Adaptive plasticity during stress and depression and the role of glutamate-nitric oxide pathways

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Abstract
Anxiety and mood disorders are amongst the most prevalent and disabling of all the psychiatric disorders. Under-diagnosis and current treatments that are often less than adequately effective, contribute to an enormous personal and economic cost to the patient, family and health-care organizations. Although distinctly separate disorders at neuropathological and phenomenological levels, brain-imaging studies in posttraumatic stress disorder (PTSD) and depression have emphasized that both 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 is 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, possibly providing a basis for relapse and treatment resistance. Similarly, even with the introduction of effective medications for PTSD, many patients remain treatment-resistant. Stress in various guises may alter synaptic connectivity in the brain by bolstering glutamatergic excitotoxic mechanisms. Understanding these mechanisms may assist in developing more effective treatment strategies. This paper will review pre-clinical and clinical evidence supporting a role for the glutamate-nitric oxide pathway as a putative mediator of the neuropathological changes evident in depression and stress-related disorders, particularly PTSD, and its potential as a novel target for psychotropic activity.

Keywords: Depression; PTSD, nitric oxide, glutamate; neuroplasticity; stress

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Introduction
The seminal discovery of the antidepressant imipramine in 1958 and of the mood-stabilising actions of lithium ion in 1949 have set the gold standard for research and treatment in mania and depression. However, since these halcyon days of psychopharmacology, 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 while lithium salts still remain the benchmark treatment for manic depression. The advent of extremely sophisticated techniques in molecular neuroscience and molecular psychiatry have uncovered 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 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.

Although the monoamine hypothesis of depression has proved to have significant construct validity in that it has formed the basis for the development of our current armamentarium of antidepressant drugs, their delayed onset of action, that they seldom exceed an expected rate of remission of 50%, and having an unacceptably high incidence of relapse, has paved the way to a more intractable form of the illness. Despite their improved side effect profiles and 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. While the focus over the past three or more decades has been 5HT, noradrenaline (NA) and dopamine (DA), the rapid advancement of neuroscience has in the past years revealed that brain function is also regulated by many other neurochemicals and neuronal messengers, some acting as transmitters in their own right, while others modify the actions of the more traditional transmitters.

In recent years, increasing evidence has begun to indicate that major depressive disorder, as well as severe stress disorders such as post traumatic stress disorder (PTSD), are associated with neuronal structural remodeling and possibly also neural damage and cell death. Recent brain imaging studies in PTSD patients have highlighted that if not treated aggressively and adequately, the illness may over time induce damaging effects on brain areas important in regulating the stress response, especially the hippocampus. Thus, multiple exposures to trauma will result in hippocampal shrinkage and cognitive changes, which would appear to be a direct consequence of the illness as both are reversible with effective drug treatment. Similar structural changes have been observed in depression, including cognitive changes. As in PTSD, recurrent depressive episodes may act as the driving force for cumulative hippocampal atrophy and possibly permanent damage.

The successful management of both depression and PTSD with the SRI’s 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. 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. While controversy still prevails as to whether the above brain changes are pathological or adaptive, the mechanisms underlying the changes would appear to be a sequel to stress and may present an important target for antidepressant action. Stress-induced messengers, such as glutamate, that regulate cell excitability and survival are therefore of considerable importance.

**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, e.g. 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 allostatics. 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 allostatics may lead to allostatic load and ultimately a mental illness.

The hypothalamic-pituitary-adrenal (HPA)-axis and the monoaminergic-sympathetic nervous system play an important role in how an animal deals with stress. Where the catecholamines facilitate the availability of energy to vital organs, glucocorticoids released from the adrenals 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. 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. These structural changes may follow the up-regulation of excitatory pathways that mediate synaptic plasticity, a decrease in cell proliferation and shrinkage of neuronal networks. This deficit in synaptic connectivity lays the foundation for the development of psychiatric illness.

**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 and PTSD. Glutamatergic NMDA pathways play an important role in neural plasticity and modifying existing neural networks. 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. GABA occupies a critical role in inhibiting glutamatergic transmission via pre-synaptic GABA-B heteroreceptors, such that stress-induced GABA release in the hippocampus may play an important protective mechanism to curb excessive glutamate activation.

**The nitric oxide (NO)-pathway**

In the central nervous system (CNS), glutamate activation of the NMDA receptor mediates the release of the pleiotropic messenger molecule, NO, via activation of the Ca++-
Dependent neuronal NO synthase (nNOS) (Figure 1). Constitutive nNOS and endothelial (eNOS) produce receptor-regulated pulses of NO, while the inducible (iNOS) enzyme in macrophages and microglia produces sustained levels of NO in response to cytokines. Inducible NOS is primarily involved in inflammatory reactions, producing sustained cytotoxic concentrations of NO. Receptor-regulated NO release has important neuroregulatory properties in the cardiovascular system (via eNOS), while in the CNS (via nNOS), NO is implicated in a variety of higher functions, including learning, memory, and emotionality. In the context of this paper, it is the neurotoxic actions of NO as well as its effects on 5HT, memory, and emotionality. NO is small gaseous, paramagnetic molecule with great lipid solubility. These properties allow it to rapidly move within and between cells where it binds to and stimulates the soluble guanylate cyclase leading to an increase in the second messenger, cyclic guanosine monophosphate (cGMP; Figure 1). Important neuronal effects of cGMP include activation of G-kinase, activation or inhibition of phosphodiesterase (PDE) and subsequent effects on cyclic adenosine monophosphate (cAMP), effects on ion channels and G-proteins and neurotransmitter release. All these actions exert a significant effect on neuronal function. The effects of cGMP are terminated by the PDE family, among which PDE 3 and PDE 5 are considered specific for cGMP.

High concentrations of NO are found in brain regions involved in the modulation of anxiety and defensive behaviour. 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. Although NO normally acts to conserve neural function, under conditions of allostatic load and increased glutamate-NOS activity, excessive release of NO will react with many cellular proteins resulting in neuronal damage or death. Thus, targeting glutamate and NO may play an important role in treating anxiety-related behaviors. Indeed, drugs acting at various targets within the NMDA-NO pathway, including guanylate cyclase inhibitors, NMDA receptor antagonists, and NO inhibitors 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.

**Depression, antidepressant compliance and nitric oxide**

Major depression is a mood disorder that invariably follows chronic environmental adversity and stress. Ordinarily, the body will initiate adaptive mechanisms to cope with psychosocial stressors, as described earlier. However, depending on the susceptibility of the person, e.g. genetic predisposition, and the nature and duration of the stressor, these coping mechanisms may become over-whelmed leading to a maladaptive response, structural brain changes and the gradual development of depressive behavior. Typical neuroendocrine changes include an increased circulating cortisol 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.

Studies in rats, however, have shown that structural and damaging brain changes appear only after extreme chronic stress. This has not been supported by clinical and experimental observations in humans, although post-mortem studies in depressives show adaptive and reversible changes in structural parameters in the hippocampus after stress. 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 still plays an important role in the symptoms of depression, but may also have more insidious effects by evoking the release of glutamate.

Evidence from imaging studies would concur that recurrent depressive episodes appear to be a driving force for cumulative hippocampal atrophy. Patients with depression and anxiety disorders all too often discontinue antidepressants prematurely, while patients discontinuing antidepressants have a 2 to 4 times greater risk of relapse than those maintained on medication. Similarly, greater number and longer duration of episodes of depression are associated with a higher risk of later relapse and recurrence. While corroborating clinical data are difficult to generate for ethical reasons, inappropriate antidepressant discontinuation may prove to be a driving force that prompts subsequent episodes that 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. Antidepressant withdrawal is associated with increased stress...
The characteristic physical and psychological symptoms of antidepressant withdrawal, including dizziness, nausea, gastrointestinal distress, headache, anxiety and gait instability\(^{52,58}\) are typical symptoms associated with increased serotonergic activity.\(^{59}\) Excessive SHT activity may therefore drive both the behavioral as well as neurochemical changes following antidepressant withdrawal. Indeed, concomitant administration of a SHT\(_{2A/C}\) receptor antagonist prevents imipramine withdrawal associated stress-responsiveness and NOS hyper-function\(^{51}\), suggesting that increased NOS activity post-withdrawal is mediated through a mechanism involving SHT\(_{2A/C}\) receptor activation. This is of considerable relevance not only because of the important role for SHT in depressive symptomology, but concurs with earlier studies that raised synaptic SHT levels may be detrimental to neuronal function and integrity.\(^{60}\) Indeed, 5-HT released during stress may contribute to neuroplastic events by enhancing NMDA receptor efficacy.\(^{22,61}\) Thus, antidepressant withdrawal not only evokes troublesome side effects via increased SHT 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.

In agreement with a role for glutamate in depression, post-mortem studies have found changes in the NMDA receptor complex in the frontal cortex,\(^{62}\) and reduced NMDA receptor expression in the hippocampus of depressives.\(^{63}\) Platelet glumatergic receptor super-sensitivity is also evident in patients with depression,\(^{64}\) while NMDA receptor antagonists, such as amantadine and ketamine, have been found to be remarkably effective in severe, treatment resistant depression.\(^{56,57}\) Recent clinical studies have also confirmed altered activity of the NO pathway in depression,\(^{65,66}\) as well as bipolar affective disorder,\(^{67,68}\) while lithium too has distinct activity on the NO pathway.\(^{69,70}\) These data suggest that alternate disturbances in mood, as well as anti-manic and antidepressant drugs of various classes modulate the NO-pathway.

**PTSD and the role of nitric oxide**

PTSD is a severely disabling anxiety disorder that may occur following exposure to an acute traumatic event.\(^{71}\) 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, clinical studies on HPA-axis activity during PTSD have been inconsistent.\(^{72}\) The evidence for a hyper-responsivity of the negative feedback inhibition of cortisol on glucocorticoid receptors in the HPA-axis resulting in hypocortisolemia\(^{18}\) suggest that individuals who develop PTSD respond to a stressor through a mechanism involving 5HT\(_{2A/C}\) receptor activation. This is of considerable relevance not only because of the important role for 5HT in depressive symptomology, but concurs with earlier studies that raised synaptic 5HT levels may be detrimental to neuronal function and integrity.\(^{60}\) Indeed, 5-HT released during stress may contribute to neuroplastic events by enhancing NMDA receptor efficacy.\(^{22,61}\) Thus, antidepressant withdrawal not only evokes troublesome side effects via increased SHT 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.
induced sympathetic nervous system responses. This lack of HPA axis control over the stress response may be a consequence of hippocampal damage/atrophy in the acute period post stress. Circulating glucocorticoids exert a bidirectional effect on hippocampal function, eg memory, such that while hippocampal damage may be associated with exposure to excessive levels of glucocorticoids, too low levels post stress are also detrimental for normal hippocampal function. The incidence and severity of PTSD is dependent on multiple exposures to trauma that is compounded by hippocampal damage, a perpetuating stress-response and a dysfunction in memory formation. These are evinced by hypo- or hypercortisolemia, re-experiencing of trauma memories and attenuation of explicit memory function.

Although there is evidence of decreased hippocampal volume pre-dating PTSD, the hypothesis that PTSD leads to hippocampal atrophy and associated memory deficits is supported by both clinical and pre-clinical research where associated memory changes (and decreased hippocampal volume in patients) in PTSD and in animals can be reversed with SRI’s. Associative learning and other behavioral processes mediated by the hippocampus and that play a role in PTSD, involve glutamate NMDA receptors. Dysfunctional brain glutamatergic systems, particular the NMDA receptor, have been suggested as an important neurobiological component of PTSD, perhaps contributing to adaptive hippocampal changes. Animal studies implicate the hippocampus in emotional processing as well as explicit memory, where stress-related hippocampal structural changes appear related to the neurotoxic effects of glucocorticoids and the subsequent release of glutamate, thus implicating a possible role for down-stream messengers of the NMDA receptor.

Inducible NOS has been found to be activated following acute severe stress in rats. Using time-dependent sensitization (TDS) stress, a putative animal model of PTSD, we have found that repeated trauma causes a sustained elevation in iNOS-mediated NO synthesis in the hippocampus. That stress-induced NO release is inhibited by ketoconazole, a steroid synthesis inhibitor, further confirms the role of stress-induced glucocorticoids in this response. iNOS is activated by cytokines, pivotal modulators of inflammatory processes. Psychological stress in humans is associated with increased secretion of pro-inflammatory cytokines, such as interleukin-6, and high levels of cerebrospinal fluid interleukin-6 have been measured in patients with PTSD. Since interleukin-6 secretion is suppressed by glucocorticoids, conditions of hypocortisolemic as often noted in PTSD may allow an unopposed increase in interleukin-6 secretion, with subsequent increase in iNOS activation. These data make the startling suggestion that PTSD may be an inflammatory disease, possibly underlying the structural changes that have been noted in the disorder.

TDS stress has also been found to significantly increase the accumulation of hippocampal NOx, the stable oxidative metabolite of NO. Of note is that this response can be blocked by an nNOS inhibitor or an inhibitor of nuclear factor kappa beta (NFκ-β), an important transcription factor mediating the induction of iNOS. Interestingly, the glutamate NMDA receptor antagonist, memantine was found to be ineffective, possibly due to a down-regulated state of the NMDA receptors following stress. The cGMP-specific PDE inhibitor, sildenafil, however, significantly augmented stress-induced NOx accumulation. These data conclude that increasing neuronal cGMP acts as a protagonist in driving stress-related events, while both nNOS and iNOS 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.

It is noteworthy that a recent clinical study has described the involvement of the NO-pathway in patients with acute PTSD, while the drugs of choice in the treatment of PTSD, the SRI’s, have been found to inhibit hippocampal NOS. Moreover, D-cycloserine, a partial agonist at the glycine regulatory site on the NMDA receptor has demonstrated clinical efficacy in treating PTSD. These studies provide a strong rationale that the NMDA receptor and its down-stream events represent an important pharmacological and therapeutic target for the treatment of PTSD.

**Conclusion**

Anxiety disorders are the most prevalent, disabling and chronic of psychiatric disorders and their under-treatment contributes to enormous personal and economic costs. 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. This paper has only discussed the CNS role of NO in depression and stress. However, NO occupies an extremely important regulatory role in the cardiovascular system and, considering the long-term detrimental effect of stress on this system, it is well worth making mention of an important point in this regard. Stressing rats is associated with an increase in atrial sensitivity to circulating catecholamines. More interesting, however, is that NMDA receptor blockade may prevent stress-induced cardiotoxicity, suggesting that glutamatergic pathways are mobilized during severe stress and may also be driving the peripheral autonomic manifestations of stress. Clearly, 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.

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. 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 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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