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Stress and the brain:

A new challenge for psychopharmacology

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Stress and the brain: A new challenge for psychopharmacology

INAUGURAL LECTURE BY

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The Pursuer

Abstract:

Anxiety and mood disorders such as panic, obsessive compulsive disorder (OCD), posttraumatic stress disorder (PTSD) and depression, are amongst the most prevalent, disabling, and chronic of all the psychiatric disorders. Neuropsychiatric disorders comprise the second largest component of the burden of disease in South Africa. To compound the problem, under-diagnosis and current treatments that are in most cases less than adequately effective, contributes to an enormous personal and economic cost to the patient and his/her family. Recent brain imaging studies in PTSD and depression have emphasized that these illnesses may induce damaging effects on regions of the brain involved in regulating the response to stress. While controversy prevails as to whether these changes represent an adaptive process or are indeed pathological, they are associated with marked changes in memory and other cognitive functions. In depression, a history of prior episodes are correlated with a higher risk of relapse, while poor compliance with antidepressants not only predicts later relapse, it may result in a more rapid shrinkage of the above-mentioned brain regions. Perhaps the structural change caused by prolonged depressive illness explains why recurrent episodes are less responsive to treatment. Similarly, even with the introduction of effective medications for PTSD, many patients remain treatment-resistant. However, does stress damage the brain and if so, are the mechanisms and prompts for its induction different for these disorders? Clearly, understanding their pathological basis will assist in developing more effective treatment strategies. This paper will highlight at least one potential new avenue for future psychotropic drug research in depression and anxiety disorders, viz. the glutamate-nitric oxide-cGMP pathway.

1. Introduction

For at least 5000 years, man has had a close association with mind altering drugs. From circa 3000BC, the Sumerians were exploiting the mood elevating effects of poppy juice, while from 2000BC, the Egyptians were deploying the opium poppy for its analgesic properties (Figure 1; Leonard, 2003). The discovery of codeine in 1832 and ether and nitrous oxide in 1846, set the scene for a surge in central nervous system (CNS) drug discovery during the early to mid 20th century (Figure 1), such as phenobarbital, reserpine and phenytoin that are still used today. However, this noteworthy progress was nothing compared to the halcyon days between 1950 and 1960, regarded by most as the era of psychopharmacology (Figure 1). During a period of unprecedented discovery, man made his most significant steps into understanding the complex workings of the brain and psychiatric illness with the discoveries of the neuroleptic, chlorpromazine in 1952, the benzodiazepine anxiolytic, chlordiazepoxide in 1957 and the antidepressant, imipramine, in 1958. An equally important happening was the discovery of the mood-stabilising actions of lithium ion in 1949. With these discoveries was coined the word "psychopharmacology", a term representing the study of drug action in the brain. These discoveries have set the gold standard for research and treatment in almost all of the most severe psychiatric illnesses, including schizophrenia (chlorpromazine), anxiety disorders (chlordiazepoxide) and the mood disorders, mania (lithium) and depression (imipramine). However, one is left with the burning question: With 50 years of research behind us since these first discoveries, how much further have we progressed in our knowledge of these psychiatric illnesses, and are our new drugs better than the prototypical drugs?

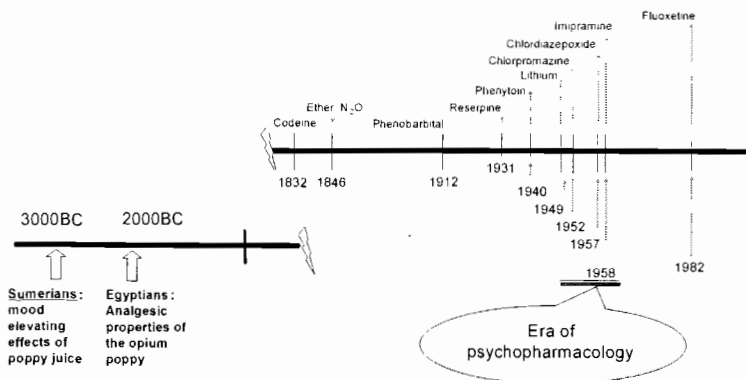


Figure 1: Man's association with mind-altering drugs: A brief history and seminal discoveries. Refer to the text for detailed discussion.

Sadly, however, drug discovery has not realized its full potential and in many ways is decades behind our understanding of psychiatric illness. Our newest drugs, for example the serotonin (5HT) reuptake inhibitors (SRI) such as fluoxetine (Prozac®), are not any more effective than imipramine in the treatment of depression (Geddes JR et al, 2000), as are the new generation atypical antipsychotics, such as olanzapine, in the treatment of schizophrenia (Krausz, 2002), while lithium salts still remain the bench mark treatment for manic depression (Compton and Nemeroff, 2000). The advent of extremely sophisticated techniques in molecular neuroscience and molecular psychiatry have helped in uncovering a wealth of knowledge pertaining to the development, susceptibility and progression of a psychiatric illness, as well as identifying new candidate target molecules for drug action. However, integrating this into a single working hypothesis upon which drugs can be developed and marketed has proved the biggest challenge. Contrary to our early beliefs and hopes in the 1960's, a psychiatric illness is not a single neurotransmitter disorder, but represents a continuum of environmental, genetic and neurochemical determinants that all occupy a variable yet distinct role in the etiology, progression and treatment response of disorders as apparently distinct as depression on one end, to psychosis on the other.

Early evidence from studies using the monoamine depletor, reserpine, describing its ability to induce depressive behavior in patients, and the later work on the tricyclic antidepressants demonstrating their inhibitory action on monoamine uptake, was convincing enough proof that the clinical efficacy of these drugs was strongly correlated to their ability to increase the synaptic concentrations of 5HT, noradrenaline (NA) and dopamine (DA). This subsequently led to the proposal and development of the monoamine hypothesis of depression (Harvey, 1997). Although this hypothesis has proved to have significant construct validity in that it has formed the basis for the development of all currently used antidepressant drugs, the delayed onset of action of our current armamentarium of antidepressant drugs, and that they seldom exceed an expected rate of remission of 50% (Kocsis, 2003), and having an unacceptably high incidence of relapse has paved the way to a more intractable form of the illness (Harvey et al, 2003a). While these newer drugs have improved side effect profiles with distinct benefits for long-term outcome due to improved patient compliance, it is becoming increasingly clear that these drugs are not targeting the neurobiological underpinnings of the disorder. The brain is a primordial "soup" of neurochemicals and neuronal messengers all with a distinct function, yet our focus over the past three or more decades has been 5HT, NA and DA. What role, if any, do these "other molecules" play, and how do these relate to the most basic driving force in the development of depression and anxiety disorders, namely stress and stress responsiveness (Van Praag, 2005). A major part of this paper will be dedicated to one of these novel candidate molecules.

In recent years, increasing evidence has begun to indicate that major depressive disorder, but also severe stress disorders such as post traumatic stress disorder (PTSD), are associated with neuronal structural remodeling and possibly also neural damage and cell death (Rajkowska, 2000; D'Sa and

Duman, 2002). Recent brain imaging studies in PTSD patients have highlighted that if not treated aggressively and adequately, the illness may induce damaging effects on the hippocampus over time (Elzinga and Bremner, 2002), including hippocampal shrinkage and cognitive changes. These structural and cognitive changes would appear to be a direct consequence of the illness as both are reversible with effective drug treatment (Vermetten et al, 2003). Similar structural changes have been observed in depression, including cognitive changes (McEwen, 1999; MacQueen et al, 2003). Recurrent depressive episodes may act as a driving force for cumulative hippocampal atrophy and possibly permanent damage (Sheline et al, 1999; MacQueen et al, 2003). While controversy still prevails as to whether these changes are pathological or adaptive (Gilbertson et al, 2002; Swaab et al, 2005), the mechanisms underlying the changes would appear to be a sequel to severe stress (eg PTSD) or chronic stress (eg depression).

2. Stress: Can't live with it, can't live without it

The stress response is geared to enhance the probability of survival by promoting the development of coping mechanisms to resist the stressor, and to improve the response to a later similar stressor. Typical responses would include behavioural changes, such as anxiety and aggression, and involuntary actions such as increased heart rate and hormonal changes, eg. adrenaline and cortisol. Stress therefore can be viewed as a challenge that requires behavioral, psychological and physiological adaptation that initiates a series of responses that are vital for survival. This process is referred to as allostasis. However, a balance (homeostasis) is extremely important to ensure harmony between internal regulatory mechanisms and external/internal stressors. This balance is constantly challenged by repeated physical/psychosocial threats, such that disharmony in allostasis may lead to neuropsychiatric dysfunction and ultimately a mental illness (McEwen, 2004).

The hypothalamic-pituitary-adrenal (HPA)-axis and the monoaminergic-sympathetic nervous system play an important role in how an animal deals with stress (Heuser and Lammers, 2003). Where the catecholamines facilitate the availability of energy to vital organs, glucocorticoids released from the adrenals act as "anti-stress" hormones that help to contain the neural responses initiated by the stressor. Allostasis would involve adaptive responses to stress, typically manifested by a short-term activation of the HPA-axis resulting in involuntary response mechanisms, including the mobilisation of energy to the brain and muscles, sharpening/focusing attention on the perceived threat, enhanced heart rate and respiration to improve oxygenation, modulation of the immune response, inhibition of reproductive behavior, decreased feeding and appetite and the laying down of memory related to the event. Allostatic load describes a maladaptive response to the stressor, resulting in over- or under-production of stress hormones and a failure to terminate activation of the HPA-axis (McEwen, 2004). Under the latter non-physiological conditions, cumulative brain changes take place, described as a process of "wear and tear", that culminate in structural and functional brain changes. The resulting neuropsychiatric dysfunction would be

dependent on the severity and duration of the stressor, for example in the development of depression versus PTSD.

3. Glutamatergic mechanisms in stress and neuropsychiatric illness

Without dispelling the important role of catecholamines and indoleamines in the aetiology of affective illness, there is now significant evidence implicating excitatory N-methyl-D-aspartate (NMDA)-glutamate and inhibitory gamma amino butyric acid (GABA) pathways in depression (Shiah and Yatham, 1998; Kendell et al, 2005) and PTSD (Oosthuizen et al, 2005; Chamber et al, 1999). Indeed, regulation of NMDA glutamatergic mechanisms is implicated in both the behavioral and adaptive neuronal response to antidepressants and may have a key role in both the neuropathology and treatment of affective illness (Skolnick, 1999). GABA occupies a critical role in inhibiting glutamatergic transmission via pre-synaptic GABA-B heteroreceptors (Yamada et al 1999), such that stress-induced GABA release in the hippocampus may play an important protective mechanism to curb excessive glutamate activation (Oosthuizen et al, 2005). Our work over the past 7 years has been to focus on the GABA-glutamate interactions in stress-related disorders.

The successful management of both depression and PTSD with the SRI's (Harvey, 1997; Stein et al, 2000) has increased attention to the role of 5HT in the neurobiology and treatment of stress-related disorders. However, in both instances, these drugs have distinct shortfalls in efficacy (Stein et al, 2000; Kocsis, 2003). Many patients therefore have lasting anxiety and other manifestations resulting in chronic psychosocial malfunction with significant socio-economic implications. Improved pharmacotherapeutic interventions are thus urgently needed. A better understanding of the stress response under varying conditions, namely chronic low grade stress versus acute severe stress, and the biological mediators involved in these responses, will highlight new targets for drug development. In recent years, increasing evidence has accumulated for the role of glutamate and its down-stream messenger, nitric oxide (NO), in anxiety and stress such that investigating the role of the glutamate-NO-pathway in suitable animal models would be a viable novel route of investigation. Studies performed in our laboratory have highlighted the important contribution of glutamate and NO to the neurobiology and treatment of anxiety and stress-related disorders.

3.1. The nitric oxide (NO)-pathway

NO synthase (NOS) exists in three different isoforms that are either constitutive or inducible (Table 1). The activity of the constitutive NOS depends on Ca^{2+} and calmodulin, whereas the inducible NOS is independent of Ca^{2+} .

Table 1. Physiological characteristics of the NOS isoforms (reproduced from Oosthuizen et al, 2005).

	Neural (nNOS, type 1)	Inducible (iNOS, type 2)	Endothelial (eNOS, type 3)
Cell type first identified	Neurons	Macrophages	Endothelium
Other cells expressing	Myocytes Astrocytes	Astrocytes Microglia	Neurons
Intracellular localization	Soluble or membrane bound	Soluble or membrane bound	Largely membrane bound
Ca ²⁺ dependency	Activity depends on elevated Ca ²⁺	Activity is independent of elevated Ca ²⁺	Activity depends on elevated Ca ²⁺
Expression	Constitutive Inducible under certain circumstances e.g. trauma	Inducible	Constitutive
Amounts of NO released	Small, pulses	Large, continuous	Small, pulses
Proposed function	Regulatory	Host defence	Regulatory
Activators	Glutamate Noradrenaline	Lipopolysaccharide	Acetylcholine

Endothelial eNOS is mainly located in the cell membrane, neuronal nNOS in neuronal cells, while inducible iNOS is located in macrophages and glial cells (Oosthuizen et al, 2005). All NOS isoforms are dependant on NADPH (β -nicotinamide adenine dinucleotide phosphate) and calmodulin. In iNOS, calmodulin is present in a tightly bound form, such that iNOS produces NO in a sustained manner in the presence of adequate substrate. Calcium-calmodulin binds to the constitutive enzyme in a reversible manner, but binds irreversibly to the inducible enzyme, so that neurons and endothelial cells containing the constitutive enzyme produce receptor-regulated pulses of NO, while the inducible enzyme in macrophages and microglia produces sustained levels of NO in response to cytokines that are not regulated by receptors (Table 1).

In the CNS, glutamate activation of the NMDA receptor represents the most important stimulus for the release of NO via activation of the Ca²⁺-dependent nNOS isoform (Figure 2). In the presence of calmodulin, Ca²⁺ activates NOS that converts the amino-acid L-arginine to N ω -hydroxy-L-arginine, which is further converted to NO and L-citrulline (Oosthuizen et al, 2005). A small gaseous molecule (MW 30 Da) with a biological half-life of minutes, NO is rapidly degraded to nitrites and nitrates. However, its great lipid solubility affords it the unique ability to move quickly within and between cells. Once NO is released, it binds to and stimulates the soluble guanylate cyclase leading to an increase in the second messenger, cyclic guanosine monophosphate (cGMP; Figure 2; Dawson and Dawson 1995). Important neuronal effects of cGMP include activation of G-kinase, activation or inhibition of phosphodiesterase and subsequent effects on cyclic adenosine monophosphate (cAMP), effects on ion channels and G-proteins and neurotransmitter release (Harvey, 1996). All these actions exert a significant effect on neuronal function. The effects of cGMP are terminated by the

phosphodiesterase (PDE) family, among which PDE 3 and PDE 5 are considered specific for cGMP.

High concentrations of NOS are found in brain regions involved in the modulation of anxiety and defensive behaviour (Vincent and Kimura 1992), and exposure to stressful stimuli has been found to induce the activation of NO-producing neurons in the amygdala, hypothalamus, peri-aqueductal grey, and pedunculopontine tegmental nucleus (Krukoff and Khalili 1997). The activation of neuronal NOS and other Ca^{2+} -dependent enzymes account for many of the deleterious effects associated with excessive glutamate NMDA receptor activation (Dawson and Dawson, 1995). Moreover, NO not only has important neurotoxic effects if excessively activated, but also plays an important role in anxiety-related behaviors (Oosthuizen et al, 2005), as well as being closely involved in the laying down of memory (McLeod et al, 2002). Thus, NMDA receptor antagonists (Padovan et al., 2000), guanylate cyclase inhibitors (Eroglu and Caglayan, 1997; Heiberg et al, 2002), and NOS inhibitors (Volke et al, 2003a) all demonstrate significant anxiolytic properties in animal models of anxiety, while increasing cGMP in the brain with the selective PDE-5 inhibitor, sildenafil, causes anxiogenic effects (Volke et al, 2003b; Kurt et al, 2004). Clearly, these data argue strongly that closer study of the NO pathway in anxiety disorders is warranted. However, to do this requires the development of an animal model that closely emulates the human condition in its symptom presentation and response to drug treatment.

4. Depression studies, and the role of nitric oxide

A feeling of depression occurs in the course of any active, eventful life and is considered a key emotional experience in early development and a predictor of emotional maturity in later life. These ephemeral states of "depression" begin with early emotional experiences often linked to failed expectations, ambitions and goals. Both depression and anxiety are signals that help guide an adult through the complexities of life. However, pathologic depression is evident when these feelings interfere with normal functioning - such as with the maintenance of important relationships, work performance and economic self-support. Epidemiological studies show a high co-morbidity of anxiety and depression with up to two-thirds of patients with depressive symptoms having prominent anxiety symptoms (Rodriguez et al, 2005), suggesting that the presence of the one condition increases the likelihood of the other occurring. In most patients, major depression is a relapsing, remitting illness. After a first episode, there is a greater than 40% risk of recurrence over a two-year period. After two episodes of depression, the risk of recurrence within 5 years is approximately 75% (Judd et al, 1998; Keller and Boland, 1998).

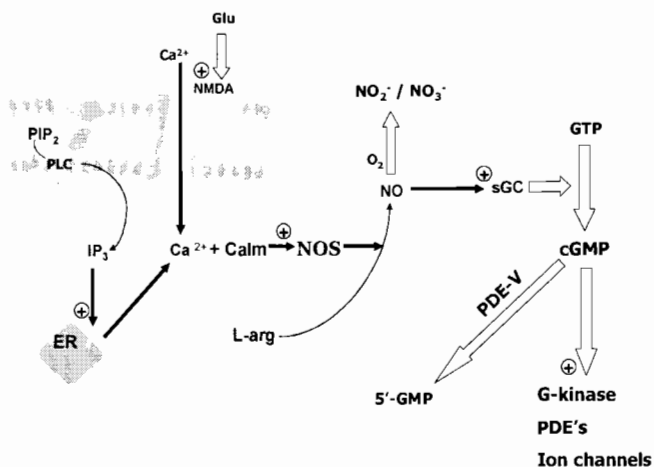


Figure 2: The glutamate-NMDA receptor mediated activation of the NO-cGMP pathway in the CNS. Upon binding of glutamate (Glu) to the NMDA receptor, the resulting influx of Ca^{2+} leads to the activation of nNOS in a calmodulin (Calm) - dependent manner and the synthesis of NO from L-arginine (L-arg). NO then binds to and activates soluble guanylate cyclase (sGC) leading to the synthesis of cGMP that is responsible for cell-specific responses. NO is broken down to nitrogen oxides (NO_2^- and NO_3^-), with the actions of cGMP terminated by type-5 phosphodiesterase (PDE-V). Another source of NOS activation indicated in the sketch is via Ca^{2+} release from the endoplasmic reticulum (ER) via metabotropic receptor induced activation of phospholipase (PLC) and the synthesis of inositol triphosphate (IP_3) from the membrane lipid, phosphatidylinositol bisphosphate (PIP_2).

Major depression is a mood disorder that invariably follows chronic environmental adversity and stress (Van Praag, 2005). Ordinarily the body will initiate adaptive mechanisms to cope with psychosocial stressors, as described earlier. However, depending on the susceptibility of the person, i.e. genetic predisposition, and the nature and duration of the stressor, these coping mechanisms may become over-whelmed leading to a maladaptive response and the gradual development of depressive behavior. Typical neuroendocrine changes include an increased circulating cortisol (Swaab et al, 2005) and variable monoamine changes, one being the gradual depletion of synaptic monoamines, and up-regulation of monoamine receptors due to a maladaptive over-use, in keeping with the monoamine hypothesis of depression (Harvey, 1997; Leonard, 2003).

As has been alluded to earlier, various clinical studies in depression have highlighted that major depressive disorder is associated with neuronal structural remodeling (Rajkowska, 2000; D'Sa and Duman, 2002), especially in the hippocampus. Although studies in rats have shown that high levels of cumulative corticosteroid exposure and rather extreme chronic stress induce

neuronal damage that selectively affects hippocampal structure, this has not been supported by clinical and experimental observations in humans. In a few recent postmortem studies in patients treated with corticosteroids and patients who had been seriously and chronically depressed no indications for neuropathology, massive cell loss, or loss of plasticity could be found, while the incidence of apoptosis was extremely rare and only seen outside regions expected to be at risk for steroid overexposure (Swaab et al, 2005). In addition, no massive cell loss in the hippocampus following exposure to stress or steroids could be found, but rather showed adaptive and reversible changes in structural parameters after stress (Swaab et al, 2005). Thus, while the HPA-axis in depression is moderately activated, possibly due to the initial (primary) hippocampal degeneration in this condition, the ensuing structural changes are likely to be adaptive in nature. Nevertheless, cortisol may still play an important role in the signs and symptoms of depression via the actions of cortisol on the release of glutamate (Harvey et al, 2003a).

Although the causal relationship between structural brain changes and the development of depression requires further confirmation (Gilbertson et al, 2002), evidence from imaging studies would concur that recurrent depressive episodes appear to be a driving force for cumulative hippocampal atrophy (Sheline et al, 1999; MacQueen et al, 2003). Patients with depression and anxiety disorders all too often discontinue antidepressants prematurely (Basca and Rush, 1995). This may happen for various reasons, including poor tolerance, the stigma of using an antidepressant or simply using the drug as a periodic "lift-me-up"-er. This has major negative implications for the longitudinal outcome of the illness. Depressed patients who have responded to antidepressants and who are switched to placebo relapse 2 to 4 times more often than those maintained on medication (Nierenberg, 2002). Similarly, greater number and longer duration of episodes of depression are associated with a higher risk of later relapse and recurrence (Judd et al, 1998; Keller and Boland, 1998). Clearly, inappropriate antidepressant discontinuation will prompt subsequent episodes that invariably are of longer duration, more severe and less treatment-responsive.

Chronic stress is recognized as one of the most important initiators of the cellular events that pre-date the development of depression and its associated neuropathology (Van Praag, 2005). Recent evidence suggests that antidepressant withdrawal is associated with a stress response (Michelson et al, 2000). Acute antidepressant withdrawal in patients receiving chronic treatment, particularly of short half-life compounds, is associated with increased stress system activity involving the hypothalamic-pituitary growth axis and the sympathetic nervous system, specifically elevations in plasma insulin-like growth factor-1 (IGF-1) and heart rate (Michelson et al, 2000). The latter reflect stress-related dysfunction of the hypothalamic-pituitary-adrenal and growth axes that are features of depression (Chrousos and Gold, 1992). Precious little is known regarding the neurobiological mechanisms that are activated following antidepressant withdrawal, and of their implications for the course and prognosis of depression. However, preliminary evidence suggests that patients with a history of switching are associated with a more rapid decline in hippocampal volume (MacQueen et al, 2003), providing impetus for

further study in this area. We set out to examine this question using an animal model of antidepressant withdrawal (Figure 3).

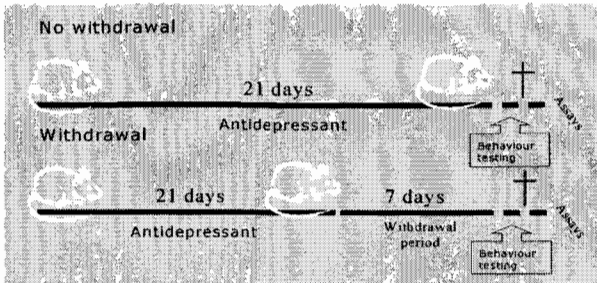


Figure 3: The antidepressant withdrawal paradigm. Rats are treated chronically with imipramine for 21 days, or for 21 days plus a 7-day withdrawal period during which time the animals receive saline injection. Two parallel controls are run, viz. a 21 and 28 day saline treatment group. Behavioral and neurochemical analyses are then performed immediately after the chronic treatment period and again after the 7-day withdrawal period.

4.1. The forced swim stress (FST) model

The ability of both humans and animals to exert control over their environment is dependent on “coping behavior” that prevents the deleterious effects of stress. In humans, and as best that it can be measured in animals, this represents an important homeostatic mechanism against the development of depression. Thus, animals subjected to an inescapable stressor, such as forced swim stress, will demonstrate increased immobility once they perceive a lack of control over the situational stressor. This represents a state of “learned helplessness” or “behavioural despair” in which active coping mechanisms are eventually attenuated. Effective antidepressants counter the “stress-induced” immobility response. In clinical depression, this trait is evident in symptoms such as decreased volition (drive), apathy, lack of self-worth and esteem and locomotor retardation. The resulting behavioural changes can be exploited to associate a given neurochemical and/or structural abnormality in the brain to altered behaviour that can be quantified.

The FST model was originally developed by Porsolt and colleagues (1979). We have used this procedure to assess the animal’s response to an unconditioned situational stressor. During a preconditioning period, the animals were placed into Perspex cylinders containing 18cm of water and observed for 15 minutes. Immediately after drying, each animal was returned to its cage and housed overnight. The following day, each rat was returned to the water-filled cylinders for 5 minutes where immobility was recorded and timed using video cameras and in-house observers. The animal was judged to

be immobile when it remained afloat making only the necessary movements to keep its head above the water.

4.2. The glutamate-NO pathway in depression

In the antidepressant withdrawal paradigm in rats using swim stress as an unconditioned situational stressor (Figure 3), we found that acute imipramine withdrawal after chronic treatment was associated with a loss in situational stress responsiveness induced by imipramine (Harvey et al, 2002; Figure 4). These behavioral changes were accompanied by an increase in hippocampal glutamate NMDA receptor density and a subtle, possibly compensatory, increase in hippocampal GABA levels (Harvey et al, 2002). Antidepressant withdrawal, therefore, increases the number of NMDA receptors with an increased probability of receptor binding by available synaptic glutamate. Since major depressive illness may be associated with increased circulating glutamate levels (Altamura et al, 1995; Maes et al 1998; Mauri et al, 1998), while antidepressant response involves a dampening of NMDA receptor function (Boyer et al, 1998; Skolnick, 1999; Harvey et al, 2002), the increase in NMDA receptor density described after antidepressant withdrawal reflects a primary increase in glutamatergic tone subsequent to removal of the inhibitory effect of the antidepressant. In the same study, we found these behavioral and neurochemical effects of imipramine withdrawal to be reversed by an NMDA receptor antagonist, thereby supporting a role for prior NMDA receptor activation (Harvey et al, 2002; Figure 4).

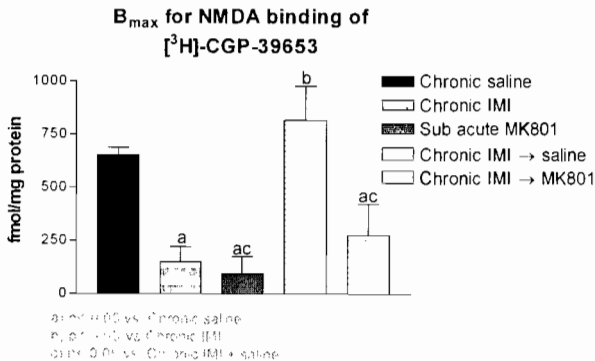


Figure 4: Chronic antidepressant treatment with and without withdrawal and effects on NMDA receptor density in the rat hippocampus (data reproduced from Harvey et al, 2002). Chronic imipramine induces a profound decrease in receptor binding that is significantly reversed upon treatment withdrawal (IMI+saline). These receptor effects are reversed by the NMDA receptor antagonist, MK-801, administered during the withdrawal period (IMI+MK801).

More recently we have extended these findings by looking at the effect of antidepressant withdrawal on events down-stream of the NMDA receptor, specifically NOS. In this study, we found that IMI withdrawal was associated with a significant increase in swim stress-induced immobility together with a significant increase in hippocampal NOS activity compared to both control and IMI-treated groups, confirming that antidepressant discontinuation increases stress responsiveness together with disinhibition of hippocampal NOS (Harvey et al, 2005a). The resulting increased glutamate and nitergic activity may have significant implications for depressive illness and its treatment (Harvey, 1996; Harvey et al, 2003a). A mechanistic hypothesis is depicted schematically in Figure 5.

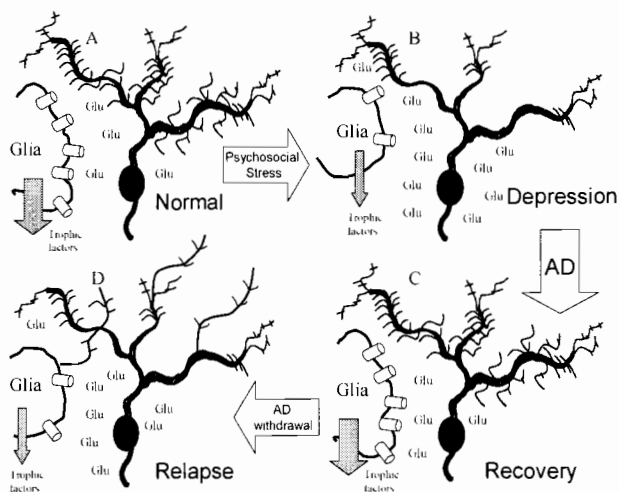


Figure 5: Schematic representation of the impact of depressive illness and inappropriate antidepressant withdrawal on neuronal structure (figure reproduced from Harvey et al, 2003a). Psychosocial stressors provoke depressive illness by impacting negatively on neurogenesis and cellular resilience through attenuated expression of essential neurotrophins leading to the gradual loss of glial cells and reduced number of glial glutamate transporters (Figure 5 A-B). The result is a build up of synaptic glutamate and activation of NOS, and the development of depression and neurodegenerative sequelae (Figure 5 B). Successful antidepressant treatment will reverse this pathology (Figure 5 C). Similarly, inappropriate antidepressant withdrawal will evoke conditions of overt glutamate activity (Figure 5 D), with increased NOS activity as a consequence. Although not depicted in the latter figure, also present in this scenario is an increase in neuronal glutamate NMDA receptor density. This, and the loss of neurotrophic cover, will underlie synaptic and neuronal remodeling that may be different to that of the previous episode. The result is a recurrent episode of depression that presents differently to the previous episode, possibly requiring higher dose or alternative treatment.

That chronic antidepressant treatment decreases hippocampal NOS activity, and which is reversed upon drug withdrawal, confirms the role of NOS in the pharmacological action of imipramine. Taking this further, other pre-clinical studies have confirmed the antidepressant-like properties of NOS inhibitors (Harkin et al, 1999) while in an *in vivo* microdialysis study in rats (Wegener et al, 2003), we have confirmed that NOS inhibition represents an important pharmacological property of various clinically effective antidepressants, and that may play an important part in their antidepressant activity. In fact, Harkin and colleagues (2004) have demonstrated that NOS inhibitors can augment the antidepressant action of typical antidepressants, suggesting that the less than adequate efficacy of current antidepressants in treating depression may be due to a relative shortfall in NOS activity. To bring the relevance of this work to the clinical setting, recent clinical studies have confirmed that depression is associated with altered activity of the NO pathway (Suzuki et al, 2001; Xing et al, 2002).

Some of the characteristic physical and psychological symptoms of antidepressant withdrawal include dizziness, nausea, gastrointestinal distress, headache, anxiety and gait instability (Schatzberg et al, 1997; Zajecka et al, 2001). Since these symptoms suggest increased excitability of serotonergic neurons (Coupland et al, 1996), we investigated the role of excessive 5HT activity as a possible driving force for the above neurochemical and behavioral changes following antidepressant withdrawal. Using the selective 5HT_{2A/C} receptor antagonist, ritanserin, we found that ritanserin re-established the anti-immobility effects and reversed NOS hyper-function during IMI withdrawal (Harvey et al, 2005a), suggesting that increased NOS activity post-withdrawal is mediated through a mechanism involving 5HT_{2A/C} receptor activation. This is of considerable relevance since raised synaptic 5HT levels may be detrimental to neuronal function and integrity (Vaidya et al, 1999). Indeed, 5-HT released during stress may contribute to neuroplastic events by enhancing NMDA receptor efficacy (McEwen, 1997), and studies have found that lowering synaptic 5HT levels with the serotonin re-uptake enhancer, tianeptine, prevents stress-induced atrophy of dendrites of CA3 pyramidal neurons (McEwen et al., 1997). Thus, antidepressant withdrawal not only evokes troublesome side effects via increased 5HT activity that are ultimately self-limiting, but through neuronal release of NO more insidious long-term complications may be realized that may underlie structural brain changes evident in brain imaging studies in depression, as well as manifest as treatment resistance and repeated relapse (Figure 5). While the possibility that depression is a neurodegenerative disorder remains speculative at best, these studies have provided convincing proof that the glutamate-NO pathway is an important new neurobiological target that needs serious consideration in antidepressant drug development.

5. PTSD studies, and the role of nitric oxide

PTSD is a severely disabling anxiety disorder that may occur following exposure to an acute traumatic event (APA, 1994). The disorder is characterized by re-experiencing and hyperarousal symptoms, as well as by

avoidance symptoms and problems with explicit recall of memories. Re-experiencing symptoms include intrusive recollections of the original trauma in the form of recurrent daytime memories, nightmares and flashbacks. Avoidance and amnesic symptoms include "feeling numb" and gaps in memory that may last from minutes, hours, even days.

While the mediating role of glucocorticoids, particularly cortisol, in the psychobiology of PTSD is well-recognized (Schelling et al, 2004; Raison and Miller, 2003), clinical studies on HPA-axis activity during PTSD have been inconsistent (Rasmusson et al, 2003). Evidence for hypocortisolemia in PTSD (Yehuda et al, 2000) is the antithesis of that in depression and particularly interesting, such that the exact role of cortisol in trauma and later development of PTSD remains unclear. The HPA response to stress is ultimately terminated by the negative feedback inhibition of cortisol via glucocorticoid receptors in the pituitary, hypothalamus and extra-hypothalamic brain sites (Yehuda et al, 2000). The evidence for a hyper-responsivity of this negative feedback system in PTSD, resulting in hypercortisolemia (Yehuda et al, 2000), suggests that the stress response is no longer able to remain in a state of homeostasis. Indeed, low cortisol levels are noted in the presence of high catecholamine levels in patients with PTSD (Yehuda, 1997). Some authors have argued from these data that individuals who develop PTSD respond to a traumatic event by failing to release sufficient levels of cortisol for a long-enough period of time to shut down stress-induced sympathetic nervous system responses (Yehuda and Harvey, 1997), speculating that there is an exaggerated conditioned autonomic reaction to thoughts related to the trauma, such as increased heart rate, skin conductance, raised blood pressure and anxiety.

Lack of HPA axis control over the stress response, due to negative feedback hyper-responsivity, may be a consequence of hippocampal damage/atrophy in the acute period post stress, such that normal regulatory mechanisms that should terminate an inappropriate stress response are no longer functional. Although hypercortisolemia may underlie the initial hippocampal damage, both hypo- and hypercortisolemia may underlie the memory dysfunction evident in the immediate aftermath of trauma, and in chronic PTSD. A narrow window of circulating glucocorticoids is critical for normal functioning of the hippocampus, such that bi-directional effects on memory function are displayed. Thus, while hippocampal damage may be associated with exposure to excessive levels of glucocorticoids, too low levels of glucocorticoids are also detrimental for normal hippocampal function (de Kloet et al, 1999). This may place into perspective the apparent paradoxical observation of hypocortisolemia and diminished memory function in PTSD.

Although there is evidence of decreased hippocampal volume predating PTSD (Pitman et al, 2001), the hypothesis that PTSD leads to hippocampal atrophy and associated memory deficits is supported by recent work that during treatment of PTSD, there is an increase in hippocampal volume (Vermetten et al, 2003). Associative learning and other behavioral processes mediated by the hippocampus and that play a role in PTSD, involve glutamate NMDA receptors (Heresco-Levy and Javitt, 1998). Dysfunctional brain

glutamatergic systems, particular the NMDA receptor, have been suggested as an important neurobiological component of PTSD, perhaps contributing to adaptive hippocampal changes (Chambers et al, 1999). Animal studies implicate the hippocampus in emotional processing as well as explicit memory (Brown et al, 1999), where stress-related hippocampal structural changes appear related to the neurotoxic effects of glucocorticoids and the subsequent release of glutamate (McEwen, 1999; Sapolsky, 2000). In a recent pilot study Heresco-Levy and colleagues (2002) report on the clinical evidence for efficacy of D-cycloserine, a partial agonist at the glycine regulatory site on the NMDA receptor, in the treatment of PTSD. These data suggest that investigating down-stream events linked to the NMDA receptor may be of pharmacological significance in PTSD. In order to accomplish this, we first set out to develop an animal model that closely emulates the human condition in its development and symptoms presentation, neuroendocrine changes and its response to drug treatment.

5.1. The time-dependent sensitization (TDS) model

Animal models of PTSD have utilized intense stressors, aversive challenges, and situational reminders of a traumatic stress, in an attempt to model long-term effects on behavioral, autonomic, and hormonal responses seen in humans with PTSD. TDS stress in rodents is an animal model that emphasizes repeated trauma (Figure 6). In this model, animals are exposed to single session of prolonged stress (eg, 2hr restraint followed by a 20 minute forced swim, followed by exposure to ether or halothane vapors). The animals are allowed to recover for a week, where after they are subjected to a brief re-stress on day 7 (30 minutes of restrain stress or 20 minutes swim stress). The rationale being that the frequency of exposure to situational reminders contributes to the maintenance over time of fear-related behavioral disturbances (Uys et al, 2003).

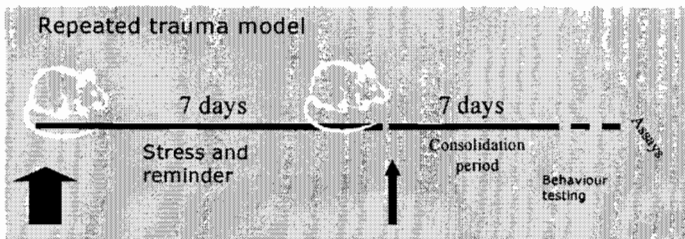


Figure 6: The time-dependent sensitization stress protocol. Animals are subjected to a severe stressor on day 1 of the protocol, and then left undisturbed before being subjected to a less severe re-stress procedure on day 7. The latter allows consolidation of fear-related memory that may be accompanied by long lasting behavioral and neurochemical changes. The latter determinations are performed at various times after the re-stress, eg. 7 days, 14 days and 21 days post re-stress.

Novel exploratory studies performed in our laboratory aimed at validating the model have demonstrated that TDS stress induces important phenomenological and psychobiological features reminiscent of PTSD, including increased aversive behavior and a dysregulated monoamine and HPA-axis response (Brand et al, 2005). Moreover, in keeping with the evidence for memory disturbances in the disorder, we found that TDS induces pronounced spatial memory defects (Figure 7) together with hypocortisolemia (Harvey et al, 2003b). In agreement with the clinical efficacy of serotonergic drugs in the treatment of PTSD (Stein et al, 2000), TDS induces distinct serotonergic receptor abnormalities in the hippocampus and prefrontal cortex (PFC; Harvey et al, 2003b). Of great importance, TDS stress-induced 5HT-receptor and memory changes can be reversed with the SRI, fluoxetine, but not by the 5HT depleting agent, p-chlorophenylalanine (PCPA; Figure 7; Harvey et al, 2004a). A particularly important preliminary observation has been the reversal of TDS stress-induced bio-behavioral changes with the adrenal steroid synthesis inhibitor, ketoconazole, thereby suggesting the pre-requisite involvement of stress-induced glucocorticoid synthesis in these changes (Brand et al, 2004).

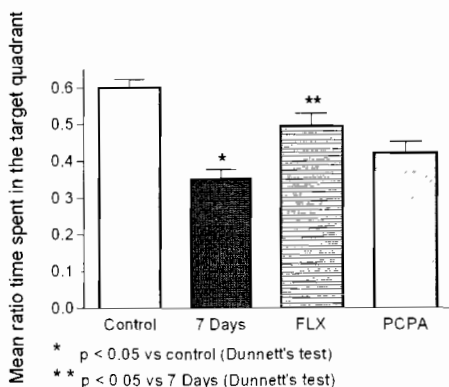


Figure 7: The effect of TDS stress on spatial memory as determined in the Morris water maze, and its modulation by serotonergic-active drugs (data reproduced from Harvey et al, 2004a). As determined on day 7 post re-stress, TDS stress evoked a pronounced decrease in spatial memory performance compared to control (data presented is that of the probe trial only). This response was significantly reversed by fluoxetine (FLX), but not by p-chlorophenylalanine (PCPA).

The above studies have provided an important measure of the validity of TDS as an animal model of PTSD. Firstly, the model has proven to resemble the clinical situation with accurate face validity, in other words how well the model resembles the psychiatric condition. Secondly, the model demonstrates strong

construct validity, or how well the model is consistent with theoretical rationale, and strong predictive validity in that the model responds to drugs used in humans with PTSD. These may be summarized as follows:

Face validity: TDS evokes pronounced spatial memory deficits together with lowered plasma corticosterone (Harvey et al 2003b), consistent with clinical findings in PTSD. More recently, Kahn and Liberzon (2004) also describe the ability of the model to engender an increased startle reflex, another typical symptom of PTSD. The TDS model also emphasizes the role of prior trauma in predicting subsequent dysfunction and allows for the study of bidirectional expression of symptoms, such as enhanced or reduced behavioral responsiveness to environmental stimuli and variable changes in monoamines and glucocorticoids following stress (Brand et al, 2005). These represent important phenomenological and biological correlates of PTSD.

Construct validity: The model is useful for studying HPA abnormalities relevant to PTSD in that animals subjected to TDS display the enhanced sensitivity to negative glucocorticoid feedback that is often characteristic of PTSD (Liberzon et al 1997; Liberzon et al 1999; Yehuda et al, 2000). Further, the model leads to changes in hippocampal 5HT_{1A} and PFC 5HT_{2A} receptors (Harvey et al 2003b), brain areas that are intimately involved in memory and stress responsiveness. These data also correlate positively with the accepted role of 5HT in the pathology of the disease and its responsiveness to SSRI's (Stein et al, 2000).

Predictive validity: The effects of TDS-induced stress on spatial memory and its reversal by fluoxetine (Harvey et al 2004a), and by the steroid synthesis inhibitor, ketoconazole (Brand et al, 2004), provide strong predictive validity. These data correlate positively with the putative causal role of 5HT and glucocorticoids in PTSD-associated memory changes.

The above findings on the validity of the model have helped to establish the model as an extremely useful paradigm with which to study new hypotheses and/or new neurobiological targets of possible relevance in PTSD, in particular the role of the glutamate-NO system in PTSD.

5.2. The glutamate-NO-pathway in PTSD

A significant finding from our studies was that TDS stress causes a significant and sustained elevation in NO synthesis in the hippocampus that is reversed by an inhibitor of the inducible isoform of NO synthase (iNOS) but not with an inhibitor of the neuronal (nNOS) isoform (Figure 8; Harvey et al, 2004b). Since iNOS is predominantly linked to inflammatory conditions, these data suggest that TDS stress may involve the activation of an inflammatory mechanism in the brain, possibly through cytokine release (Harvey et al, 2004b, Oosthuizen et al, 2005). The observation that stress-induced NO release was inhibited by ketoconazole, a steroid synthesis inhibitor, further confirmed the role of stress-induced glucocorticoids in this response (Figure 8; Harvey et al, 2004b). These data make the startling suggestion that PTSD may be an inflammatory disease (Oosthuizen et al, 2005), possibly underlying the structural changes

that have been noted in the disorder. Clearly, more studies are needed to confirm these results.

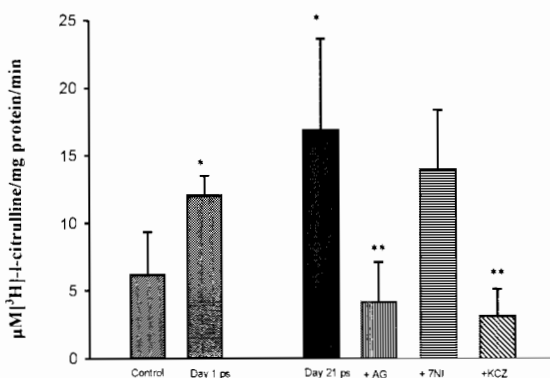


Figure 8: The effects of TDS stress on hippocampal NOS activity and its modulation by various drugs. TDS stress engenders a sustained elevation in NOS activity on day 1 after acute stress (Day 1 ps) and on day 21 after re-stress (Day 21 ps). This response is driven primarily by the iNOS isomer due to its select inhibition by the iNOS inhibitor, aminoguanidine (AG) and not the nNOS inhibitor, 7-nitroindazole (7-NI). The response is also dependent on circulating glucocorticoids, evinced by its inhibition by ketoconazole (KCZ). * $p < 0.05$ versus Control; ** $p < 0.05$ versus Day 21 ps.

In order to study this phenomenon further at a pharmacological level, we investigated the effect of TDS stress on the hippocampal NO-cGMP signalling pathway, specifically the release of the stable oxidative metabolites of NO, viz. nitrogen oxides (NO_x), and its modulation by drugs with selective actions on the glutamate NMDA receptor, as well as more down stream sub-cellular events, including nNOS, cGMP and nuclear factor K-β (NFK-β). NFK-β is an important nuclear transcription factor involved in the activation of iNOS, but also is involved in glutamate-NMDA receptor-mediated events (Burr and Morris, 2002). TDS stress significantly increased hippocampal NO_x which was blocked by either the nNOS inhibitor, 7-nitroindazole sodium salt or the NFK-β antagonist, pyrrolidine dithiocarbamate. Interestingly, the glutamate NMDA receptor antagonist, memantine was without effect. The cGMP-specific PDE inhibitor, sildenafil, however, significantly augmented stress-induced NO_x accumulation (Harvey et al, 2005b). From these data we were able to conclude that increasing neuronal cGMP acts as a protagonist in driving stress-related events, while both nNOS (neuronal NOS) and iNOS (inducible/immunological NOS) may represent a therapeutic target in preventing the effects of severe stress. While the value of NMDA receptor antagonism appears limited, targeting its sub-cellular linkage to the NO-cGMP pathway may have important implications for the pharmacological treatment of PTSD. The lack of response to memantine requires further study, but may be that shortly after acute stress NMDA receptors are down-regulated (Harvey et

al, 2004b) with the result that blocking the receptor some time after the initial trauma may have limited benefit.

While the efficacy of SRI's, such as paroxetine and fluoxetine, in the treatment of PTSD (Stein et al, 2000; Vermetten et al, 2003) are undoubtedly linked to their serotonergic properties, we wondered whether at least some of the efficacy of these drugs rests on an inherent ability to attenuate NOS. Earlier studies have found that various classes of antidepressants inhibit the functioning of the glutamate NMDA ion channel (Skolnick, 1999) providing a strong rationale that down-stream events of the NMDA receptor, such as the activation of NOS, may be modified by antidepressants. Using *in vivo* microdialysis (Wegener et al, 2003) as well as *in vivo* chronic treatment (Harvey et al, 2005a) we were able to demonstrate that various classes of antidepressants inhibit NOS activity in the rat hippocampus. Of more significance, this attenuation of NOS was correlated with a reversal of stress-related aberrant behavior (Harvey et al, 2005a). While the clinical relevance of NOS inhibition in patients with PTSD still requires investigation, it is of note that a recent clinical study has described the involvement of the NO-pathway in patients with acute PTSD (Yeh et al, 2002).

6. Conclusion

Anxiety disorders are the most prevalent, disabling and chronic of psychiatric disorders (Kessler et al, 1994; Murray & Lopez, 1996) and their under-treatment contributes to enormous personal and economic costs (Dupont et al, 1996; Greenberg et al, 1999). In particular, mood and stress-related disorders are becoming a growing global health burden, while current treatments are in most cases less than adequately effective. Understanding the mediating psychobiology of these disorders will assist in developing effective interventions, while such advances can be drawn upon to encourage early detection and effective treatment of the anxiety disorders (Stein et al, 2005).

This paper has presented the important role of stress and the nature and duration of the stress in the initiation of disorders such as depression and PTSD, their progression and response to treatment. However, many aspects relating to variation in individual response to the stressor, and the underlying neurobiology is poorly defined. Despite early advances in the drug treatment of depression and anxiety some 50 years ago, and more recent developments that have identified new putative neurobiological targets in depression and PTSD, the currently available drugs are disappointing in their long-term efficacy. Further, there is a limited pipeline of new drugs, despite a plethora of possible drug targets that have been identified. While targeting monoamine transmission has been successful, this has only been partially so and one is left with the question whether we are targeting the correct processes with our drugs. Moreover, are these "less than effective" drugs maybe not worsening the illness through incorrect usage, leading to the escalating relapse rates and chronic morbidity associated with these disorders? New targets for drug treatment, and novel ideas for psychotropic drug design are urgently needed

and this paper has highlighted at least one potentially useful avenue of investigation, viz. the glutamate-NO-pathway.

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