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Major depression and models for new generations effective antidepressants

Prof CB Brink

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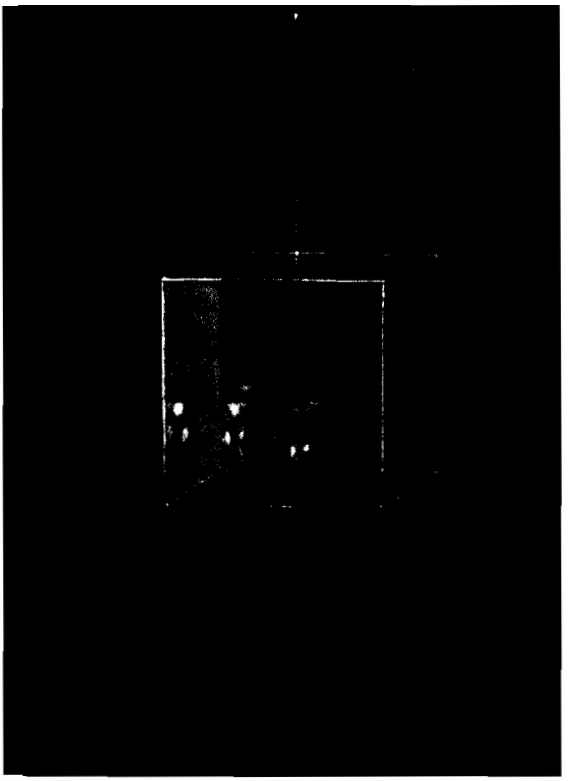
Preface

Research is a uniquely intriguing thing: it is sometimes a sole journey, yet intensely collaborative; it obeys strict rules, yet it is very creative; it provides us with answers, yet it leaves us with even more questions. This publication is about my journey in the world of science.

Madam the Rector, members of the Management Committee, Dean of the Faculty of Health Sciences, Deans of other Faculties, Directors, distinguished guests, colleagues, family and friends, it is my honour and privilege to share some of my experiences and insights with you today. Thank you for your presence and interest in this momentous occasion in my life.

I wish to dedicate this publication to my family who has been the jewels, cornerstone and love of my heart for many years: my wife Corné, my daughter Anchenique and my two sons Theowald and Henco.



"The Man in the Box"

Ben Schwarcz, acrylic on canvas.

(http://www.alternativedepressiontherapy.com/art-therapy-for-depression.html, accessed online on 28 August 2009)

Table of Contents

Abstract

(Afrikaans follows English / Afrikaans volg Engels)

Major depression is a severe mood disorder, affecting the lives of more than a hundred million people world-wide, including in Africa. Yet existing effective treatment options, including antidepressant drugs, are insufficient, prompting and leaving many challenges for research.

Major depression is a multi-factorial disorder, affecting multiple systems of the body. Even when focusing only on the central nervous system (brain - as it is primarily a psychiatric disorder by definition), there is a multitude of neurobiological functions that are affected in this disorder. Consequently there are several hypotheses to explain the neurobiological basis of depression and antidepressant action, and many approaches are needed to study this complex disorder and its treatment. This overview focuses on various pre-clinical approaches and techniques in neuropsychopharmacological research, particularly those implemented by the author in his research. It will review recent data on the effects of antidepressants on monoamine receptor signalling, the implementation of ozone inhalation by rats as a model to investigate the role of oxidative stress in antidepressant action, and on a 'bench-to-behaviour' approach to investigate novel targets for antidepressant action (specifically the role of modulators of cGMP signalling).

Secondly, the overview discusses current needs for capacity building in neuropsychopharmacology on the African continent, and strategies to address these needs. It reviews initiatives and projects with which the author have been involved to promote the sciences on the African continent, referring to international, continental and national role players. While there are several success stories and hope shared, the need to assist Africa to help itself, and to identify and support leaders in science, is highlighted.

It is concluded that success in science ultimately lies in compassion with your fellowman.

Key words: Depression; antidepressants; neuroplasticity; oxidative stress; ozone; receptor signalling; cGMP; African initiative

Abstrak

Afrikaanse titel: *Major depressie en modelle vir nuwe generasies effektiewe antidepressante*

Major depressie is 'n ernstige gemoedversteuring, wat die lewens van meer as 'n honderdmiljoen mense wêreldwyd affekteer, insluitend in Afrika. Bestaande effektiewe behandelingsopsies, insluitend antidepressant-geneesmiddels, is egter onvoldoende, wat navorsing aanspoor en baie uitdagings laat.

Major depressie is 'n multifaktoriële versteuring, wat verskeie sisteme van die liggaam affekteer. Selfs wanneer daar slegs op die sentrale senuweestelsel (brein) gefokus word (omdat dit per definisie primêr 'n psigiatriese versteuring is) is daar 'n verskeidenheid van neurobiologiese funksies wat in hierdie versteuring aangetas is. Gevolglik is daar verskeie hipoteses om die neurobiologiese basis van depressie en antidepressantwerking te verklaar, en vele benaderings om hierdie komplekse versteuring te ondersoek. Hierdie oorsig fokus op verskeie pre-kliniese benaderings en tegnieke in neuropsigofarmakologiese navorsing, veral daardie wat deur die outeur in sy navorsing geïmplementeer is. Dit verskaf 'n oorsig van onlangse data oor die effekte van antidepressante op mono-amien-reseptorseining, die implementering van osooninhalasie deur rotte as 'n model om die rol van oksidatiewe stres op anti-oksidantwerking te bestudeer, en op 'n 'bank-tot-gedrag'-benadering om nuwe teikens vir antidepressantwerking te ondersoek (spesifiek die rol van moduleerders van cGMP-seining).

Tweedens bespreek die oorsig huidige behoeftes aan kapasiteitsbou in neuropsigofarmakologie op die Afrika-kontinent, en strategieë om hierdie behoeftes aan te spreek. Dit gee 'n oorsig oor die inisiatiewe en projekte waarby die outeur betrokke was om die wetenskappe op die Afrika-kontinent te bevorder, met verwysing na internasionale, kontinentale en nasionale rolspelers. Terwyl daar verskeie suksesstories en hoop gedeel word, word die behoefte om Afrika te ondersteun om homself te help, en om leiers in die wetenskap te identifiseer en te ondersteun, uitgelig.

Daar word tot die slotsom gekom dat sukses in die wetenskap uiteindelik geleë is in deernis met jou medemens.

Sleutelwoorde: Depressie; antidepressante; neuroplastisiteit; oksidatiewe stres; osoon; reseptorseining; cGMP; Afrika-inisiatief.

1 Introduction & Context

Major depression is a severe and debilitating mood disorder with a high prevalence in all populations. According to the World Health Organisation (WHO), depression affects about 121 million people world-wide. Projections from current trends in its epidemiology suggest that it will become the 2nd most burdening disease after cardiovascular disease by 2020, as calculated across all age groups and across gender, while for ages 15-44 it is already ranking 2nd (World Health Organization, 2009). Importantly, depression is a serious, potentially chronic and recurring disorder, causing suffering (to the individual and loved ones) and disability in normal daily functioning, while it is also a leading cause for suicide. Yet, about 75% of the world population do not have access to effective treatment, while the disorder is also grossly under diagnosed (World Health Organization, 2009). In addition, currently available drug treatments are plagued with bothersome side-effects, treatment resistance and delayed onset of action.

Eventhough there have been noteworthy advances in our understanding of the biological basis of depression in the past two decades, and in particular the last, the mere complexity of the disorder also became apparent. Therefore, to better manage and treat this disorder, there is an urgent need for ongoing extensive and focussed research on the biological basis of depression and antidepressant treatment.

In Africa, urbanization and modernization, as elsewhere in the world, have been associated with an increase in the incidence of depression. At the same time, socio-political instability and the general economic status of the continent, as well as a lack of knowledge and understanding of the disorder, have limited accessibility to effective treatment. With Africa as the continent struck hardest by the HIV-AIDS pandemic, this disease also contributes to the prevalence of depression, i.a. due to the emotional trauma of the disease and stigmatization, and complicated by antiretroviral side-effects and the neurocognitive complications (dementia) associated with HIV-AIDS (Owe-Larsson et al., 2009). In South Africa, the prevalence of depression (9.7% life-time prevalence) has been reported to be slightly lower than in the United States, but higher than in Nigeria (Tomlinson et al., 2009), supporting such explanations, as provided above, for trends in the prevalence of depression.

Besides the promotion of public awareness and engagement, ways to assist the people of the continent include enhanced awareness of the role of science, the promotion of training in the management and drug treatments of depression (pharmacology) and basic and clinical pharmacology research. This publication will review both general experimental approaches in psychiatric research and current strategies to promote health sciences on the African continent.

2 Research on Depression & Antidepressants

2.1 Problem statement

2.1.1 Depression as complex disorder

There is currently an increasing recognition and a growing body of evidence that depression is a complex disorder, affecting multiple systems of the body (Krishnan et al., 2008). However, it's defining and most prominent symptoms are associated with altered mood (i.e. psychiatric symtoms). By definition, the disorder is characterised by i.a. depressed mood, a loss of interest or pleasure in most activities, feelings of guilt or low self-worth, disturbed sleep and/or appetite, low energy, and impaired cognitive function, particularly concentration and memory (American Psychiatric Association, 1994).

Based on various experimental and clinical observations, it is apparent that the aetiology of major depression is associated with a combination (and interplay) of genetic susceptibility (i.e. biological factors of predispositioning) and environmental trigger(s) (i.e. psychological stressor(s)). To explain the biological factors involved, several (related/overlapping) hypotheses of the biological basis of depression have emerged and evolved over the years, including the monoamine hypothesis, cholinergic super sensitivity hypothesis, hypothalamic-pituitary-adrenal (HPA) axis hyperactivity hypothesis, neuroplasticity hypothesis and epigenetic modifications hypothesis of depression. In addition, major depression has been described as a metabolic encephalopathy, thereby recognising its well-described association with hypoxia, hypoglycaemia, oxidative stress and/or inflammation (Harvey, 2008). A short description of the various hypotheses of the biological basis of depression, and the scientific evidence in support thereof, are explained below:

TEXT BOX 1: Philosophy of science and the development of psychiatry & pharmacology

Fundamental in our understanding of the neurobiology, psychiatry and psychopharmacology of major depression (and other psychiatric disorders), is the view that the physical and psychological dimensions of the human being (body and mind) are integrated and interdependent. This view implies that the psychological has a biological basis, and consequencially that drugs may alter, for example, mood. This is in stark contrast to so-called dualism, viewing body and mind as two separate and strictly independent dimensions. On the otherhand, there has also been caution to avoid neurological reductionism, whereby the human mind/psyche is viewed as a mere extention of the electro-biochemistry of the physical brain.

Monoamine hypothesis

The monoamine hypothesis of depression, postulating a dysfunction of monoaminergic signalling in the brain, is the best studied hypothesis of the neurobiological basis of depression (Baldessarini, 2006; Elhwuegi, 2004). This involves primarily a postulated neuronal dysfunction in serotonergic and noradrenergic signalling, but also of dopaminergic signalling. While the original/classical hypothesis was based on the observations that drugs that

deplete monoamine stores (e.g. reserpine) cause depression, and that drugs that enhance monoamine signalling (e.g. monoamine oxidase inhibitors and pre-synaptic reuptake inhibitors) alleviate the condition, it was soon realised that this scenario represents an oversimplification of the monoaminergic dysfunction associated with depression. Later modifications of the monoamine hypothesis postulated longterm changes in pre- and post-synaptic monoamine receptors, to modulate receptor signalling (Blier, 2003). Still in support of this hypothesis and in search of genetic traits associated with depression, it has been demonstrated that a polymorphic variant of the serotonin-transporter–linked polymorphic region (5-HTTLPR), affecting the promoter of the serotonin-transporter gene, is strongly associated with a predisposition to depression (Caspi et al., 2003). To date, all clinically available antidepressants affect monoamine neurotransmission in some way or another.

Cholinergic super sensitivity hypothesis

The cholinergic super sensitivity hypothesis postulates that an imbalance between cholinergic and adrenergic signalling/tone (i.e. decreased adrenergic signalling and enhanced cholinergic signalling) may be associated with depression (Dilsalver, 1986). Studies indicated that muscarinic acetylcholine receptors in the nucleus accumbens may be involved in depression and antidepressant action (Chau et al., 2001). In particular, the antimuscarinic drug scopolamine has been shown to exert robust antidepressant effects, even in treatment-resistant patients (Furey et al., 2006; Janowsky, 2007). Some clinically available antidepressants possess antimuscarinic activity, but which is also associated with unwanted side-effects. However, all anticholinergic drugs are not consistently antidepressant and it is believed that, while anticholinergic properties may contribute to the antidepressant effect of certain drugs, these properties *per se* are not sufficient to explain antidepressant action.

HPA hyperactivity hypothesis

It has been well established that the hypothalamic-pituitary-adrenal (HPA) axis of patients with major depression is hyperactive, leading to chronically elevated cortisol levels and desensitisation of glucocorticoid receptors (Ehlert et al., 2001; Holsboer, 2001; Leonard, 2001). This, in turn, affects the immune system (including inflammation), monoaminergic neurotransmission, neuroplasticity and metabolic dysfunction, all of which are associated with major depression. Non-steroidal anti-inflammatory drugs with central action, for example celecoxib and reboxetine, have been shown to exert antidepressant action as adjunctive therapies in treatment resistant patients, when added to the standard antidepressant treatment regime (Akhondzadeh et al., 2009; Muller et al., 2006). As a model, the HPA axis hypothesis unifies various hypotheses (or at least accommodates the multi-factorial characteristics) of the biological basis of depression. However, its role in the aetiology of depression is not clear.

Neuroplasticity hypothesis

It is now well-recognised that major depression (as several other stress-related disorders) is associated with impaired neuroplasticity, which is reversed by antidepressant treatment (Manji et al., 2001; Zarate et al., 2006). Using magnetic resonance imaging scan technology, it has been demonstrated that patients with severe major depression have significantly reduced right hippocampal volume compared to controls (Bell-McGinty et al., 2002). In addition, and in contrast to earlier theories, there is now evidence of continued neurogenesis, and particularly synaptogenesis (neuronal sprouting), in the adult brain (Chun et al., 2009; Imayoshi et al., 2009; Lee et al., 2009; Suh et al., 2009), which is believed to be impaired during major depression. For example, neuroprotective brain-derived neurotrophic factor (BDNF) has been shown in many studies to play a central role in neuroplasticity and synaptoplasticity, whereas long-term treatment with all antidepressants increases its expression (Racagni et al., 2008). In addition, the administration of BDNF exerts antidepressant-like activity in animal behavioural models of depression (Shirayama et al., 2002). It is believed that the antidepressant activity of the atypical antidepressant tianeptine is primarily due to its neuroprotective properties, while the long-term treatment with other antidepressants may also exert neuroprotective effects.

Epigenetic modification hypothesis

Interindividual variations in germ line genetics (i.e. inheritable genetic traits, with changes in DNA code, such as genetic polymorphisms or mutations) provide a logical basis for inter-individual differences in neurological plasticity, and therefore variable susceptibility to psychiatric disorders. However, long-lasting 'genetic scars' can be also induced by, for example, early-life stress, and this is believed to potentially render an individual more vulnerable/susceptible to depression (Krishnan et al., 2008). Such epigenetic modifications do not alter DNA code, but may include covalent changes to DNA (e.g. DNA methylation), post-translational modifications of histone N-terminal tails (e.g. acetylation and methylation) and non-transcriptional gene-silencing mechanisms (e.g. micro-RNAs) (Tsankova et al., 2007). In depression this may be associated with changes in the promoter gene for BDNF (Tsankova et al., 2007). In this regard, demonstrating its relevance to depression, it has been demonstrated that pre-stressed animals respond differently to stressful situations than stress-naïve controls (Wegener et al., 2009).

Metabolic encephalopathy hypothesis

Major depression is a multi-factorial disorder and its manifestations include systemic symptoms, such as a dysfunctional immune system and metabolism, as well as common co-morbidities such as cardiovascular disorders (Clarke et al., 2009; Perlmutter et al., 2000). While metabolic encephalopathy is associated with hypoxia, hypoglycaemia, oxidative stress

and/or inflammation in its aetiology, these are shared with major depression - arguably a form of metabolic encephalopathy (Harvey, 2008).

While all of the abovementioned hypotheses can be viewed as different frameworks to understand the biological basis of depression, they are not mutually exclusive, but rather are complimentary in understanding the whole picture of the complexity of this multi-factorial mood disorder. The current challenge is to find a common or unifying factor in all hypotheses; the common causal factor in the aetiology of all the observed neurobiological and systemic manifestations of major depression. By identifying this, it may be easier to identify drug targets for the prevention and treatment of major depression. However, it is also very likely that there are more than one subtype of major depression with different biological aetiologies, and hence different drug targets, so that there should be a continuous awarenes of potential oversimplification, with rigorous scrutiny of hypotheses.

2.1.2 Depression and treatment strategies

Being a multi-factorial disorder, the successful treatment of depression needs a holistic approach. The treatment approaches for major depression include psychotherapy, antidepressant drug treatment and diverse treatment interventions (in particular electroconvulsive shock, but also vagal nerve stimulation, deep brain stimulation, etc.). Life-style adjustments, such as exercise (Mead et al., 2009) and a healthy diet, also contribute to management and therapeutic response. While major depression is a severe and serious disorder, drastic medical intervention, typically including antidepressants, is usually necessary for the effective treatment of severe major depression. Typically about one-third of patients respond to a first drug, and about two-thirds after three drugs have been tried (Little, 2009). Due to large inter-individual variation in response, success is (unfortunately) usually achieved on a trial-and-error basis.

Whilst effective in some patients, current antidepressant drugs are still plagued with common bothersome side-effects, delayed onset of action and a high frequency of partial response or treatment resistance. All of the currently available drugs modulate monoamine transmission in the brain in some or other way, while for tianeptine and agomelatine the claim is made, respectively, that they work primarily via neuroprotective activity and a positive effect on the sleep-wake cycle. Experimental antidepressants also affect the NMDA/NO/cGMP pathway or inflammation.

2.2 Research approaches and techniques

2.2.1 Principles of approaches in research

As alluded to above, current antidepressant drug treatments are associated with many deficiencies, including a large frequency of treatment resistance.

Consequently, there is an urgent need to better understand the neurobiological basis of major depression, and in particular of the putative subtypes of depression that seem to be resistant to current treatments.

Eventhough clinical studies (i.e. studies involving the use of humans) are important to obtain final answer in terms of the true clinical efficacy and safety of drugs, such studies have obvious limitations. Foremost, there are several ethical considerations that limit studies possible in humans, while clinical studies are also excessively expensive. Pre-clinical studies are therefore implemented to study drugs and to predict effective and safe drugs and drug targets. Pre-clinical studies of psychiatric disorders and treatments implement *in vitro* (e.g. cultured cells and test tube-type assays), *ex vivo* (e.g. brain tissue of treated animals) and *in vivo* (e.g. behaviour of treated, live animals) techniques in experimental designs.

Using *in vitro* techniques usually provide a useful means to perform multiple screening experiments and/or to investigate disease neurobiology or drug action mechanistically at a biochemical, molecular (e.g. drug-receptor and signalling) and genetic level. However, these techniques are commonly reductionistic and do not account for integrative, interrelated and interdependent responses in whole, intact systems. *Ex vivo* techniques provide the first-line bridge to overcome this barrier, whereby live, intact animals are first treated to yield a system response, whereafter the animals are sacrificed and the tissue isolated to perform biochemical analyses, as with *in vitro* techniques. The only way to evaluate drug effects in the intact system (closest to the clinical situation in humans) remains the use of intact, live animals, albeit *in vivo* techniques. The most critical problem with these techniques is that they are based on the assumption of interspecies similarity, and therefore the need to extrapolate animal data to the human situation. While this assumption is generally true and remarkably accurate for many biological systems, it is well known that there may be vast (and sometimes unexpected) differences between the human and animal response.

So in the final analyses, techniques used in pre-clinical studies are indispensible and may provide us with valuable information, while their limitations should never be underestimated. Usually combinations of pre-clinical models are implemented to obtain answers with good predictive value for the clinical human situation. For example, a combination of *in vitro* studies (including on isolated human tissue) plus *ex vivo* biochemical and *in vivo* behavioural animal studies are more useful than either alone.

2.2.2 Specific experimental techniques and assays

Basic principles of techniques and assays for pre-clinical studies on the biological basis of depression and antidepressant action, as have been implemented at the North-West University, are described briefly below.

Cultured cells

Cells (biological tissue) can be isolated and grown *in vitro* under artificial physiological conditions. For example, many cells with special proliferating properties are sometimes maintained and grown in specially formulated culture medium (containing all physiological nutrients needed for survival and growth) at typically 37°C and physiological pH. Under these conditions the cells can also multiply, allowing differential treatment of multiple cells. In psychopharmacological experiments, cells are typically treated with drugs and then analysed to observe the biological effects of these drugs.

Animal behaviour

While animals and humans are not biologically equal, they share many characteristics. Several animal models have been developed (including validated behavioural animal models) that allow for the evaluation of certain aspects of human behaviour that correlate with that of the animal (Lapiz-Bluhm et al., 2008). Animal models are usually validated according to their face validity (i.e. extend to which the model correlates with symptoms of human condition), predictive validity (i.e. extend that the model can predict certain effects in humans), construct validity (i.e. extent of correlation with underlying (neuro-)biology/pathology in humans).

Animal brain dissections

The brain tissue of, for example, drug-treated animals can be dissected and analysed *ex vivo* to observe changes in biomarkers. By following this approach we may learn more about the effects of drugs on the neurobiological processes in the brain and on neuropathology in psychiatric disease.

Biochemical assays

Whether we use *in vitro* approaches (such as cultured cells) or *ex vivo* approaches (such as animal brain tissue), these tissues need to be analysed further to observe biological effects/events. Many of these events happen at the level of cell signalling (biochemistry, intracellular and intercellular communication). Typical assays can measure pharmacological receptor expression and function, and the properties of signalling molecules at the cell membrane, cytoplasm, organelles and nucleus. Such measurements reflect on resulting cell function.

2.2.3 Ethics for the use of animals in research

The need for the use of animals in research has been explained in par. 2.2.1. However, this does not take away the responsibility of the researcher to use animals with the greatest respect and discretion. The South African Research Council published guidelines on the use of animals in research and training (SA MRC, 2004), while research institutions have Ethics Committees who review and supervise the responsible use of animals for research. In this regard, I have steered the recent development of the currently used Research Ethics

Applications Form of the North-West University, guiding researchers and members on the evaluation panels of the Ethics Committee to review ethics applications for new research projects, including the use of animals in research (NWU, 2008).

In general, the importance of the research question must justify the use of animals. For each experiment the potential benefit must outweigh the potential harm. Therefore, in context of the current topic (i.e. depression and antidepressants), any discomfort, stress or pain imposed on the animals must be proportionate to the potential alleviation of human suffering expected from the outcome of the study.

In addition, good care should be taken of animals kept in captivity, including, for example, providing for the appropriate social and environmental needs of the animals (SA MRC, 2004).

All studies must also comply with national legislation (South African Animals Protection Act No. 71 of 1962) and with the institutional guidelines where the study is performed, whichever requirements are the highest (SA MRC, 2004).

TEXT BOX 2: Ethics guidelines for the use of animals in research: Three "Rs"

As a world-wide consensus, the use of animals is guided by the principles of the so-called three "Rs", namely "Replace, Reduce & Refine". These principles can be explained as follows:

Replace

Whenever the use of animals are considered for a particular experiment, it should be considered whether or not there is any non-sentient (e.g. computer model or *in vitro* technique) alternative that may address the research objective/question.

Reduce

The number of animals used for the study should always be minimum required to obtain valid information with meaningful statistically analyses. The use of either too many or too few animals is unethical.

Refine

The sourcing and care of animals and experimental protocols should be designed such that physical and psychological distress to the animals is mimimised.

2.3 Findings from Our Pre-Clinical Studies

At the North-West University a research focus group on neurodegenerative, and specifically anxiety-related disorders was established about a decade ago. In collaborative projects, pre-clinical research has been done following a "bench-to-behaviour" approach, thereby incorporating complimentary *in vitro* techniques with drug-treated cultured cells, *ex vivo* analyses on the brain tissue of drug-treated animals and *in vivo* behavioural studies with drug-treated animals. This approach has proven to be very effective in addressing current issues and contributing to our current understanding of depression and its treatment. Key findings from our research, demonstrating the effectiveness of our "bench-to-behaviour" approach in neuropsychopharmacological studies, are discussed in this section.

2.3.1 *Antidepressants & cell signalling*

According to the monoamine hypothesis of depression (see par. 2.1.1 above), antidepressant action is attributed to its ability to modulate monoaminergic neurotransmission. While the original hypothesis postulated that these drugs enhance the levels of norepinephrine (noradrenalin) and/or serotonin, it is now recognised that there are several other ways to modulate monoamine neurotransmission, including the up- or down-regulation of pre-synaptic autoreceptors. Membrane-bound monoamine receptors are of the so-called G protein-coupled receptor type, and upon binding to norepinephrine or serotonin they are 'activated' (i.e. active conformation promoted) to signal via G proteins, thereby to initiate or promote a cascade of intracellular events (see Figure 1). These intracellular events are eventually responsible for the cellular/biological responses we observe.

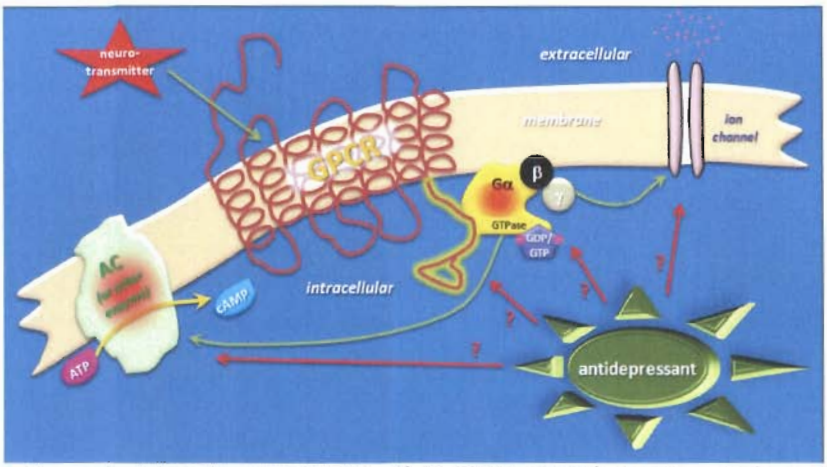
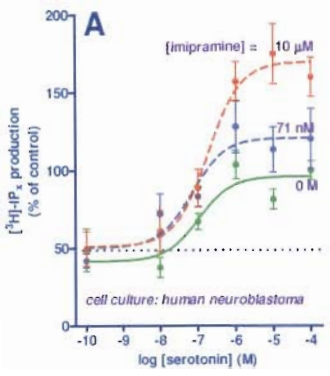


Figure 1: Schematic representation of G protein-coupled receptors, such as monoaminergic norepinephrine or serotonin receptors, and their intracellular signalling cascades. It now remains to be answered where in these cascades antidepressant may modulate signalling, besides the traditional modes. (Figure adapted (Brink et al., 2004a)).

In this context, as shown in Figure 2 below, it was found that, when cultured human neuroblastoma cells (a neuronal cell line) were incubated for 24 hours with the classical antidepressant imipramine, signalling via serotonin $5HT_{2A}$ receptors was enhanced. Similarly, when these cells were incubated for 24 hours with the classical antidepressant fluoxetine, muscarinic acetylcholine receptor number was decreased (Brink et al., 2004b).



Concentration-effect curves measuring [^{3}H]-IP$_x$ accumulation in transfected human neuroblastoma SH-SY5Y cells, following 24 hour pretreatment with the indicated antidepressant. Data are averages of triplicate measurements from three independent and comparable experiments, and data points are expressed as mean ± standard error of the mean.

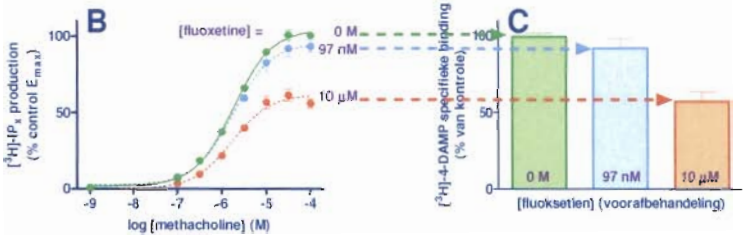


Figure 2: Concentration-dependent modulating effects of (A) imipramine on serotonin 5HT$_{2A}$ receptor function and (B & C) fluoxetine on muscarinic acetylcholine receptor function and expression. (Figure adapted (Brink et al., 2004b)).

Both of these abovementioned mechanisms reflect modulation of G protein-coupled receptor signalling by antidepressants and may contribute to antidepressant action. In the same study it was demonstrated that *myo*-inositol, an endogenous glucose derivative with antidepressant activity, also modulates G protein signalling (data not shown) (Brink et al., 2004b). Taken together, these data demonstrate that the mechanism of action of antidepressants may include modulation of monoamine receptor signalling, and that it is more complex and multifaceted than originally believed. In addition, the data also suggest cues (leads) for additional drug targets of antidepressant action.

2.3.2 Ozone as model of oxidative stress in depression

Accoding to the neuroplasticity and metabolic encephalopathy hypotheses of depression (see par. 2.1.1 above), oxidative stress plays an important role in depression. We investigated whether the inhalation of ozone by rats, at concentrations typically found in industrialised cities, may render a model to investigate the antidepressant action of drugs in oxidative stress. Figure 3

shows a typical experimental setup for such experiments to expose rats to ozone.

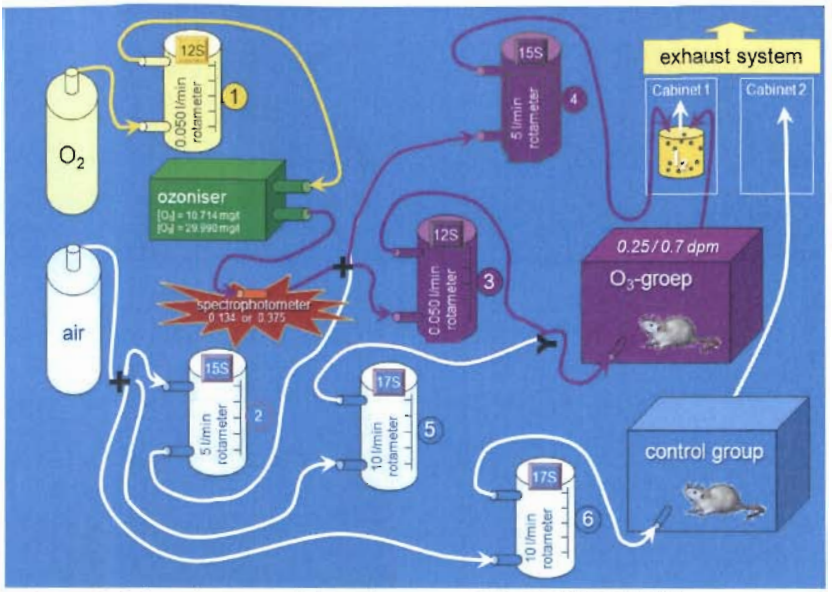


Figure 3: Schematic representation of an ozone inhalation setup for rats.

We exposed rats to 0.25 ppm ozone, 4 hours/day for 30 days, and then administered the classical antidepressant imipramine to observe the effect of ozone exposure on antidepressant response. This concentration of ozone corresponds to that found in peak traffic in large industrialised cities.

As shown in Figure 4A below, chronic exposure of rats to ozone alone does not alter behaviour. However, the response to the imipramine is inhibited by chronic ozone exposure. In Figure 4B it is shown that ozone induces a state of oxidative stress, independent of the presence of the antidepressant in the rat hippocampus. Figure 4C shows that imipramine effectively reverses the ozone-induced increase in oxidative damage in the rat hippocampus.

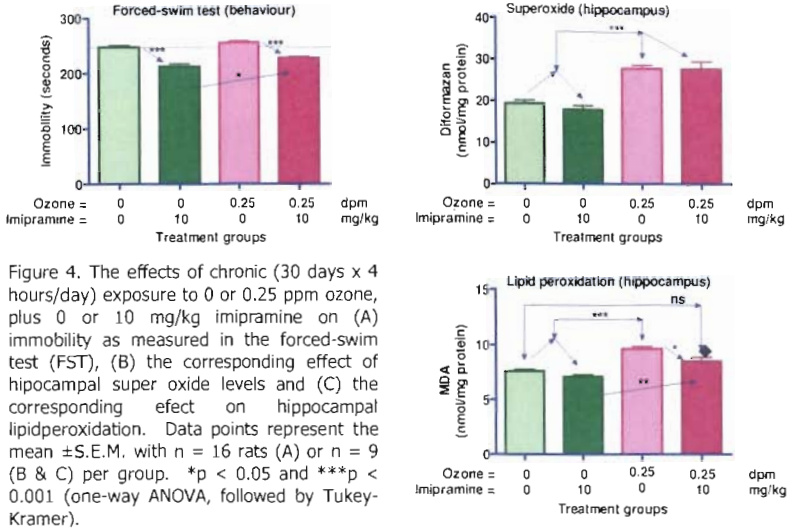


Figure 4. The effects of chronic (30 days x 4 hours/day) exposure to 0 or 0.25 ppm ozone, plus 0 or 10 mg/kg imipramine on (A) immobility as measured in the forced-swim test (FST), (B) the corresponding effect of hipocampal super oxide levels and (C) the corresponding efect on hippocampal lipidperoxidation. Data points represent the mean ±S.E.M. with n = 16 rats (A) or n = 9 (B & C) per group. *p < 0.05 and ***p < 0.001 (one-way ANOVA, followed by Tukey-Kramer).

Figure 4: The effect of ozone on rat immobility (antidepressant-like activity), hippocampal superoxide levels (oxidative stress) and hippocampal lipid peroxidation (oxidative damage) (Mokoena et al., 2009).

Importantly, when comparing the data to other brain regions (data not shown), it was demonstrated that the protective effect of imipramine was selective in the hippocampus, a brain area specifically commonly associated with the neurobiology of depression (and select other stress-related disorders). It was concluded from this study that the lack of response in behaviour from ozone alone may be due to uncontrolled (lack of) stress susceptability. Rats with a genetic susceptibility to stress, or with induced early-life stress, may give a more pronounce response to ozone and that further studies should investigate this plausible working hypothesis. Also, it may be concluded that ozone exposure may be a viable model to investigate oxidative stress in depression and antidepressant action (Mokoena et al., 2009).

2.3.3 Modulators of the NO/cGMP pathway

According to the neuroplasticity hypothesis of the biological basis of depression (see par. 2.1.1 above), the glutamate/nitric oxide (NO)/ cyclic guanosine monophosphate (cGMP) signalling pathway plays a key role in neuroplasticity in depression and antidepressant action. Accordingly we investigated potential novel antidepressant action of the phophodiesterase type 5 inhibitor sildenafil. Initial *in vitro* experiments, using cultured cells, revealed (unexpectedly) that sildenafil enhances muscarinic acetylcholine receptor signalling (data not shown), which theoretically predict depressogenic activity. However, such

depressogenic effects are not observed clinically with the use of sildenafil. We therefore postulated that sildenafil may possess an antidepressant activity that is masked by its pro-cholinergic property (i.e. its antidepressant effect is negated by an opposing depressogenic effect), and that its antidepressant property may be unmasked if it is combined with an antimuscarinic drug.

This working hypothesis was tested in an *in vivo* rat behavioural model for antidepressant action. We employed the Flinders Sensitive Line (FSL) rat model, consisting of rats that were genetically bred to exhibit depressive-like symptoms. In Figure 5 it can be seen that sildenafil in combination with the anticholinergic drug atropine, exerts an antidepressant-like effect in these rats, as measured by immobility in the forced-swim test.

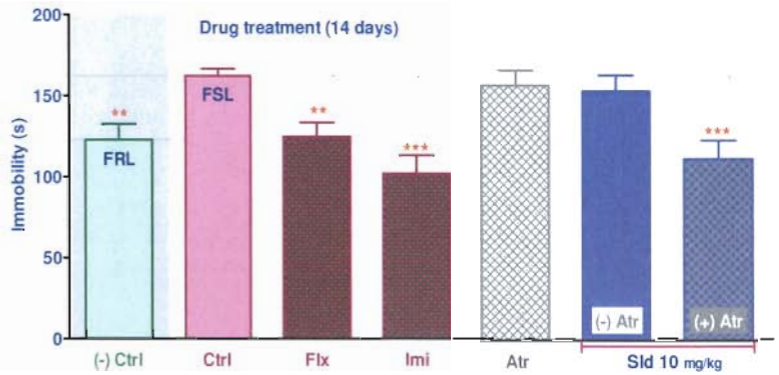


Figure 5: The effect of a 14-day drug treatment of Flinders Sensitive Line rats with various drugs on immobility (antidepressant-like activity), as measured in the forced-swim test. Data represent the mean ±S.E.M. for 18 rats per group, except for the vehicle-treated FSL control group which consisted of 60 rats. **P<0.01 and ***P<0.001 indicates a significant difference relative to FSL control (Tukey's post-test). FRL = Flinders Resistant Line control rats; FSL = Flinders Sensitive Line rats; Ctl = vehicle control; Flx = fluoxetine; Imi = imipramine; Sld = sildenafil; Atr = atropine (Liebenberg et al., 2009).

It was concluded from the data in Figure 5 that sildenafil indeed possesses an antidepressant-like activity in rats, which is revealed only in the presence of a centrally acting antimuscarinic drug. By following this approach we demonstrated not only a novel and clinically viable drug target for antidepressant action, but this also demonstrates the successful application of our 'bench-to-behaviour' approach mentioned in par. 2.2.1 above.

2.4 Conclusions and Recommendations

With our pre-clinical studies at the North-West University we have been able to evaluate the neurobiological basis of antidepressant drug action at molecular-biological level, using *in vitro* and *ex vivo* techniques, as well as at behavioural level, using *in vivo* techniques. We have been able to contribute to the current understanding of drug action, identify new drug targets for antidepressant action and develop new models to test and evaluate antidepressant mechanisms. These studies contribute to the neuropsychopharmacological sciences internationally, whereby the effective treatment of i.a. major depression may be improved to alleviate the suffering of millions of people world-wide.

3 Neuropsychiatric Research in Africa

3.1 Challenges in Africa

While the need for quality psychiatric care in Africa is comparable to the rest of the world, Africa also has a number of unique challenges. These include the accessibility of professional services, as well as the burden and consequences of the HIV-AIDS pandemic, poverty, and other diseases uniquely prevalent in Africa. In addition the socio-political instabilities in Africa have resulted in underdevelopment of not only political powers, but also of societies and scientific communities (and sciences/research) within these societies.

As a result, there are but a few true centres of excellence of research in Africa, and where they are found, they are usually build upon the expertise of an individual researcher. South Africa is probably the only country in Africa where research infrastructure and support systems are well developed and established, followed by less scientific resources of excellence found in Egypt, and then even less found in a select few other African countries.

In addition, existing scientific communities in most of Africa are not well organised, if at all. For example, only three learned societies of pharmacology on the African continent are members of the International Union of Basic and Clinical Pharmacology (IUPHAR), being the South African Society for Basic and Clinical Pharmacology (SASBCP), the Egyptian Society for Pharmacology and Experimental Therapeutics (ESPET) and the West African Society of Pharmacology (WASP) (IUPHAR, 2009). Until recently, there was no existing database with information or contact details of pharmacologists on the continent, so that nobody had any idea of how many pharmacologists there are, where they are and what they do. All of these facts clearly indicate a need to assist Africa to organise its learned societies and/or to assist pharmacologists to form such societies at regional or national level (see par. 3.2.1 below).

The importance of capacity building building in Africa is accordingly well recognised (see par. 3.2.1 & 3.2.5 below). This implies a continued plan to empower young and established researchers to improve the quality of their expertise and scientific output, as well as to establish local and international networks and collaborations.

3.2 Strategies for Capacity Building

3.2.1 PharfA

As an initiative of the South African Society for Basic and Clinical Pharmacology (SASBCP), the 'Pharmacology for Africa' initiative (PharfA) was launched in 2006 (PharfA, 2009). Together with Prof Douglas Oliver, I have been a co-chair of the steering committee to establish this initiative, which aims to organise and promote pharmacology on the African continent. The initiative set as first priority to establish a database of pharmacologists on the continent. A major objective was to stimulate and coordinate the formation of national societal organisation. It also focuses on teaching and training, in order to eventually promote basic and clinical research. In addition, it aims to be truly representative of all countries and regions in Africa.

PharfA was soon officially supported by the International Union of Pharmacology (IUPHAR) and the South African National Research Foundation (NRF). A website with a subscription form and database was launched in 2007, and today, after only two years, there are already 138 pharmacologists on the database. Although this number may be small according to international criteria, it is a large number for Africa and a big step forward. The database has already been used for effective and regular communication with African pharmacologists, for projects of the World Health Organization and for an ICSU-supported project of the International Union of Pharmacology (see par. 3.2.3 & 3.2.5 below).

Very recently, early in 2009, the first new national society of pharmacology was formed out of the activities of PharfA, being the Kenyan Society of Pharmacology (KeSoBAP), suggesting that this strategy may be successful in achieving its objectives.

3.2.2 WCP2014

The South African Society for Basic and Clinical Pharmacology (SASBCP) won the bid to host the 17th World Congress of Pharmacology 2014 (WCP2014) in Cape Town, South Africa (WCP2014, 2009). This will be a 1st for Africa. I have served on the Executive Committee, overseeing the national organising of the congress.

While this international meeting will showcase advances in pharmacological sciences world-wide, it is also an opportunity for capacity building in South

Africa, and an opportunity to bring African pharmacologists from all over the continent together. The meeting will make a special input to ensure active participation of African delegates.

3.2.3 IOSP

A committee of the International Union of Pharmacology (IUPHAR), called the 'Integrative and Isolated Organ Systems Pharmacology' (IOSP), is a group that promotes training (capacity building) in experimental pharmacology in developing counties. The motivational concept behind IOSP is to combat so-called "reductionism", particularly in the face of basic, molecular research in pharmacology (with sometimes invalid extrapolation to clinical human pharmacology) that became a world-wide trend, at the expense of essential systematic research. I serve on this committee as African representative.

The IOSP committee found a platform to serve with workshops in Africa via PharmA, mentioned in par. 3.2.1 above. A grant from the International Council for Sciences (ICSU) was received to hold workshops in Africa to promote the objectives of the IOSP committee and to train African pharmacologists in experimental pharmacology. Accordingly, a 1st IOSP School (workshop) were held in September 2009 in Potchefstroom, South Africa, attended by Kenyan and Nigerian pharmacologists. Feedback indicated that this workshop indeed transferred valuable skills and knowledge to stimulate enhanced quality of research in the respective African countries, whilst also establishing sound networks for future colloboration.

This workshop will be followed up by a workshop in Egypt and eventually by one to be hosted by Kenya in 2010 (and hopefully others beyond), so that this becomes a true capacity building, legacy project for African pharmacologists.

3.2.4 IUPHAR Teaching Section

The Teaching Section of the International Union of Pharmacology (IUPHAR) is an active section that promotes effective teaching (education) in pharmacology world-wide. It also strives to create opportunities for poor countries with limited resources, including in Africa. Besides interactive local and international meetings, it also makes available electronic resources and software to assist educators in pharmacology. I am currently serving as Secretary of this Section.

As a Pre-Satellite to the WorldPharma2010 congress in Denmark, the Teaching Section will hold a workshop. I serve as co-chair of the Programme Committee and have stimulated the introduction of Early Educators' Awards, as well as travel awards for poor countries to this meeting (IUPHAR Teaching Section, 2009). This will stimulate capacity building amongst the younger generation of educators, also for Africa.

3.2.5 ICSU-Africa (ROA)

The International Council for Sciences (ICSU) took a decision to establish regional offices world-wide, including one such an office in Africa. Africa was the first to respond and the Regional Office for African (ICSU-ROA) was established in 2005, based in Pretoria, South Africa (ICSU ROA, 2009). ICSU ROA strives to promote excellence in science via policy making and sustainable socio-economic development in Africa, while it also promotes equitable access to scientific data and information and scientific capacity building. Our contact and interaction with ICSO ROA has been instrumental in the progress seen with PharmA.

3.2.6 North-West University

The North-West University contributes significantly to capacity building in neuropsychopharmacology, i.a. via the training of postgraduate students at masters, doctoral and postdoctoral level (NWU, 2009a). Research is mostly pre-clinical, done within the Research Unit for Drug Research and Development. The vision is to contribute to the health care needs of the people of South Africa through research, to develop and expand the human resource capacity in the field of health care, develop current knowledge and technologies, discover new scientific knowledge, products and technologies, and apply it in the pharmaceutical, medical and industrial health science areas. By training upcoming researchers and leaders in scientific problem solving, and specifically the understanding of neuropsychopharmacology, we contribute to this vision and contribute to provide in the needs of our country in terms of human resource development and the building and provision of expertise.

In addition, the North-West University presents a distance-learning web-based Hons. B.Sc. Programme in Pharmacology (NWU, 2009b) for medical practitioners and pharmacists as continued professional development course. I have been instrumental in the development of this programme and is currently the programme manager. Students from all over South Africa, the rest of Africa and globally have enrolled for this programme. This creates accessibility for practitioners to the latest information, thereby enabling them to practice more effective pharmacotherapy in community medicine.

3.3 Africa, hope and tomorrow

Taken together, Africa is a continent with an enormous need for capacity building, in order for it to be able to help itself. True solutions for the challenges in Africa can only come from within. South Africa is well positioned to make a difference in Africa, better than any other country in the world, and has already done so in the field of

TEXT BOX 3: Opportunities
Some do not notice or use opportunities; others make the most of them and develop; a few who have developed then begin to create opportunities for themselves; and eventually a very few see the light to develop opportunities for others. (adapted from words of wisdom by Prof Douglas W Oliver, NWU, 2009)

health sciences, and in particular in the speciality of pharmacology. The North-West University, via its faculty staff, is in the forefront of the latest developments to assist Africa to become part of the international scientific community.

At this stage it is important to continue with current strategies and to get identified leading African pharmacologists more actively involved in the capacity building strategies. Africa needs continued support, and the recognition of successful scientists who excell ("heroes", if you will).

4 Summary and Final Conclusions

Science, experiments and a "call for leadership" could summarise key points of this publication. Most importantly, however, an essential drive of research should revolve around a true compassion for people, their needs and their cries. When, in the final analysis, science becomes an ivory tower or objective in itself, or a vehicle for self-serving objectives, and is not motivated by compassion and an attitude that embraces the concept of "love thy neighbour", it may all be in vein. The metaphorical powerful ship may then take the wrong course, arriving at unintended shores, or even get shipwrecked, leaving a line of affected or hurt passengers and bystanders along the way. Undoubtedly leading scientists need to be driven by solid values and compassion, and, I believe, inspired by the Spirit of a loving God who leads by example.

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