the model. However, despite its ability to evoke an antidepressant-like response, the 30 mg.kg⁻¹ dose was found to induce a significant suppression of general locomotor activity, as tested in the Digiscan® Animal Activity Monitor (see Chapter 4, Figure 4-6). Considering the known sedative effects of imipramine that is dose-dependent, it was decided forthwith to only use the 20 mg.kg⁻¹ dose for future investigations. On the other hand, pioglitazone still did not show any significant effect on immobility in the FST. As alluded to earlier, a possible explanation for this lack of effect could be a pharmacokinetic issue associated with the oral route. Based on these findings, it was therefore decided to undertake another pilot study, this time administering the test drugs via the subcutaneous route. In this case, imipramine was used as positive control at a dose of 20 mg.kg⁻¹ and pioglitazone at its highest dose of 120 mg.kg⁻¹. A 3% methylcellulose
solution was again used as vehicle control. The layout for this study is presented in Table 3-3 below and schematically represented in Figure 3-5:

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>n-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>n/a</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Imipramine</td>
<td>20 mg.kg(^{-1})</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Pioglitazone</td>
<td>120 mg.kg(^{-1})</td>
<td>8</td>
</tr>
</tbody>
</table>

- **Group 1**: The control group received only the vehicle (3% methylcellulose dissolved in 0.9% saline) via the subcutaneous route of administration.
- **Group 2 + 3**: These groups received their assigned drug treatment at the indicated doses. Imipramine was dissolved and pioglitazone suspended in the methylcellulose/saline vehicle.

![Figure 3-5: Schematic illustration of the treatment timeline for chronic experimental study in FSL and FRL rats](image)

As evident in the results presented in Chapter 4, Figure 4-11, imipramine, but not pioglitazone, proved to be an effective antidepressant following s.c. administration.

### 3.3.4.3 Main experimental study

In the main experimental study, treatment parameters were selected based on the foregoing pilot studies, but this time applied in a chronic treatment regime. Although
antidepressant efficacy can be demonstrated following active treatment, the acute FST is essentially a screening tool for possible antidepressant activity and is not definitive proof thereof (Detke et al. 1997). This is usually overcome by using a pathological animal model such as the FSL rat (see Harvey et al. 2011), or, alternatively, the chronic FST is advocated. In the latter case, the drugs under evaluation are tested in the FST following a chronic treatment period. Indeed, this has greater predictive validity for depression, since all known antidepressants are ineffective following acute administration, only showing efficacy after 3-4 weeks of treatment (Harvey 1997).

The pilot studies have convincingly demonstrated the face validity of the FSL rat for depression in comparison with its FRL control; while at the same time have demonstrated the antidepressant-like effects of imipramine in reversing these depressive-like behaviours in the FST. Consequently, the FSL rat was selected as the animal model to be used in the chronic treatment study, whereas imipramine was selected as the positive control (at a dose of 20 mg.kg$^{-1}$). Unfortunately, pioglitazone showed no evidence for significant antidepressant effects across a wide dosage range (30-120 mg.kg$^{-1}$) following either oral or subcutaneous administration. This was an unexpected finding, especially given the earlier published data describing an antidepressant response achieved with sub-chronic treatment with rosiglitazone (Eissa Ahmed et al. 2009). The relevance and implications of the findings of the current study are discussed in detail in Chapter 5.

However, as alluded to in Chapter 1 and more fully described in Chapter 2, there is substantial evidence in support of a role for insulin and the PPARγ pathway in mood regulation and antidepressant response. It is therefore possible that another way to test the response to pioglitazone, albeit not a definitive indication of an antidepressant effect, is to assess its ability to either augment or attenuate the antidepressant effect of a known antidepressant following chronic treatment. The main experimental study will therefore investigate this question by looking at whether drugs that are known to modulate insulin receptor sensitivity, namely PPARγ-agonists such as pioglitazone, and insulin releasing agents such as gliclazide, can alter the antidepressant response of imipramine. Although using a PPARγ antagonist (e.g. GW9662 –
Sadaghiani et al., (2011) would be a more appropriate negative comparator for pioglitazone, the study made use of gliclazide, an insulin releasing agent, being more readily available whilst mechanistically the two drugs will have the opposite effect on insulin receptor sensitivity (see Chapter 5 for more detail). Seeing that none of the doses of gliclazide as tested in the dose ranging study demonstrated any remarkable effect on behaviour (as anticipated), and that no supportive evidence of psychotropic effects could be obtained in the literature, the mean dose of 10mg.kg\(^{-1}\) (Serradas et al. 1989, Vallejo et al. 2000) was chosen to be used in subsequent experiments. The treatment groups for this study are outlined in Table 3-4 and schematically represented in Figure 3-6, whereas the findings are presented and discussed in Chapters 4 and 5. A 3% methylcellulose solution was again used as vehicle and control. A FRL group receiving vehicle was used as control for the FSL response.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>n-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle (FSL)</td>
<td>n/a</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle (FRL)</td>
<td>n/a</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Imipramine (FSL)</td>
<td>10 mg.kg(^{-1})</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Pioglitazone (FSL)</td>
<td>120 mg.kg(^{-1})</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Gliclazide (FSL)</td>
<td>10 mg.kg(^{-1})</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>Pioglitazone + Imipramine (FSL)</td>
<td>120 mg.kg(^{-1}) 20 mg.kg(^{-1})</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>Gliclazide + Imipramine (FSL)</td>
<td>10 mg.kg(^{-1}) 20 mg.kg(^{-1})</td>
<td>12</td>
</tr>
</tbody>
</table>

- Group 1: The control group received only the vehicle p.o. via oral gavage
- Group 2: The FRL-control group received only the vehicle p.o. via oral gavage
- Group 3, 4 + 5: These groups received their assigned drug treatments at the indicated doses p.o. via oral gavage
- Group 6+7: Gliclazide and pioglitazone was administered p.o. via oral gavage at the indicated dosages in combination with imipramine
3.3.5 Statistical analysis of data

As mentioned in the study layouts above, each experiment consisted of 4 animals per test group. Data from all behavioural studies in the experimental study were for three comparable experiments in the experimental study (total number of animals observed thus adding up to n=12) and two experiments in the dose-ranging and pilot studies (total number of animals observed thus amounting to n=8). Fewer animals were used in the latter studies in order to conserve the number of animals used, and because earlier FST studies undertaken in our laboratory have found that n-values as low as 5/group are adequate for statistically meaningful results, eg Harvey et al., 2010. For all behavioural data (i.e. general locomotor activity, FST) observations were made individually for each rat.

GraphPad Prism® version 5.00 for Windows (GraphPad Software®, San Diego, California, USA, www.graphpad.com) was used for statistical analysis of data. For comparison of two values, the nonparametric Student’s t-test was implemented. For multiple comparisons, a one-way analysis of variance (ANOVA) was performed followed by either the Dunnett’s post-test (for comparing experimental groups to control) or the Tukey-Kramer or Bonferroni post-test (for comparing experimental groups to each other). For all reported statistical probability values, significance was denoted by “*” (p<0.05), “**” (p<0.01) and “***” (p<0.001).
Various clinical (Kemp et al. 2009, Kemp et al. 2011) and preclinical (Eissa Ahmed et al. 2009, Sadaghiani et al. 2011) studies have provided evidence for the involvement of PPARγ in depression and for PPARγ-agonists in antidepressant response.

A recent preclinical study demonstrated the antidepressant-like effects of the insulin receptor sensitizer, pioglitazone, after acute treatment in rats (Sadaghiani et al. 2011). This study, however, did not consider whether these effects could be replicated in a chronic treatment paradigm, generally regarded as the more definitive test for antidepressant action (Detke et al. 1997). The study also did not consider whether pioglitazone modulates the effects of a known antidepressant drug. Indeed, as discussed in section 2.2.3, recent clinical studies have demonstrated that pioglitazone exhibits antidepressant effects in patients with metabolic syndrome (MetS) and co-morbid depression (Kemp et al. 2009, Kemp et al. 2011). The primary objectives of this study therefore centred on investigating whether pioglitazone is capable of evoking an antidepressant effect in a chronic treatment protocol and, secondly, whether chronic treatment with pioglitazone is able to augment the effects of the known antidepressant compound, imipramine. An additional secondary objective includes a comparison with gliclazide, a compound that, unlike PPARγ-agonists, increases insulin release, which may lead to a down-regulation in insulin receptors (Garvey et al. 1985). Moreover, while previous studies using PPARγ-agonists have been performed in healthy animals, the current study will investigate the effects of pioglitazone in a pathologic animal model of depression, the Flinders sensitive line (FSL) rat. Consequently, another primary objective included validation of the FSL rat with respect to its depressive-like behaviour compared to healthy FRL controls, and its response to a known antidepressant. The study and treatment protocol were conducted as outlined in Chapter 3. Results that will be presented here include effects of the administered agents on locomotor activity followed by behavioural assessment in the forced swim test (FST).
4.1 Pilot study: A dose-ranging analysis

In this series of experiments, the goal was to confirm earlier findings using thiazolidinediones as putative antidepressants in animal models, and then to establish appropriate doses for both pioglitazone and gliclazide following acute dosing under our conditions of study and that will be used later in the chronic treatment study. Furthermore, it was necessary to confirm the antidepressant-like effects of the positive control, first fluoxetine then imipramine, in FSL rats using the FST. This pilot study first used standard Sprague Dawley rats and later FSL rats, as outlined in Chapter 3 (see Section 3.3.4). Drugs were administered orally in this pilot study. The dosage range of pioglitazone (1-20 mg.kg\(^{-1}\)) was based on earlier studies with rosiglitazone (6-12 mg.kg\(^{-1}\); Eissa Ahmed et al. 2009), since, at the time, no similar studies had been undertaken with pioglitazone.

4.1.1 Acute treatment: Sprague Dawley rats

4.1.1.1 General locomotor activity

As depicted in Figure 4-1, neither the positive control, fluoxetine, nor any of the doses of pioglitazone markedly affected locomotor behaviour compared to the vehicle-treated control animals. Results of various doses of gliclazide are represented in Figure 4-2, and also show no significant effects when compared to the vehicle-treated control group.
4.1.1.2 Forced swim test

As illustrated in Figure 4-3 and Figure 4-4, neither the positive control, fluoxetine, nor any of the doses of pioglitazone (Fig. 4-3) or gliclazide (Fig. 4-4) produced any significant effects compared to the vehicle treated control animals.
To our knowledge, there is no published data implicating gliclazide in antidepressant or other psychotropic action and the lack of results observed with gliclazide was therefore expected. On the other hand, however, the data with pioglitazone, and especially also with fluoxetine, was troubling. Since fluoxetine was expected to decrease immobility in the FST, it
was decided to introduce a number of adaptations to the study protocol in order for the FST to detect the antidepressant activity of a known antidepressant drug, as highlighted below.

Due to a lack of significant results in the acute dose-ranging study in Sprague Dawley rats, especially for the positive control, fluoxetine, it was firstly decided to test these drug responses in a more stress-sensitive animal (viz. FSL rats). Secondly, a drug known to have a more pronounced antidepressant-like effect in the FST (viz. imipramine) was used, while a chronic treatment protocol was now applied (7 days; see Chapter 3, par. 3.3.4.1.2). In addition, since pioglitazone is approximately 10 times less potent than rosiglitazone (Junichi et al. 2000), it was forthwith decided to explore higher doses of pioglitazone (up to 120 mg.kg$^{-1}$). These doses are nevertheless well below the minimum toxicity values for this drug (LD50>1814 mg.kg$^{-1}$ in rats) (Ito et al. 2004). In order to establish an effective dose for imipramine in the FST, two doses were tested (viz. 20 mg.kg$^{-1}$ and 30 mg.kg$^{-1}$). Finally, the Flinders resistant line (FRL) rat was used as reference control for the FSL rat. All drugs were now to be administered orally for 7 days, as described in Section 3.3.4.1.2. Gliclazide, however, was set aside for later use in the main experimental study. These results are presented below in 4.1.2.

### 4.1.2 Chronic treatment: FSL rats

#### 4.1.2.1 General locomotor activity

As seen in Fig. 4-5, no significant differences were observed in the locomotor activity of vehicle-treated FSL and FRL rats and FSL rats treated with the higher doses of pioglitazone.
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Figure 4-5: Locomotor activity of FSL rats treated with pioglitazone for 7 days, compared to vehicle treated FSL and FRL rats. ns p>0.05 (one-way ANOVA: Dunnett’s post-test). n=8 for all groups.

Figure 4-6: Effect of various doses of imipramine (20 mg.kg⁻¹-30 mg.kg⁻¹) after 7-day treatment, on general locomotor activity in FSL rats as compared to vehicle treated control FSL rats. *p<0.05 vs. FSL; **p<0.01 vs. FSL (30 mg.kg⁻¹) (one-way ANOVA: Tukey post-test). n=8 for all groups.

However, as illustrated in Fig. 4-6, imipramine had a pronounced dose-related inhibitory effect on general locomotor activity. It reduced the number of horizontal beam breaks from 2150 ± 139 in vehicle treated FSL rats to 1558 ± 145 in rats treated with 20 mg.kg⁻¹ imipramine and 1314 ± 177 in rats treated with 30 mg.kg⁻¹. This phenomenon can be explained by the
dose-dependent sedative effect of tricyclic antidepressants (Tucker et al. 1986). However, this was not viewed as a potentially confounding factor in the antidepressant response described below since the sedative effects of these antidepressants are well-described. Furthermore, even though locomotor activity was diminished, it did not lead to increases in immobility in the FST (as may be expected) (see Figure 4-8).

4.1.2.2 Forced swim test

First, it was necessary to confirm the depressive-like phenotype of the FSL rat relative to its healthy control, the FRL rat. To do this, groups of FSL and FRL rats were compared with respect to their performance in the FST, as described in paragraph 3.3.4.1.2. These data are presented in Fig 4-7.

As illustrated in Fig. 4-7, treated FSL rats presented with significantly more immobility (218.6 ± 6.3 seconds) than their FRL-counterparts (60.0 ± 14.8 seconds), thus reaffirming the depressive phenotype of FSL animals. It was now necessary to confirm that these behaviours could be reversed by a known antidepressant agent, in this case imipramine.
As illustrated in Fig. 4-8, oral administration of imipramine dose-dependently decreased immobility in FSL rats subjected to the FST from $218.6 \pm 6.3$ seconds in vehicle treated animals to $95.0 \pm 19.2$ seconds in animals treated with $20 \text{ mg.kg}^{-1}$ imipramine and $57.5 \pm 26.7$ seconds in $30 \text{ mg.kg}^{-1}$ treated animals, thus confirming the antidepressant-like response of imipramine in FSL rats. The difference in immobility between the two administered doses of imipramine, however, was not significant.

![Graph showing effect of imipramine on immobility in FSL rats](image)

**Figure 4-8**: Effect of imipramine ($20 \text{ mg.kg}^{-1}$) vs. imipramine ($30 \text{ mg.kg}^{-1}$), after 7-day treatment, on immobility in the FST in FSL rats compared to vehicle treated control FSL rats. **p<0.01 vs. FSL; ***p<0.001 vs. FSL (one-way ANOVA: Bonferroni post-test). n=8 for all groups.
Turning to pioglitazone, while oral imipramine 20 mg.kg\(^{-1}\) was an effective antidepressant in this model, oral administration of high-dose pioglitazone on the other hand had no significant anti-immobility effect in the FST at any of the doses tested when compared to vehicle-treated FSL rats (Fig 4-9).

Comprehensive bioavailability studies were beyond the scope of this study, and hence could not be used to corroborate a possible pharmacokinetic basis for the lack of pharmacological effect with pioglitazone. Nevertheless, since oral administration offers a number of pharmacokinetic hurdles that may limit absorption, such as bioavailability and first pass elimination, all drugs were subsequently re-tested but this time following administration via the subcutaneous (s.c.) route, also for 7 days. These data are presented below.
4.1.3 Chronic treatment via the subcutaneous (s.c) route

4.1.3.1 General locomotor activity

Following s.c. administration for 7 days, imipramine (20 mg.kg⁻¹) significantly suppressed locomotor activity in FSL rats (1506 ± 174 horizontal beam breaks) compared to vehicle treated FSL rats (2115 ± 256 horizontal beam breaks) (Fig 4-10). Pioglitazone (120 mg.kg⁻¹), however, did not adversely affect locomotor activity compared to the vehicle-treated control rats (Fig 4-10).

![Figure 4-10: Locomotor activity of FSL rats treated s.c. with imipramine (20 mg.kg⁻¹) and pioglitazone (120 mg.kg⁻¹) for 7 days, as compared to vehicle treated (s.c.) FSL rats. *p<0.05 vs. FSL (one-way ANOVA: Dunnett’s post-test). n=8 for all groups.](image)

4.1.3.2 Forced swim test

When considering the response in the FST, imipramine (20 mg.kg⁻¹ s.c. x 7 days) produced a significant anti-immobility effect in FSL rats, reducing immobility time seen in vehicle treated control animals from 211.9 ± 10.0 to 23.8 ± 10.2 seconds (Fig 4-11). Pioglitazone (120 mg.kg⁻¹), however, did not significantly alter immobility scores in the FST when compared to the vehicle-treated FSL control group (Fig 4-11).
4.2 Main experimental study

The inherent immobility behaviour of the FSL rat in the FST (as opposed to its FRL-counterpart), and the anti-immobility effects of the positive control, imipramine (20 mg.kg\(^{-1}\) and 30 mg.kg\(^{-1}\)), were now established and confirmed in this model. However, despite various modifications to the original protocol, including alterations to the route of administration, dose and duration of treatment, pioglitazone failed to illicit an antidepressant-like effect in the FSL model.

The use of augmentation strategies in partial responders is a fairly popular practice in antidepressant therapy, mainly due to an improvement in antidepressant response when certain non-antidepressant drugs are added to therapy, as opposed to just switching a patient to another antidepressant and losing improvements seen with initial antidepressant treatment (Nelson 2003). It is therefore important to also assess the potential of drugs that do not present with antidepressant effects themselves, but that nevertheless act on certain molecular targets purported to be involved in the neurobiology of depression as a possible augmentation strategy. As alluded to earlier, clinical and pre-clinical studies have found that rosiglitazone and pioglitazone improve mood (Kemp et al. 2009, Kemp et al. 2011), while there is also a strong
association between depression and T2DM (Anderson et al. 2001, Capuron et al. 2004, Krishnan et al. 2008). Consequently, pioglitazone was tested at its highest dose for its ability to bolster the antidepressant effects of imipramine. In addition, gliclazide was reintroduced into the study as a negative control for pioglitazone, at a dosage based on the original dose response studies, as well as the literature (refer to § 3.3.4.1.1).

In the main experimental study, it was investigated whether co-administration of pioglitazone (120 mg.kg⁻¹) or gliclazide (10 mg.kg⁻¹) together with imipramine (20 mg.kg⁻¹) over a chronic treatment period of 7 days would in any way alter the existing anti-immobility effects of the latter antidepressant drug.
4.2.1 General locomotor activity

Figure 4-12: Locomotor activity of FSL rats chronically treated with imipramine (20 mg.kg$^{-1}$ p.o.), pioglitazone (120 mg.kg$^{-1}$ p.o.), gliclazide (10 mg.kg$^{-1}$ p.o.) and imipramine (20 mg.kg$^{-1}$ p.o.) co-administered with either pioglitazone or gliclazide for 7 days at the same doses as compared to vehicle treated FSL and FRL animals. ***p<0.001 vs. FSL (one-way ANOVA: Dunnett’s post-test). n=12 for all groups.

FRL and FSL rats did not differ with respect to general locomotor activity (Fig 4-12). All imipramine-treated groups presented with a significant inhibition of locomotor activity (Fig 4-12) when compared to groups not treated with imipramine (including vehicle-treated FSL and FRL rats).
4.2.2 Forced swim test

As illustrated in Figure 4-13, FSL rats presented with significantly more immobility in the FST than their FRL controls (Fig 4-13), while imipramine significantly attenuated this immobility versus vehicle-treated FSL rats. However, neither pioglitazone nor gliclazide had any significant anti-immobility effects in FSL rats.

Now considering the pioglitazone combination treatments, when compared to vehicle-treated FSL rats (216.4 ± 4.5 seconds), animals treated with pioglitazone as single drug treatment (181.3 ± 20.7 seconds) did not present with any change in immobility behaviour (Fig 4-14). However, significant anti-immobility effects were observed in animals treated with either imipramine alone or receiving pioglitazone co-administered with imipramine (90.5 ± 17.6 seconds) (Fig 4-14). However, the combination of pioglitazone plus imipramine did not differ significantly from animals treated only with imipramine (95.4 ± 13.9 seconds) (Fig 4-14). Immobility in the pioglitazone alone group (181.3 ± 20.7 seconds) was also no different to FSL (216.4 ± 4.5 seconds) and significantly higher than that in the imipramine (95.4 ± 13.9 seconds) and imipramine plus pioglitazone treated groups (90.5 ± 17.6 seconds) (Fig 4-14).
Figure 4-14: Effect of chronically administered imipramine (20 mg.kg⁻¹ p.o.), pioglitazone (120 mg.kg⁻¹ p.o.) and pioglitazone co-administered with imipramine for 7 days, on immobility in the FST in FSL rats as compared to vehicle treated FSL rats. ***p<0.001 vs. FSL (one-way ANOVA: Tukey post-test). n=12 for all groups.

Now considering the gliclazide combination treatments, a different profile emerged (Fig 4-15).
Figure 4-15: Effect of chronically administered imipramine (20 mg.kg\(^{-1}\) p.o.), gliclazide (10 mg.kg\(^{-1}\) p.o.) and gliclazide co-administered with imipramine for 7 days, on immobility in the FST in FSL rats as compared to vehicle treated FSL rats. **p<0.01 and ***p<0.001 vs. FSL (one-way ANOVA: Tukey post-test). n=12 for all groups.

As illustrated in Figure 4-15, while imipramine alone (95.4 ± 13.9 seconds) was effective in reducing immobility time in the FST versus FSL control (216.4 ± 4.5 seconds), gliclazide alone (198.8 ± 14.1 seconds) failed in this regard. Gliclazide (198.8 ± 14.1 seconds) was also no different versus FSL (216.4 ± 4.5 seconds) and significantly different versus imipramine alone and imipramine plus gliclazide (120.8 ± 18.7 seconds). Although still significant versus FSL control, the gliclazide-imipramine combination was now a less effective antidepressant than imipramine alone. Although imipramine versus imipramine plus gliclazide did not differ (Fig 4-15; NS), when compared to the vehicle-treated FSL group, the extent to which immobility was suppressed in the GLZ+IMI group (p<0.01) was less than that seen in animals treated with imipramine as single drug treatment (p<0.001).
5.1 Introduction

Depression is a debilitating disorder with increasing prevalence (§2.1.1). Current treatments are less than adequate, while there is a desperate need for novel antidepressant agents as well as new molecular targets of relevance in the regulation of mood (Vetulani et al. 2000, Skolnick et al. 2001). Various hypotheses have attempted to elucidate both the pathophysiology and neurochemistry underlying the disease (Nestler et al. 2002, Krishnan et al. 2008, Vetulani et al. 2000). Although a number of useful and valid arguments have been put forward, none have been able to fully explain all the facets of depression (§2.1.3). Indeed, the cause of depression is multifactorial and its pathology complex (§2.1.2). In this regard, depression is highly co-morbid with a number of metabolic disorders, including metabolic syndrome (MetS), cardiovascular disorders and type 2 diabetes mellitus (T2DM), all of these sharing some mutual underlying biochemical feature with depression (§2.2). It seems that the common denominator presents itself as chronic inflammation, a symptom that is central to all of these co-morbid diseases. Levels of proinflammatory cytokines (prominent inflammatory mediators) are elevated in all these diseases (Anisman et al. 2003, Anisman et al. 2008, Capuron et al. 2002, Capuron et al. 2008, Dantzer et al. 2008) and may be interpreted by the brain as stressors (Anisman et al. 2003), leading to abnormalities in neurotransmitter metabolism, neuroendocrine function, synaptic plasticity and behaviour (Danner et al. 2003, Ford et al. 2004, Raison et al. 2006, Tiemeier et al. 2003, Miller et al. 1999, Musselman et al. 2001). This highlights the importance of inflammation in chronic diseases and that this may contribute significantly to an increased occurrence of depression in such patients. It also raises the need for more efficacious pharmacotherapeutic approaches that will target both the metabolic disorder and its effect on mood.

Both clinical and pre-clinical studies have described the antidepressant-like effects of PPARγ-agonists and insulin-sensitizing drugs in rodents (Eissa Ahmed et al. 2009, Sadaghiani et al. 2011) and in human subjects (Kemp et al. 2009), whereas Kemp and colleagues (2011) has
described anti-depressant effects of pioglitazone in patients with MetS. Indeed, Rasgon et al. (2010) also reported a decline in the severity of depressive symptoms in depressed, insulin-resistant patients who were prescribed rosiglitazone as an addition to their existing antidepressant/mood-stabilizing drugs. Furthermore, antidepressant treatment has also been demonstrated to be beneficial for co-morbid diseases in patients suffering from depression, e.g. certain SSRIs in cardiovascular disease and T2DM (also see Chapter 2, § 2.2.2.2) (Halaris 2009, Connolly et al. 1995, Daubresse 1996, Gray et al. 1992, Sauer et al. 2001, Sauer et al. 2003).

Preclinical studies in FSL rats, a genetic animal model of depression, have demonstrated that a long-term high-fat diet exacerbates depressive-like behaviour in these animals (Abildgaard et al. 2011), also presenting with decreased endogenous cardio-protective mechanisms and a resultant increase in infarct size, compared to healthy FRL control rats (Solskov et al. 2010). Results from a study by Buhl et al. (2010) established that SSRIs not only correct disturbances in HPA-axis activity, but also improve insulin resistance as seen in adult rats born with low birth weights. It has also been proposed that metabolic disturbances, e.g. oxidative stress and inflammation, associated with systemic disorders may lead to a form of metabolic encephalopathy, resulting in structural brain damage and neuropsychiatric symptoms, e.g. depression (Harvey 2008). Recent studies in sub-Saharan Africa concluded that there was a strong correlation between depressive symptoms and the presence of cardiovascular disease in humans. The presence of MetS was also associated with an increased incidence of co-morbid atherosclerosis and depression in black Africans (Hamer et al. 2011). In addition, depressive symptoms were linked to deviations in blood pressure associated with disturbances in circadian rhythm in both black and caucasian Africans (Hamer et al. 2012).

The above-mentioned results reinforce the need to consider the mood-metabolic interface as a new and important area of research into depression and antidepressant action.

The primary objectives of the current study was to investigate the proposed antidepressant-like effects of the insulin-sensitizing PPARγ-agonist, pioglitazone, in the FSL rat, a genetic animal model of depression, in both an acute and a chronic treatment protocol. This was extended to the secondary objectives which included insulin-releasing drug gliclazide, as described in
section 1.2.2. The behavioural assessments that were performed included the FST and general locomotor activity.

This chapter will discuss the results as presented in Chapter 4 as follows:

- Results of the acute treatment study
- Inherent depressive-like behaviour of FSL rats compared to FRL rats
- Chronic antidepressant treatment reverses depressive-like behaviour in the FSL rat
- Investigation into the antidepressant-like effects of the PPARγ-agonist, pioglitazone, in FSL rats
- Investigation into gliclazide- and pioglitazone-induced modification of the antidepressant response in imipramine-treated FSL rats

### 5.2 Results of the acute treatment study

In an attempt to determine the appropriate doses for both pioglitazone and gliclazide to be used in the chronic treatment study, various doses were administered to the different groups of animals, each group consisting of eight male Sprague Dawley rats.

Following assessment of general locomotor activity in the DigiScan® Animal Activity Monitor, results indicated that none of the vehicle, fluoxetine, pioglitazone (Fig. 4.1) or gliclazide (Fig. 4.2) treated groups presented with significantly altered locomotor activity. Thus, performance in the FST could be interpreted without a confounding influence of locomotor performance.

In the FST, none of the acute treatments (Fig 4.3 & Fig 4.4) exhibited any significant variation in immobility. This was unexpected, seeing that neither pioglitazone, nor the positive control, fluoxetine, showed any significant anti-immobility effects. An acute dose-response protocol was initially tested in order to confirm whether acute dosing would indeed be a viable treatment option, especially since the FST is widely recognised to be sensitive to acute
treatment. Moreover, recent studies using acute and sub-chronic treatments with rosiglitazone and pioglitazone have demonstrated the antidepressant-like potential of these drugs (Eissa Ahmed et al. 2009, Sadaghiani et al. 2011). Similarly, being an established antidepressant, fluoxetine was expected to reduce immobility and thus justify its inclusion in the study as a positive control. While the failure of pioglitazone to produce an antidepressant response is disappointing (but nonetheless possible), the failure of the positive control to attenuate immobility led us to believe that the treatment protocol was inappropriate, which would negatively bias the testing of pioglitazone under these conditions and possibly explain its lack of effect in the FST.

Studies with rosiglitazone and pioglitazone have demonstrated apparent antidepressant effects in rodents following 1 and 5 days of treatment (Eissa Ahmed et al. 2009, Sadaghiani et al. 2011), which motivated the initial intention to assess the behavioural effects of pioglitazone with fluoxetine as positive control using an acute treatment protocol. This despite evidence in the available literature that fluoxetine (5 mg.kg\(^{-1}\)) was found to only reduce immobility after a treatment period of 7 to 14 days, but not after one day (Vázquez-Palacios et al. 2004). In fact, this latter data coincides with the drug’s inability to demonstrate an immediate onset of antidepressant effect in humans. That pioglitazone elicits antidepressant-like effects following both acute and sub-chronic treatment (Eissa Ahmed et al. 2009, Sadaghiani et al. 2011) seems unusual but nonetheless interesting. It was clear that further study using a chronic treatment protocol, as well as introducing a new positive control into the study, would be essential. To further enhance the chances of success, it was also decided to replace Sprague Dawley rats with FSL rats, a genetic animal model of depression. FSL rats present with many of the behavioural and biological characteristics of depression (see § 2.2.6.1) and hence provide added validity and sensitivity to antidepressant-drug testing. However, the depressive-like character of these animals needed to be confirmed under the current laboratory conditions before further study could be considered.
5.3 Inherent depressive-like behaviour of FSL rats compared to FRL rats

Considering the lack of results in the acute treatment strategy, it was decided to employ a more stress-sensitive animal, the FSL rat, and use its more resilient counterpart, the FRL rat, as control.

The general locomotor activity of FSL versus FRL groups of animal were assessed and no significant differences were observed (Fig. 4-5), which indicates that any reduction of immobility behaviour in the FST would not be the result of inherent deficits in locomotor activity in FSL animals. With locomotor activity now confirmed to be unaltered in these animals versus their healthy control, further study focussing on the effects of drug treatment on behaviour could now be considered.

Following locomotor assessment, the animals were introduced to the FST. As depicted in Fig. 4-7, the FSL rats presented with significantly more immobility than the FRL controls. In the FST, increased immobility denotes an increased depressive-like state (refer to §2.1.9.3), thus confirming the depressive-like nature of FSL rats compared to their healthy control. The data also confirms the face validity of this animal both in the FST and as a genetic animal model of depression. It can therefore be concluded that the FST could be used as a reliable model to assess any possible antidepressant-like effects following treatment with pioglitazone, and the new positive control, imipramine.

5.4 Chronic antidepressant treatment reverses depressive-like behaviour in the FSL rat

As an additional measure to elicit a more robust response in the FST, fluoxetine was replaced with the tricyclic antidepressant (TCA) drug, imipramine. This is not meant to imply that imipramine is a more effective antidepressant than fluoxetine but it is known that imipramine demonstrates a more reproducible result in the FST compared to the SRIs (Kornstein et al.
2000, Mason et al. 2009). It is for this reason that several modifications have been implemented in the traditional Porsolt-FST (refer to Chapter 3, §2.2.7.3). Indeed, earlier studies have demonstrated that imipramine is an effective positive control in the FST, engendering a significant antidepressant response in the FST after both acute and chronic treatment (Harvey et al. 2010, Frankowska et al. 2010, Hirani et al. 2002, Nowak et al. 1991). Finally, the behavioural effects of treatment in the FST were now assessed in FSL rats over a chronic treatment period of 7 days.

As illustrated in Fig. 4-6, general locomotor activity was progressively inhibited by the administration of increasing doses of imipramine (20 and 30mg.kg\(^{-1}\)) – a phenomenon that is explained by the well-known sedative effects of TCAs (Tucker et al. 1986). The suppression of locomotor activity, however, did not result in increased immobility in the FST as could be expected, thus precluding adverse effects on locomotor activity as a possible confounding effect in the event of a demonstrable loss of antidepressant effect in the FST. In fact, imipramine treatment at both doses induced a significant decrease in immobility behaviour (Fig. 4-8), despite an apparent lowering of locomotor performance. This data confirms that imipramine exerts an antidepressant-like action after chronic treatment, attenuating the depressive-like behaviour exhibited by the FSL rats. These findings reaffirm the predictive validity of the FSL rat as a behavioural animal model of depression.

The above studies with imipramine therefore re-established an appropriate positive control of antidepressant efficacy to be used as reference in all subsequent experiments in the chronic treatment study. However, since 20 mg.kg\(^{-1}\) imipramine daily p.o. delivered an adequate and significant antidepressant-like effect, this dose was used forthwith to minimize dose-dependent locomotor complications.
5.5 Investigation into the antidepressant-like effects of the PPARγ-agonist, pioglitazone, in FSL rats

Since the potency of pioglitazone is approximately ten times less than that of rosiglitazone (refer to §4.1.1.2), the dose of pioglitazone was increased to approximately ten times that described for rosiglitazone, viz. 6-12mg.kg\(^{-1}\) (see Eissa Ahmed et al. (2009)). None of the doses of pioglitazone tested across this range (viz. 70-120 mg.kg\(^{-1}\)) induced any significant decrease in immobility in FSL rats (Fig. 4-9). Despite this, it was decided to use pioglitazone at a dose of 120 mg.kg\(^{-1}\) in all subsequent experiments as it represented the projected upper end of its dosage based on its potency compared to rosiglitazone.

As is evident in Figure 4-13, pioglitazone failed to engender an antidepressant-like response in FSL rats following chronic treatment. Clinically, it has been demonstrated that pioglitazone exerts antidepressant effects in patients with co-morbid MetS (refer to §2.2.3), while in animal models of depression, both rosiglitazone and pioglitazone have been shown to exert antidepressant-like effects in two different animal models of depression, namely Sprague Dawley rats and mice (§2.2.3).

Results from the current study using the FST indicate that the effects of treatment with both the vehicle and imipramine correspond closely to values obtained in earlier studies performed in our own laboratory and elsewhere (Abildgaard et al. 2009, Mokoena et al. 2011, Overstree et al, 2005). It is therefore highly unlikely that the lack of anti-immobility effects observed after pioglitazone treatment is a result of flawed method.

Considering then the data from both acute and chronic treatment studies, the presented evidence refutes the claim that pioglitazone has antidepressant activity. However, a new paper published (Sadaghiani et al. (2011)) while the present study was being undertaken, seems to contradict our findings. The authors did indeed observe an antidepressant-like effect in the FST following treatment of mice with pioglitazone. There are, however, some noteworthy differences in study design between the latter study and ours. First and probably most obvious
of these are the contrasting animal models used during these studies, viz. NMRI mice (a traditional outbred strain of mice used extensively in a wide range of preclinical studies) in the Sadaghiani study vs. a genetic animal model of depression, the FSL rat, in the current study. In the Sadaghiani study, animals only received acute treatment orally with pioglitazone before being exposed to the FST. Moreover, doses used (5, 10, 20 and 30 mg.kg\(^{-1}\)) were much lower than that used in the current chronic study (30-120 mg.kg\(^{-1}\)), doses that we had earlier demonstrated to be ineffective (see Figure 4-9). Sadaghiani et al. found that doses of 20 and 30 mg.kg\(^{-1}\) were effective in exerting antidepressant-like effects in the FST 2 and 4 h after administration, but not when the drug was administered 1 and 8 h prior the FST. This is an unusual finding. The authors of the paper do not explain this anomaly, but it may be postulated that this time-dependant effect is associated with pharmacokinetic factors. It may therefore be argued that a delay of one hour is insufficient for the drug to reach the necessary blood concentrations to mediate its effects, whereas after eight hours the drug may have been metabolized to such an extent that blood concentrations have attained sub-effective levels. On the other hand, however, the short-lived anti-immobility effects seen after acute treatment may also predict a loss of antidepressant-like effects after chronic treatment. Probably the greatest shortcoming of the Sadaghiani et al. study is the failure to extend the anti-immobility effects observed after acute treatment to similar testing following chronic treatment. Even though decreased immobility in the FST after acute treatment is indicative of probable antidepressant effects, it remains crucial to prove that the anti-immobility effects seen after acute treatment can in fact be ascribed to antidepressant-like activity, keeping in mind that chronic but not acute studies are generally regarded as the definitive test for antidepressant action (Detke et al. 1997). If this is in fact the case, the results presented by Sadaghiani et al may even be more supportive to the lack of antidepressant-like effects presented in the current study. Another difference between the two studies worth noting is the choice of animals used, viz. mice versus rats, and secondly healthy versus “sick” animals in the case of the FSL rat model.

If pharmacokinetic issues, however, did indeed result in the loss of anti-immobility effects, it would support the initiative to explore another route of administration, viz. the subcutaneous
route (§4.1.3), which bypasses first-pass metabolism and may in fact cause delayed metabolism and retention of the drug in circulation (Beránková et al. 2007, De Souza et al. 1991) – factors that may be beneficial, especially during chronic treatment. Therefore, in order to exclude a possible pharmacokinetic basis for the failure of pioglitazone to induce an antidepressant-like effect (as mentioned above), it was decided to test the drug via another route of administration.

A new group of FSL animals was therefore treated with pioglitazone via the subcutaneous route (120mg.kg\(^{-1}\)) and compared to animals treated with subcutaneous vehicle and imipramine (20mg.kg\(^{-1}\)), all at the same doses used in the oral treatment protocol described above. The results of this experiment, however, were congruent with that seen in orally treated animals, with only imipramine producing a significant anti-immobility effect in the FST (Fig. 4-11). Except for the impairment of locomotor activity observed in imipramine-treated animals, general locomotor activity was unaffected (Fig. 4-10). This indicates that the route of administration, per se, cannot exclusively offer an explanation for the lack of anti-immobility effects seen subsequent to chronic oral administration. It was therefore decided to resume oral administration during the experimental study.

5.6 Investigation into gliclazide- and pioglitazone-induced modulation of the antidepressant response in imipramine-treated FSL rats

Although chronic pioglitazone treatment failed to demonstrate any antidepressant-like response in the FST, there remains the distinct possibility that it may possess the ability to augment the effects induced by a known antidepressant treatment, such as imipramine. Examples of successful augmentation strategies include the addition of thyroxin to both SSRIs and tricyclic antidepressants (Barak et al. 1996, Łojko et al. 2007, Spoov et al. 1998) and also the addition of bupropion, buspirone and lithium to existing sub-efficacious treatment (Rush et al. 2004).
Gliclazide was now reintroduced into the chronic combination-treatment study as a negative control. Gliclazide is a hypoglycaemic agent. During a review of the literature, no evidence could be found that it interacts with PPARγ, while in fact it may actually compromise insulin receptor sensitivity, opposite to pioglitazone (Garvey et al. 1985).

When analyzing Table 4-1 and Figure 4-12, it is evident that a decrease in general locomotor activity could be observed in all drug-treated groups, but once again only imipramine-treated animals demonstrated a significant inhibition of locomotor activity compared to vehicle-treated FSL rats, although it did not appear to compromise performance in the FST.

When considering the results in the FST, pioglitazone did not exert any significant effect on the anti-immobility effects induced by imipramine (Fig. 4-14), which suggests that, apart from its inability to induce antidepressive effects of its own in FSL rats after chronic treatment, it does not reverse or counter the antidepressant-like effects of imipramine. Furthermore, a possible explanation for the lack of pioglitazone to bolster imipramine’s effects could be that an already maximal anti-immobility response by imipramine was already present under the current conditions of study, thus allowing little room for any augmenting action to be visible.

*However*, in the gliclazide-imipramine combination study, gliclazide presented with a trend (did not reach statistical significance) to *decrease* the anti-immobility effects of imipramine compared to vehicle-treated animals, even though anti-immobility effects observed in the imipramine-gliclazide combination remained significantly lower than that observed in the vehicle treated controls (Fig. 4-15). This suggests that the underlying effects on insulin receptor sensitivity, and possibly other metabolic factors, mediated by gliclazide may attenuate the ability of imipramine to mediate its anti-immobility effects at an optimal level. As mentioned earlier (see Chapter 2, §2.2.4), depression has been associated with insulin resistance. Increased insulin release, as mediated by the stimulating effects of gliclazide on β-cells in healthy FSL rats, may not only lead to hyperinsulinaemia, but possibly reduce insulin sensitivity (Garvey et al. 1985) in both the periphery and in the CNS (Mendelson 2008). Since only one dose of gliclazide was tested, viz. 10 mg/kg, it raises the interesting caveat that more
pronounced inhibition of imipramine’s antidepressant effects could have been attained at higher dosages. Further studies in this regard are necessary.

Hyperinsulinaemia may also affect neurotransmitter release and reuptake (Sauter et al. 1983) and also have debilitating effects on cognitive function (Kalmijn et al. 1995, Stolk et al. 1997). Furthermore, the effect of increased insulin levels may also affect the brain indirectly through modifying the regulation of glucose uptake (Biessels et al. 2002). Keeping in mind that normal brain function greatly relies on the presence of glucose, a lack thereof could be a predictor of diminished brain function, modified behaviour and subsequent depression (Rasgon et al. 2004, Wright et al. 1978). It is therefore proposed that the proposed ability of gliclazide to decrease the anti-immobility effects of imipramine when co-administered is based either directly or indirectly on the effects of gliclazide on insulin release.

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In summary, the FSL rat was reaffirmed to be an effective animal model of depression and imipramine a suitable positive control for antidepressant-like effects in the FST – both therefore suitable for use during this experimental study.

Pioglitazone, however did not present with antidepressant-like effects in the FST after a 7-day chronic treatment protocol. Even though this finding is contrary to results following acute treatment in mice published elsewhere (Sadaghiani et al., 2011), it is plausible since antidepressant-like efficacy can only be confirmed following a chronic treatment protocol. Furthermore, data presented by the latter study leads us to belief that pioglitazone’s antidepressant-like effects are highly variable, dependent on a number of confounding factors such as dose, duration of treatment, pharmacokinetics as well as the animal model used. Additionally, unlike gliclazide, pioglitazone did not adversely affect the anti-immobility effects of imipramine in the FST. Even though the current study failed to convincingly demonstrate the antidepressant-like effects of pioglitazone after chronic treatment in an animal model of depression, the results obtained in both clinical and other preclinical studies, as discussed in the literature review, are enough motivation to further explore a possible antidepressant role for
drugs acting on PPARγ. Further investigation with respect to augmentation is especially warranted.

The data presented with respect to the modulatory effects of gliclazide on antidepressant response is encouraging, especially since gliclazide tended to reduce the anti-immobility effects of imipramine in the FST – an effect that could possibly be attributed to the ability of gliclazide to interfere with insulin regulation and its subsequent effects on neurotransmitter regulation. This finding is in support of evidence linking insulin to mood regulation and antidepressant response discussed above and also in Chapter 2.
CHAPTER 6  Conclusion

Major depression is a highly prevalent and debilitating mood disorder of which the aetiology is multifactorial and not well understood (refer to Chapter 2, §2.1.2). It is also highly co-morbid with not only other psychiatric disorders, but chronic systemic disorders as well, e.g. atherosclerosis, type 2 diabetes mellitus and metabolic syndrome (MetS). This co-occurrence of depression with diseases containing a prominent inflammatory component has led to increasing credibility of evidence linking major depressive disorder with inflammation.

Furthermore, the shortcomings of currently available antidepressant drugs have generated investigations into novel antidepressant drugs (Skolnick et al. 2001) – some of these aimed at targeting both mood and metabolic disorder. This led to the proposal that PPARγ may play a role in depression (Rosa et al. 2008, Ji-Rong et al. 2010) and that the PPARγ-agonist insulin sensitizing drugs may exhibit antidepressant effects (Eissa Ahmed et al. 2009, Kemp et al. 2009). Indeed, this was proven to be the case for pioglitazone in depressed patients with MetS (Kemp et al. 2009, Kemp et al. 2011). Furthermore, both pioglitazone and rosiglitazone were demonstrated to evoke antidepressant-like effects in rodents. However, these effects in animal models were restricted to acute and sub-chronic treatment strategies and therefore did not serve as definitive proof of their antidepressant-like effects.

The primary purpose of this study, therefore, was to employ the PPARγ-agonist drug, pioglitazone, in a chronic treatment paradigm, and using a genetic animal model of depression, to extend on previous studies performed to investigate the mood-metabolic interface, and how this may be explored to further our understanding of depression and its possible treatment.

As a primary objective, the current study has reaffirmed the inherent depressive-like stereotype of the Flinders sensitive line (FSL) rat in comparison with its more resilient counterpart, the Flinders resistant line (FRL) rat. The results also confirmed that the known antidepressant drug, imipramine, evoke a robust antidepressant-like effect in FSL rats in the forced swim test (FST) at various dosages, over both acute and chronic treatment, and via both the oral and subcutaneous routes of administration. This confirms the validity of the FSL rat as an animal model of depression, thus presenting with robust face and predictive validity.
Chronic treatment with pioglitazone, however, did not result in antidepressant-like effects following neither acute nor chronic treatment, or when performed in two animal models, viz. healthy induced or FSL rats. Moreover, co-administration of pioglitazone and imipramine at the doses tested did not lead to an improved anti-immobility response in the FST. These results, therefore, do not confirm the antidepressant-like effects of acute pioglitazone treatment described in a previous study (Sadaghiani et al. 2011). However, data presented here and by others (Sadaghiani et al. 2011) suggests that pioglitazone’s antidepressant-like effects are dependent on a number of confounding factors such as dose, duration of treatment, pharmacokinetics as well as the animal used.

Considering the study’s secondary objectives, results did not demonstrate any influence of the insulin-releasing agent, gliclazide, on immobility in the FST, although in the gliclazide-imipramine combination study, gliclazide appeared to attenuate the anti-immobility effects exerted by imipramine. Since this finding supports evidence linking altered insulin receptor sensitivity to mood (García-Bueno et al. 2010, Sadaghiani et al. 2011), this preliminary finding requires further study, possibly looking at a broader dose range.

Even though pioglitazone failed to provide definite proof of its antidepressant-like effects, ample evidence exists in the literature to encourage further investigation into the involvement of PPARγ in mood. For example, Sadaghiani et al. (2011) demonstrated that the anti-immobility effects exerted by pioglitazone in the FST after acute treatment involved the nitric oxide (NO) cascade – one which has already been proven to be involved in the proposed pathophysiology of depression.

It is evident that that the PPARγ-agonist and insulin sensitizer, pioglitazone, does not present with antidepressant-like actions in FSL-rats following chronic administration and that it also does not enhance the antidepressant-like actions of the known antidepressant, imipramine. Moreover, gliclazide does not worsen depressive-like behaviours in FSL rats, neither does it significantly attenuate the antidepressant effects of imipramine. Our central hypotheses, as outlined in section 1.2.1, are therefore rejected. Regarding conflicting results in the literature, there remains great potential in this field of study and further study is therefore recommended.
6.1 Suggestions for future study:

- It may be beneficial to investigate whether even higher doses of pioglitazone would be able to elicit any behavioural effects in the FST, or whether such doses may more effectively bolster antidepressant response.
- Since this study did not explore any augmenting effect of pioglitazone in the face of sub-therapeutic doses of imipramine, further studies aimed at investigating augmentation of low-dose imipramine with pioglitazone may be more illuminating.
- Additional to this, it might also be necessary to investigate whether other routes of administration, e.g. intraperitoneal injection, may help to explain and confirm the lack of effect at these high doses.
- It is also important to consider whether another animal model, e.g. fat-fed FSL rats (Abildgaard et al. 2011) or obese Zucker rats, could be even more fitting considering the involvement of insulin resistance, T2DM and MetS in depression.
- Since this study demonstrated a partial attenuation of imipramine’s antidepressant response with gliclazide, further studies exploring a broader dosage range of gliclazide would be very valuable in confirming the supposition that insulin desensitizing agents abrogate antidepressant response.
- Any response to imipramine under the influence of either an insulin sensitizer or desensitizer should in future studies be combined with concomitant measurement of various peripheral and CNS markers of insulin sensitivity and glucose tolerance.
- Measuring markers of inflammation, eg. IL-6 and TNF-α may also provide an explanation for the lack of antidepressant results found after pioglitazone administration – this may also be considered for future studies.


Harvey BH, Shahid M. Metabotropic and ionotropic glutamate receptors as neurobiological targets in anxiety and stress-related disorders: focus on pharmacology and preclinical translational models. *Pharmacol Biochem Behav*. 2012 Feb;100(4):775-800


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