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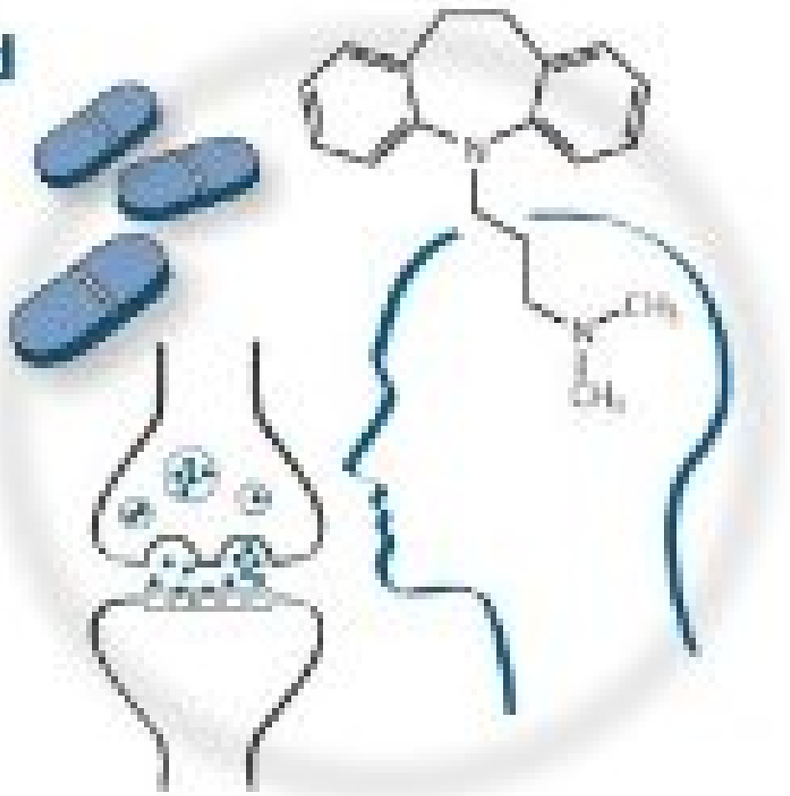
# Depression:

## From Psychopathology to Pharmacotherapy

Editors
J.F. Cryan
B.E. Leonard



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# Modern Trends in Pharmacopsychiatry

## Vol. 27

Series Editor

B.E. Leonard Galway

# Depression: From Psychopathology to Pharmacotherapy

Volume Editors

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**Modern Trends in Pharmacopsychiatry**
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# Preface

Melancholy
Sits on me as a cloud along the sky,
Which will not let the sunbeams through, nor yet
Descend in rain and end; but spreads itself
'Twixt heaven and earth, like envy between man
And man, and is an everlasting mist'
*Lord Byron*

Throughout recorded history, depression has been recognised as a disorder of the brain and the body.

In the Old Testament of the Bible, the book of Job (4th century BC) describes the severe melancholy that afflicted Job following the sudden deaths of his children and the loss of his possessions. Recurring depression also afflicted Michelangelo (1474–1564), while Martin Luther (1483–1546) described the doubts and despair, despondency, feelings of guilt and anxiety, accompanied by physical ill health, that accompanied the consolidation of the Protestant Church in Germany. Other famous theologians, philosophers, writers, composers and artists who suffered from severe depression include Ignatius de Loyola (founder of the Jesuits), the philosophers Arthur Schopenhauer and Immanual Kant, and the composers Mozart and Beethoven to name but a few. In recent times, Winston Churchill was famous for the 'black dog' that affected his mood throughout his adult life.

But perhaps the earliest and most influential description of depression was provided by the Oxford theologian Robert Burton (1577–1640). In his famous book *The Anatomy of Melancholy* of 1620, Burton gives a description of the sad fate of many scholars who, with their 'windy melancholy' (described as windy vapours that ascend to the brain there to trouble the imagination, cause fear, sorrow, dullness and many terrible conceits!), have ultimately caught nothing but wind. He concludes that such scholars have sacrificed their lives to science, but their sacrifice yielded nothing temporary for themselves and nothing lasting for the world. In evidence, he recommends 'Go to Bedlam and ask' (see the cartoon by Hogarth of the interior of Bedlam hospital of 1735).

Perhaps those of us who have spent a professional lifetime trying to understand the causes of depression and how antidepressants might work should heed Burton's

advice least the frustration caused by our limited success in understanding this disorder lead to our demise with 'windy melancholy'. It is against this background, and to prevent the onset of 'windy melancholy', that the editors have gathered a group of international researchers to demonstrate the significant advances that are being made in understanding the psychopathology of depression and the mechanism of action of antidepressant drugs, advances that will contribute to more effective treatments of this terrible disorder in the future. To achieve this, the authors have critically assessed the developments being made in both the basic and clinical aspects of depression. A brief perusal of the titles of the 15 chapters that compose this book will illustrate the breadth of the research that has been covered.

The editors hope that this book will become a reference text for basic and clinical neuroscientists, pharmacologists and psychiatrists. The editors express their appreciation to all the authors for their contributions and hope that you, the readers, will gain as much pleasure from reading the text as we have in bringing this book to fruition.

*John F. Cryan*
*Brian E. Leonard*

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# Antidepressant Compounds: A Critical Review

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**Abstract**

Major depression is a commonly encountered mental disorder in clinical practice. Although choices of antidepressant drugs appear to be many, all current antidepressant drugs have essentially similar mechanisms of action through the monoaminergic pathways. There remain a significant number of patients not benefiting from the current antidepressant compounds. Considering that major depression is likely to be a multisystem disorder, the current lack of antidepressant drugs with alternate mechanisms of action hinders treatment of drug resistance cases, residual symptoms and incomplete remission. New drugs are also needed to address many of the areas of impairment not responsive to current antidepressant drugs, such as memory impairment, impaired executive function and compromised endurance to daily life stress. To develop such new antidepressant drugs, 'out of the box' thinking is critical.



Major depressive disorder (MDD) is a common mental disorder in clinical practice. However, it is difficult to know how many patients suffering from major depression are not diagnosed or misdiagnosed and therefore not treated. Many studies suggest that major depression is much more common than reported. For example, the high prevalence of depression in college students [1–3] has been commonly noticed by school clinic physicians, but there are few systematic studies of this phenomenon or reports in medical journals. Similarly, depression associated with other medical problems, such as stroke, cardiac and gastroenterological problems, as well as somatic presentation of depression pushed into the medically unexplained syndromes, is now known to be quite common [4, 5]. Depression is already the 2nd leading cause of disability as measured by years lived with disability. It was estimated that by the year 2020, depression would reach 2nd place in the ranking of disability-adjusted life years (i.e. the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability) calculated for all ages. At the first Global Mental Health Summit in Athens, Greece, September 2009, the World Health Organization predicted that within 20 years more people would be affected by depression than by any other health problem.

For the ones fortunate enough to be diagnosed and treated, there also exist problems of inadequate treatment in terms of dosages and duration of treatment, drug noncompliance, relapses, drug side effects, incomplete resolution, residual symptoms, drug resistance and compromised ability to resist stress in life. Deficiencies in the current selection of antidepressant drugs are many. For example, MDD patients who did not respond to the first 2 antidepressant treatments had a less than 15% chance of responding to subsequent treatments [6]. Lowering of relapse rate, bipolar switching issues and resolution of residual symptoms (including executive function and memory impairments) would probably require new drug targets. Some of these issues appear to be connected. For example, a greater number of residual symptoms is associated with a greater relapse rate [7]. Bipolar switching into mania has been reported to affect as much as one third of the bipolar inpatient population [8]. While it is widely acknowledged that current antidepressants do not protect against the emergence of manic symptoms [9], the risk of switching to mania was higher during tricyclic antidepressant (TCA) treatment (36%) compared to nontricyclic treatment (17%) [8]. Memory impairment is a relatively robust finding in depressed patients. Additionally, there is preferential processing of negative affective material and exaggerated response to negative feedback in depressed subjects [10]. Many of these symptoms can be dissociated from mood and do not always resolve with current antidepressant treatment. Considering all these problems, it is clear that response to current antidepressant drugs is far from satisfactory.

Of all the problems surrounding the management of major depression, what is urgently needed is the development of new antidepressant drugs to address many of the inadequacies of the existing ones. New antidepressant compounds with efficacy in resistant cases, easing residual symptoms, and with faster onset of action are most needed. Antidepressant drugs targeting those symptoms not responsive to current medications, such as memory, energy and executive function impairment, would be very useful. New antidepressants effective in long-term maintenance with minimum side effects or adverse reactions would also help. Pharmacologically, these new antidepressant drugs should possess a core mechanism of action different from the existing ones, therefore allowing clinicians to have a real alternate choice in cases resistant to or with incomplete response to existing antidepressant drugs. This chapter reviews and discusses such a possibility.

## Traditional Paths for Development of Antidepressant Compounds

### *Exploitation of the Serotonin Transporter in Antidepressant Drug Development*

The TCAs, the selective serotonin reuptake inhibitors (SSRIs) and the serotonin-norepinephrine reuptake inhibitors (SNRIs) all block the reuptake of serotonin (5-HT) potently. The 3-dimensional molecular structure of these drugs enables them to bind

to the 5-HT transporter with high affinity. Binding to the 5-HT transporter causes conformational change of the transporter protein resulting in an interference of the passage of the 5-HT molecule through the transporter. The TCAs and the earlier SSRIs appear to bind to a common site of the 5-HT transporter protein. Some of the critical amino acids of the transporter protein contributing to this binding site have been identified [11–13]. Interestingly, there also appears to be a second site. The active enantiomer of citalopram, escitalopram, has been shown to bind to this second site and appears to cause additional changes in the kinetics of association and dissociation of the antidepressant compound itself from the first binding site. This change in the binding kinetics has been suggested to convey additional antidepressant advantage (for a review, see Tang and Helmeste [14]) making escitalopram superior to its racemic parent citalopram and other antidepressant drugs of the 5-HT and norepinephrine (NE) reuptake inhibitory class [15–18].

Evolution of the 5-HT reuptake inhibitory type of antidepressant drugs reflects attempts to develop new antidepressant compounds with higher potency and specificity and to minimize side effects, including cytochrome P450 enzyme inhibition. With good choices among the various SSRIs and SNRIs, the need for additional new SSRI compounds appears to be quite limited. There have been some attempts to develop new antidepressant compounds with triple [5-HT, NE and dopamine (DA)] reuptake inhibition [19]. It is foreseeable that such effort will not provide any additional benefits in terms of efficacy, as it will not bring in a new mechanism of action. The SSRI sertraline already possesses respectable DA uptake inhibition but has not been shown to greatly exceed other SSRIs in terms of efficacy [20].

### *Serotonin Receptors as Potential Antidepressant Drug Targets*

There are 7 known classes of 5-HT receptors. Some 5-HT receptors have been suspected to play an important role in the maintenance of mood. Also, many antidepressant drugs and anti-anxiety drugs possess moderate to high affinities for various 5-HT receptors, including 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_4$ receptors and some are not monoamine reuptake blockers. Examples of new compounds with antidepressant properties but which are not monoamine reuptake blockers include agomelatine and aripiprazole, which have affinity for 5-$HT_{1A}$ and 5-$HT_{2C}$ receptors. How these compounds produce antidepressant action through 5-HT receptors or whether the 5-HT receptor actions are essential for their antidepressant actions is far from clear [21–23]. Agomelatine, for example, is also an agonist at melatonin $MT_1$ and $MT_2$ receptors and improves sleep disturbance in depression [24].

In considering 5-HT receptors as antidepressant targets, it is important to distinguish between the acute and chronic effects as they could be quite different. The acute and chronic effects of antidepressant drugs on 5-HT receptors were examined in the early 1980s when radioactive antagonists with high specific activity became

available. Tang and coworkers [25–28] screened all the available antidepressant compounds for their binding affinities to 5-HT and NE receptors and compared their acute and chronic effects on these receptors. It was discovered that the acute and chronic effects of antidepressant compounds on 5-HT receptors were quite different indeed. Furthermore, the effects of some of these antidepressant compounds in reducing the density of 5-$HT_2$ receptors were not dependent on the presynaptic 5-HT input, as raphe lesions failed to interfere with this effect [29]. The differential acute and chronic effects of antidepressant compounds on 5-HT receptors were suspected to be related to the latency of antidepressant therapeutic action which may take up to 2 weeks or more.

The more detailed actions of antidepressant compounds on various 5-HT receptor subtypes were examined in many later experiments. It seems that at least 3 5-HT receptor subtypes are implicated in the antidepressant action of these compounds, namely 5-$HT_{1A}$, 5-$HT_{2A}$, and 5-HT $_{2C}$. Interestingly and paradoxically, both agonists and antagonists for these 5-HT receptor subtypes have been reported to have potential antidepressant effects when tested in animal models. For example, both stimulation and blockade of the 5-$HT_{1A}$ receptor may result in an antidepressant action, and both agonist and antagonistic action at 5-$HT_{2C}$ and 5-$HT_{2A}$ may also lead to an antidepressant effect. Buspirone is a 5-$HT_{1A}$ receptor agonist, trazadone is a 5-$HT_{2A/2C}$ receptor antagonist, agomelatine is a 5-$HT_{2C}$ receptor antagonist, and aripiprazole is a partial agonist with high intrinsic activity at 5-$HT_{1A}$, while being a 5-$HT_{2A}$ receptor antagonist [30–33]. This seemingly paradoxical action on 5-HT receptor subtypes reminds us of the earlier paradoxical observation that a 5-HT uptake enhancer, not a blocker, was also an antidepressant. It is quite possible that the presence of other non-5-HT inputs might play an important role in determining the final pharmacological action of these 5-HT agonists and antagonists on the same 5-HT receptor. In fact, there are experiments supporting this hypothesis.

As a recent example, agomelatine, an antidepressant that has recently been approved by the European Medicines Commission, is a potent melatonin receptor agonist with Ki values of 0.12 and 0.10 nM for the melatonin $MT_1$ and $MT_2$ receptors, respectively. It is also a potent 5-$HT_{2C}$ receptor antagonist (pKi = 6.2) in a dose that corresponds to its antidepressant and anxiolytic effects in rodents [32, 34]. This combination of melatonin receptor activation with 5-$HT_{2C}$ receptor antagonism underlies its pharmacological profile in which the early resynchronization of the sleep profile might be explained by the augmentation of the melatonergic response, while the mood effect is related to the reduction in the 5-$HT_{2C}$ receptor activity in the prefrontal cortex. Experimental studies have shown that the chronic administration of agomelatine is linked to the increase in the release of NE and DA from the prefrontal cortex without any changes in 5-HT function. This could be explained by the indirect effects on NE and DA release from this brain region by the blockade of 5-$HT_{2C}$ receptors which results in an increased outflow of these catecholamines from the frontal cortex. Recent studies have also shown that agomelatine counteracts the adverse effects of

stress on hippocampal neurogenesis in rodents [35]. Further large-scale clinical trials of agomelatine on MDD cases resistant to other antidepressant drugs will be needed to confirm its status as a new antidepressant drug with an alternate mechanism of action [36].

Other potential compounds in this category include p11 (an inducible adaptor protein), which increases localization of the serotonin 5-$HT_{1B}$ receptor at the cell surface and increases 5-$HT_{1B}$ receptor function. p11 knockout mice exhibit a depression-like phenotype and are resistant to antidepressant treatment [37].

The TREK-1 potassium channel which influences the excitability of individual neurons is another example of a new target for antidepressant drug development. TREK-1 is inhibited by therapeutic doses of SSRIs and mice lacking TREK-1 exhibit a depression-resistant profile, such as blunted cortisol response to stress. However, a reasonable worry is that if these brain proteins have too wide a distribution in the brain (and regulate other non-mood-related functions), then unwanted side effects may occur which would prevent long-term therapeutic usage. TREK-1, for example, has been implicated in neuroprotection and general anesthesia. Whether drugs targeting TREK-1 would cause undesirable side effects by disrupting other brain functions is one of many considerations in the development of such new antidepressant agents [38–40].

*The Role of GABA in Antidepressant Action*

Neuroanatomical, neurochemical, neurophysiological and behavioral data have confirmed the inhibitory action of GABAergic neurons on endogenous DA release. Dopaminergic mesolimbic and mesocortical systems are fundamental in hedonia and motivation. The inability of 5-HT to stimulate nucleus accumbens DA release has been proposed to be a factor in depression. The decrease in DA release has been suggested to be responsible for the symptoms of anhedonia and decrease in motivation. As 5-HT exerts an inhibitory action on GABA interneurons through 5-$HT_{2C}$ receptors, antagonism of 5-$HT_{2C}$ or subsensitivity of these receptors would release the NE and DA pathways from inhibition by the GABA interneurons [41]. While the release of DA and NE pathways from inhibition was proposed to be an important factor in the mood and motivation effect of antidepressant drugs, it may explain why antidepressant drugs might activate psychosis (through the DA effect) in some bipolar or schizophrenic patients [42]. However, bupropion, an antidepressant drug which possesses 'dopaminergic' action, seemed to have no effect on schizophrenic symptoms [43]. This suggests that activation of psychosis may not be a serious concern for all drugs in this category when their 5-HT action results in the enhancement of DA transmission. However, whether it is an agonistic or rather an antagonistic action that is required on the 5-$HT_{2C}$ receptor to create an antidepressant action is still far from conclusive at this point. Both agents possessing agonist or antagonist properties on the 5-$HT_{2C}$ receptor have been suggested to carry antidepressant potential [44–46]. Trazodone and nefazodone are weak 5-$HT_{2C}$ receptor antagonists and have

been shown to be useful in the treatment of milder cases of depression [47, 48]. It is important to mention that agents with single 5-$HT_{1A}$, 5-$HT_{2A}$, or 5-$HT_{2C}$ receptor action have yet to be tested clinically for true antidepressant action.

With regard to specific GABA receptor subtypes, $GABA_B$ receptor antagonists have been suggested to be antidepressants [49, 50]. SGS742, the first $GABA_B$ receptor antagonist in a clinical trial, has shown positive effect for improvement of attention and working memory in patients with mild cognitive impairment [49]. By contrast, the $GABA_B$ receptor agonist baclofen blocked the antidepressant effects of $GABA_B$ receptor antagonists in animal studies [50], and $GABA_B$ but not $GABA_A$ receptors appear to be involved in rodent models of antidepressant action [51]. Benzodiazepines have little or no antidepressant effects by themselves, but antidepressant-benzodiazepine combination therapy was found to lead to fewer dropouts and less depression severity in major depression [52].

### *Increase or Decrease in 5-HT Neurotransmission after Antidepressant Treatment*

Another area of interest in considering the 5-HT pathway as an antidepressant drug target is whether 5-HT neurotransmission is enhanced, downregulated or modulated after antidepressant treatment. According to the traditional amine theory of depression, central amine deficiency causes symptoms of major depression. 5-HT reuptake inhibition by antidepressant drugs results in a synaptic 5-HT elevation and therefore 5-HT neurotransmission enhancement. Potentiation of 5-HT neurotransmission after chronic antidepressant treatment indeed has been demonstrated in physiological paradigms. However, this simplistic interpretation ignored the potential receptor changes after chronic drug treatment. Receptor binding studies in animals treated with chronic antidepressant drugs suggested that the density of 5-$HT_2$ receptors is actually decreased after chronic antidepressant treatment [26]. A decrease in 5-$HT_2$ receptor density would suggest a decrease in 5-HT transmission through the 5-$HT_2$ receptors. The most intriguing finding in arguing against a required increase in 5-HT neurotransmission for antidepressant action is tianeptine. A 5-HT reuptake enhancer, tianeptine enhances the reuptake of 5-HT (acutely) instead of inhibiting it, thus opposite to the action of SSRIs. To reconcile these conflicting findings, changes in other important central nervous system pathways such as DA secondary to changes in 5-HT neurotransmission would need to be examined. Indeed, tianeptine has been reported to enhance the extracellular concentration of DA in the nucleus accumbens [53, 54]. The DA and glutamatergic effects of tianeptine after chronic treatment are considered to be important for its antidepressant action [55]. Furthermore, while 5-HT seems to be central to many current antidepressant drug actions, it is important to mention that presynaptic 5-HT input is not necessary for the downregulation of 5-$HT_2$ receptors in animal models [28, 29]. A better understanding of the individual 5-HT receptor changes after chronic antidepressant

treatment may help to clarify this confusion. For example, 5-$HT_{2C}$ antagonism has been suggested to result in a release of the GABA inhibition on NE and DA neurotransmission in the frontal cortex. A synergistic combination of enhanced 5-HT neurotransmission in certain brain areas combined with modulated 5-HT neurotransmission through other 5-HT receptor targets might explain this complex and seemingly paradoxical effect.

*Exploitation of the Dopamine Transporter and Receptors as Antidepressant Targets*

A small number of antidepressant drugs such as sertraline (a dual 5-HT and DA uptake inhibitor) and bupropion (a dual NE and DA reuptake inhibitor) have affinity for the DA transporter and block DA reuptake. However, there are no pure DA reuptake blockers approved for antidepressant drug use at present. Triple uptake inhibitors (5-HT, NE and DA), such as DOV 102677, have been attempted [56, 57], but, as mentioned above, it is still unclear if inhibition of DA reuptake is useful in inducing an antidepressant effect or is adding to the antidepressant effect already created by 5-HT and NE reuptake inhibition. According to Guiard et al. [58], it has yet to be demonstrated that the addition of the dopaminergic component produces more robust effects than single- or dual-acting compounds. There is certainly the continuous temptation to drive multiple signaling pathways and produce an accelerated and/or greater antidepressant response either through polypharmacy or the development of new multiple-target compounds. Although early small-scale clinical trials demonstrated efficacy of some of these multiple-target antidepressant compounds, they have yet to be proven to represent a new group of antidepressant drugs with a true new mechanism of action. Their merits will again have to be confirmed by large-scale trials on MDD cases resistant to traditional antidepressant compounds.

Although there is no existing antidepressant drug with a clear DA receptor target, the $D_3$ agonist pramipexole was observed to show efficacy comparable to that of an SSRI in a pilot study. Interestingly, this compound may also have neuroprotective action through an anti-apoptotic activity [59, 60]. Whether DA agents work through the 5-HT system or have useful antidepressant effects independently has to be explored further. So far, the DA system has not been extensively explored for the development of antidepressant drugs but it is clear that DA is involved in the maintenance of mood, and DA pathways exhibit changes after chronic antidepressant treatment [61].

DA may play an important role in working memory impairment, a disturbance which frequently occurs in patients suffering from depression. Some patients never recover from memory impairment after a depressive episode. Also, many patients continue to complain about having memory disturbances on long-term antidepressant maintenance [62].

*Monoamine Oxidase Inhibitors*

Monoamine oxidase inhibitors (MAOIs) were used more often in the old days but are no longer first-line antidepressant drugs in modern clinics due to their side effect profile. They are also potentially numerous in natural products. This group of drugs appears to induce an increase in the synaptic concentration of monoamines and whatever happens subsequent to this synaptic increase in monoamine concentration should logically be similar to the monoamine reuptake inhibitors. In a pharmacological sense, their mechanism of action is not different from that of the TCAs, SSRIs and SNRIs. It is therefore not a surprise to see that they fail to show significant efficacy above the other antidepressant drugs, although some patients do respond to them when other drugs have failed. However, the same could be said about the individual TCAs, SSRIs and SNRIs, i.e. it is a common clinical observation that some patients do respond to one particular drug and not to the others. However, because of their side effects and potentially lethal drug-drug or drug-food interactive adverse effects, unless there is a dramatic improvement in their side effect profile or speed of onset of action, it is unlikely that more MAOIs will be developed as antidepressant drugs. Overcoming treatment resistance will likely require new mechanisms of action rather than a repeat of drugs with a monoamine reuptake inhibitor profile.

In humans, MAO-A and MAO-B differ in substrate specificity, with 5-HT and NE being preferentially deaminated by MAO-A, and DA being deaminated equally by MAO-A and MAO-B. Both MAO-A and MAO-B inhibitors have been reported to have antidepressant properties. Examples include the MAO-B inhibitor l-deprenyl (selegiline), and moclobemide, a reversible MAO-A inhibitor [63–65].

## Current Antidepressant Drugs: Are They More Similar than Different?

There has not been a clinically or scientifically satisfactory classification system for antidepressant drugs to date. This problem may be due to the general lack of antidepressant drugs with distinctly different mechanisms of action. In the case of antibiotics and many other drugs in other branches of medicine, very few physicians would prescribe more than 1 drug with a similar spectrum of action over bacteria, or body chemistry. For example, it is not common practice to prescribe 2 or 3 antibiotics with an identical antibacterial spectrum, whether the compounds differ in their molecular structure or not. In psychiatric clinical practice, however, it is not uncommon to encounter polypharmacy to the extreme, in that combinations of SSRIs, SNRIs, and TCAs are prescribed. In some communities, it has been observed that different formulations of fluoxetine were prescribed [unpubl. pers. information]. While it could be attributed to the difficult nature of chronicity and the high failure rate of antidepressant treatment, a serious lack of choice of antidepressant drugs with fundamentally different core actions has been one of the important reasons.

*Groups of Antidepressant Drugs*

Currently available antidepressant drugs are grouped quite arbitrarily into 4 major groups with some that do not fit satisfactorily into any one of the groups. The 4 common groups include the MAOIs, TCAs, SSRIs and SNRIs. Many early antidepressant drugs were MAOIs or TCA drugs, a name referring to their unique 3-ring molecular structure. These drugs were inhibitors of synaptic reuptake of 5-HT and/or NE. Because of their affinities for many other neurotransmitter targets, side effects were many and they are no longer first-line choices in modern clinics. A number of antidepressant drugs developed later to target the 5-HT and/or NE transporter more specifically were grouped together as the specific 5-HT reuptake inhibitors (SSRIs) or dual 5-HT and NE reuptake inhibitors (SNRIs). The SNRI venlafaxine is in fact not a dual-action inhibitor when given at a low dose and functions only as an SSRI, with the NE reuptake inhibition only occurring at much higher doses. The more recent SNRIs, such as duloxetine, have a more balanced profile. Sertraline, which is classified into the SSRI group, is also a DA reuptake inhibitor, while paroxetine of the SSRI group also possesses good NE reuptake inhibition properties. Clomipramine, which is structurally a tricyclic, has high potency in inhibiting 5-HT reuptake similar to the other SSRIs. In general, the classification of antidepressant drugs into SSRI and SNRI based on their acute in vitro pharmacology may not reflect their in vivo and chronic actions in the brain. Many recent reports indicated that the 5-HT, NE and DA systems actually are very much interrelated in terms of drug action as discussed above.

There remain several other antidepressant compounds which do not quite fit into any of the above groups, either because of their different molecular structures or unclear or complex actions. These include the tetracyclic maprotyline, some drugs with DA actions like amoxapine, trazodone and bupropion, the $\alpha_2$-receptor antagonists mianserin and its analogue mirtazapine, the combined 5-HT and melatonin drug agomelatine, and the 5-HT uptake enhancer tianeptine.

Thus, it appears that the drug targets for all current antidepressant drugs are restricted to the 3 monoaminergic neurotransmitter systems, 5-HT, NE and DA, with 5-HT receptors and transporters being associated with the majority of the antidepressant drugs. The existing antidepressant drugs act through similar mechanisms pharmacologically.

*Major Depression and Antidepressant Drugs*

Clinically, major depression, similar to schizophrenia and dementia, is most likely to be a heterogeneous group of brain disorders with common presenting symptoms. Thus, depressed mood is similar to the symptom of pyrexia in infection and the term depression does not fully describe the illness. Mood disturbance is probably the most prevalent and prominent symptom in the majority of patients but it is not the only

disturbance seen in patients suffering from major depression. Similar to dementia, it is likely that there are patients presenting as depression cases with another etiology and not responding favorably to the current antidepressant drugs. In the absence of biological markers, it is difficult to reliably differentiate these cases according to the patients' prominent symptoms alone. However, it is well known that certain symptoms like memory impairment, sleep disturbance, poor concentration and executive function impairment are not rapidly or fully responsive to antidepressant treatment. Thus, both the term depression and the term antidepressant drug are not the best scientific terms to describe the illness and the drugs required to treat the illness. In developing new antidepressant drugs, it is important to consider that mood disturbance is not the only symptom for the drug to target.

If we accept that major depression is a multisystem disorder, then some new antidepressants efficacious in areas where current antidepressant drugs are not too effective could be developed. These include impairment in areas such as executive function, energy, sleep and memory where current antidepressant drugs tend to be less effective. Most importantly, it is possible that a single drug might not be able to correct all defects that occurred. In addition, a patient suffering from major depression may not necessarily exhibit a full complement of impairments. Following this argument, compounds targeting specific symptoms may be useful and need to be developed.

### *Models (Tissue, Animal, Structure, Receptor) for the Discovery of Antidepressant Drugs*

There are many animal models for screening and testing potential antidepressant compounds. These include the tail suspension test, forced swimming or behavioral despair test, and sucrose tasting. Whether these tests are suitable and adequate for the discovery of newer antidepressant drugs has been questioned as one could argue that using the same animal model may result in the finding of new drugs with the same old mechanism of action. Also, stressors applied to animals have been assumed to produce depression symptoms in all animals, yet we know that this assumption does not apply to all humans. Some humans are 'resistant' to stressors, others are not. In this sense, current animal stress models for screening and testing potential antidepressant compounds may be inadequate. Models are covered in detail in another chapter of this book.

### *Is It Possible that a Group of Drugs Sharing a Similar Mechanism of Action May Still Show Different Efficacy?*

There are very few antidepressant drugs with a pure single target. The TCAs, for example, are notorious in terms of specificity as they have high to moderate affinities for many sites, resulting in their well-known multiple side effect profile. Although it

is commonly observed that patients not responding to one SSRI may do so to another one, comparison of different SSRIs in clinical trials indicated that the difference appeared to be in their side effects and not in their efficacy. All first-generation SSRIs tested exhibited comparable efficacy in clinical trials. Some SSRIs or SNRIs previously claimed to show superior clinical efficacy over others in fact only demonstrated very small advantages in rating scales and most such claims failed to hold in later studies. Recently, a report on the newer SSRI escitalopram suggested that this SSRI, which binds to 2 sites on the 5-HT transporter, indeed was found to have an advantage over the earlier SSRIs [18].

### *Is the New Generation of Antidepressant Drugs as a Group More Efficacious than the Older Ones?*

Comparison between traditional TCA compounds and the later generation of antidepressant compounds such as SSRIs and SNRIs did not show significant clinical efficacy over the TCAs and some studies even found the contrary. However, for symptom management such as bipolar switching into mania, differences between TCAs and other antidepressants do appear [66]. Head-to-head comparisons of antidepressant drugs against common standards such as fluoxetine and citalopram have been unable to show one that is significantly more efficacious above others. In switching from one antidepressant drug to another in resistant cases, no single antidepressant drug has been found to be a winner either. This has been demonstrated in large clinical trials such as the STAR*D (sequenced treatment alternatives to relieve depression) study and has been well summarized recently [67]. STAR*D is a multisite, prospective, sequentially randomized, controlled trial of outpatients with nonpsychotic MDD. It uses randomization to compare various switching or augmenting strategies [68, 69]. For treatment-resistant depression, switching has generally been found to result in only small improvements. For example, in SSRI-resistant depression, switching from SSRIs to non-SSRI antidepressants (i.e. bupropion) gives very small improvement in remission rates [70].

## Other Central Nervous System Drugs Exploited as Antidepressant Drugs or Augmentation Agents

### *Atypical Antipsychotics as Antidepressant Drugs*

Some atypical antipsychotic compounds have been studied and proposed to possess antidepressant action. As early as the early 1980s, Tang and coworkers [28, 71] reported that clozapine, loxapine and amoxapine treatments had an unusual effect in reducing the density of 5-HT receptors in animals. The atypical antipsychotics

developed later demonstrated a similar action on 5-HT receptors. These atypical antipsychotics were discovered to be effective agents for bipolar affective disorders and many are also undergoing clinical trials for major depression. Recently, it has also been discovered that similar to the antidepressant drugs, they induced brain-derived neurotrophic factor (BDNF) secretion and neurogenesis in animal models [72, 73]. This raises the important question, whether neurogenesis and BDNF action are related to a drug's antidepressant properties or whether the atypical antipsychotics as a class possess antidepressant action because of their serotonergic properties. This remains to be clarified.

*Lithium, Pindolol Augmentation and Ketamine in Antidepressant Action*

The lithium ion has been found to potentiate the antidepressant effect of antidepressant drugs, converting nonresponders/partial responders to responders [74, 75]. Rapid effects observed with lithium augmentation of TCAs have been documented; the same is not always observed with SSRIs, the suggestion being that this is because SSRIs do not increase post-synaptic 5-HT receptor sensitivity [75]. Whether lithium is an antidepressant itself is unclear. Lithium as a monotherapy for bipolar disorder, on the other hand, is widely advocated alongside other recommended agents [76]. It is unfortunate that lithium has become unpopular in some communities. It is also not as heavily and aggressively promoted as many of the new mood stabilizers by the industry. In developed countries, many recent graduates even have become unaccustomed to using lithium and erroneously view it as a dangerous compound. There are significant side effects/toxicity associated with lithium which, however, do not preclude its excellent benefits as an adjunct therapy.

The NE β-receptor blockers have been tested for their effects on mood and antidepressant drug action. Propanolol has been found not to interfere with the antidepressant action of TCAs [77]. Pindolol augmentation has shown efficacy in accelerating initial SSRI treatment in some but not all studies; it has not been reported to be effective in treatment-resistant depression [75, 78].

Many compounds, such as cocaine and amphetamine, seem to have a potent transient effect on mood, but their mood effect is not lasting and some of these compounds could even become depressants when administered chronically. This coupled with the observation that antidepressant drugs require an average of 2 weeks to create a mood effect and more time to alleviate other symptoms suggests that chronic rather than acute and in vitro properties of a molecule are generally more important in testing their suitability as clinically useful antidepressant drugs. Ketamine (an N-methyl-D-aspartic acid antagonist) is a drug which has been reported to possess acute antidepressant action that is maintained for several days. It has potential use in acutely suicidal depressed patients for whom conventional antidepressants might be too slow to be clinically useful [79]. Traxoprodil (CP-101606), a selective NR2B

antagonist, has also been found to produce an antidepressant response as well as dose-dependent dissociative-like symptoms [79, 80].

### *$\alpha_2$-Receptors as a Target for Antidepressant Drugs*

$\alpha_2$-Receptors exist in 5-HT terminals and activation of these receptors by NE results in inhibition of 5-HT release. Mianserin and mirtazapine are $\alpha_2$-receptor antagonists. By blocking the $\alpha_2$-receptors, 5-HT release is enhanced, resulting in the same net effect as blocking 5-HT reuptake. Both mirtazapine and mianserin are also NE-releasing agents as a consequence of their presynaptic $\alpha_2$-receptor antagonistic action [81].

The combination of 5-HT/NE reuptake inhibition with $\alpha_2$-receptor agonism, on the other hand, has been used to develop drugs for chronic pain (USPTO Application No.: 20080153808), whereas a pharmaceutical composition comprising a 5-$HT_4$ serotonin receptor agonist and an $\alpha_2$-receptor pan-agonist has been found to be effective for treating gastrointestinal motility disorders (USPTO Application No.: 20080153829).

### *Development of Non-Monoamine-Associated Compounds*

Efforts to develop non-monoamine-associated compounds include corticotrophin-releasing-hormone-associated compounds, cortisol-associated compounds, substance-P-associated compounds, histone deacetylation inhibitors, cytokine-related drugs, opioid and cannabinoid receptor drugs. None of these approaches have been successful so far.

## Role of Brain-Derived Neurotrophic Factor and Neurogenesis in Antidepressant Action

The role of neurotrophic factors has been implicated as a possible mechanism of action for antidepressant drugs. Many antidepressant compounds have been shown to induce an elevation of BDNF levels in the brain. There are numerous correlative studies showing that BDNF levels are affected by antidepressants, by stress, and may contribute to dysfunction in depression [82, 83]. However, whether BDNF contributes directly or only peripherally in the affective disorders requires more sophisticated analysis and methodology in human subjects than has been done to date. One consideration is that BDNF mutations have been shown to interact with mutations in other genes, such as the 5-HT transporter. Double-mutant mice, which have only 1 functional allele of the BDNF gene and no functional copies of the 5-HT transporter, show increased stress-related hormones and increased anxiogenic behavior compared to wild-type mice or those with single BDNF or 5-HT transporter deletions alone [84].

Another consideration is that BDNF may regulate other pathways which contribute to antidepressant action. For example, neuropeptides induced by BDNF, such as VGF, appear to have antidepressant-like effects [85, 86]. The mechanism by which VGF acts as an antidepressant remains to be determined, but there is evidence that VGF enhances neurogenesis as well as synaptic plasticity [87].

Antidepressants increase neural progenitor cells in the human hippocampus [88] and neurogenesis has been proposed to be important in antidepressant action [89]. However, animal studies show that both the neurogenesis and BDNF effects of antidepressants are strain dependent [90]. There are regional differences in antidepressant-induced BDNF changes. Selective loss of BDNF in the dentate gyrus of the hippocampus but not the CA1 region attenuates the actions of desipramine and citalopram in the forced swimming test in rodent studies [91]. More studies are needed to understand the roles of neurogenesis and BDNF in depression and antidepressant action.

Neurogenesis and cell proliferation in the hippocampus and olfactory ventricle induced by antidepressant drugs have been reported by many. The neurogenesis effect of antidepressant drugs was at one time thought to be a new model for the development of new antidepressant drugs until later research data showed that it is a much more complicated phenomenon than first thought. For example, although hippocampal neurogenesis was found to be required for the behavioral effects of antidepressants in rodent models of depression [89], recent studies show that this is strain dependent [92]. Why hippocampal neurogenesis is required in some mouse strains and not others is unclear at the present time. There is also a debate about whether neurogenesis does occur in humans and whether the neurogenesis effect is critical to antidepressant drug action. It is now known that many other central nervous system drugs such as mood stabilizers and atypical antipsychotics also induce neurogenesis in animals. In addition, the hippocampus has been seen as an area associated with learning and memory rather than mood. How hippocampal neurogenesis may translate into mood effects is not clear, even though the hippocampus is a brain area which has received more research analysis than many other brain areas.

Interestingly, antidepressant drug treatment also reversed cortisol-induced decreases in neurogenesis and proliferation [93, 94]. Recently, the possible involvement of other neurotrophic factors has also been proposed (glial-derived neurotrophic factor). There is also a link between BDNF and 5-HT in antidepressant action, suggesting that seemingly 'novel' strategies may still return to the monoamine hypothesis after all [95].

## Discussion

Antidepressant drugs together represent a large and important group of central nervous system drugs. They are prescribed by psychiatrists as well as clinicians in other specialties and in family practice. Comparison of antidepressant drugs in terms of

efficacy has been a favorable research topic. For the psychiatrists, new antidepressant drugs are urgently needed but not available. A major reason for this frustration may be related to the very similar mechanism of action of the existing antidepressant drugs. While isolated targets such as the 5-HT transporter, NE transporter, 5-HT receptor subtypes such as 5-$HT_{2C}$ or neurotrophic factors such as BDNF have been suggested as possible targets of action, there has been no convincing unified theory for the mechanism of action. It seems that an increase in synaptic 5-HT concentration by blockade of 5-HT/NE reuptake, 5-$HT_{1A}$ receptor antagonism, MAO inhibition and/or 5-$HT_{2C}$ receptor antagonism (either by direct antagonistic or partial agonistic action or subsensitivity after chronic treatment) may still explain the action of all current antidepressants but no one single drug emerged as significantly superior to the others when resistance is met in the clinical setting. Thus, for future drug development, 'out of the box' thinking would be essential, rather than further developing monoamine types of compounds. Some alternate approaches aimed at alternate targets such as substance P analogues, corticosteroid receptor and corticotrophin-releasing-hormone antagonists have been tried but have been proven to be unsuccessful. If precise understanding of the brain pathways responsible for MDD becomes known, then development of new antidepressants would be facilitated. Until that time, however, many new developments will proceed by trial and error.

It is important to remember that depression is not simply a disorder of mood. There are multiple important symptoms of which mood disturbance is just one. Memory deficit has been explained by hippocampal dysfunction. However, existing antidepressant drugs are generally not very effective in improving memory irrespective of the finding that antidepressant drugs induce hippocampal neurogenesis in animals. Last but not least, many patients, after recovering from their current episode, continue to have residual symptoms such as somatic pain, sleep disturbances, and most importantly, continue to be unable to cope effectively with daily life stress. All these disturbances not responsive to the current repertoire of antidepressant drugs are urgently awaiting the emergence of new drugs with truly new mechanisms of action.

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# The Hypothalamic-Pituitary-Adrenal Axis in Depression

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**Abstract**

The hypothalamic-pituitary-adrenal (HPA) axis is the core endocrine stress system in humans. While corticotropin-releasing hormone (CRH) and vasopressin are the major secretagogue peptides of the HPA axis/stress system, glucocorticoids play a pivotal feedback role in the regulation of the HPA axis. Both the peak and the nadir in circulating cortisol concentrations are elevated in depression, but overall there is little reduction in amplitude of the circadian rhythm and neither is its timing significantly shifted. CRH has been administered following pretreatment with dexamethasone, which results in augmented adrenocorticotropic hormone and cortisol responses in depressed patients compared to normal controls. Studies have also shown that women who were sexually or physically abused in childhood exhibit a markedly enhanced activation of the HPA axis. To date, most large-scale studies of compounds targeting the HPA axis have produced disappointing results. Whether or not the HPA axis is an appropriate target for novel antidepressants has yet to be convincingly demonstrated.


While acute stress activates the sympathoadrenal medullary system resulting in the components of the 'fight or flight' response with a release of catecholamines, chronic stress results in alterations of the hypothalamic-pituitary-adrenal (HPA) axis with an increase in the release of cortisol. The HPA axis is the core endocrine stress system in humans [1]. It not only regulates the body's peripheral functions relating to metabolism and immunity but has also profound effects on the brain by regulating neuronal survival and neurogenesis in structures such as the hippocampus where it plays a role in memory [2].

The main regulatory centre of the axis is the hypothalamic paraventricular nucleus (PVN). Under basal conditions, corticotropin-releasing hormone (CRH) produced within the medial parvicellular division of this nucleus is the dominant regulator of the axis [3], mediating the endocrine response to stress. In situations of chronic stress, many parvicellular neurones co-express vasopressin (AVP), which plays an important

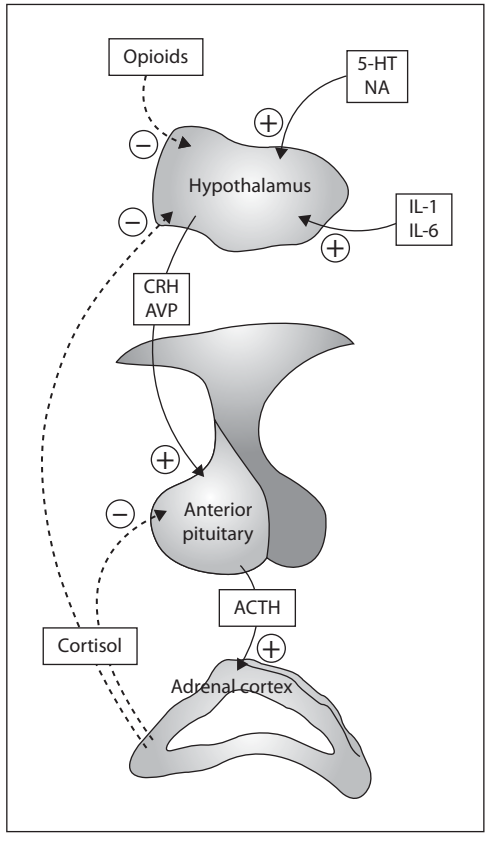


**Fig. 1.** The regulation of the HPA axis. At a hypothalamic level, classic neurotransmitters and cytokines regulate CRH and AVP release into the portal vasculature. ACTH from the corticotropes stimulates cortisol release from the adrenal cortex. A series of negative feedback loops controls the forward drive.

role in sustaining HPA axis activation through a synergistic action with CRH [4]. CRH and AVP act on the anterior pituitary corticotropes to stimulate the release of adrenocorticotropic hormone (ACTH), causing increased synthesis and release of cortisol, the main stress hormone of the axis, from the adrenal glands [5].

In response to psychological stress, CRH release is controlled by classic central neurotransmitters such as norepinephrine and 5-HT [6]. The release of CRH is under excitatory input from the amygdala and inhibitory input from the hippocampus.

In response to physical stressors, such as infection, CRH-containing neurones respond to proinflammatory cytokines such as interleukin 1, interleukin 6, and tumour necrosis factor α [7]. CRH acts through 2 different receptors, $CRH_1$ and $CRH_2$. The former mediates the release of ACTH from the anterior pituitary (fig.1).

AVP is released following a variety of stimuli including increasing plasma osmolality, hypovolaemia, hypotension and hypoglycaemia. It has powerful antidiuretic and vasoconstrictor effects. AVP is released from the magnocellular system and from the parvicellular neurones of the PVN. AVP produced by the parvicellular neurones of the PVN is secreted into the pituitary portal circulation from axon terminals projecting

to the external zone of the median eminence. The PVN serves as an important relay site. It receives projections from ascending catecholaminergic pathways including noradrenergic projections from the nucleus of the solitary tract and from the locus coeruleus. The PVN also receives input from areas of the limbic system, notably the bed nucleus of the stria terminalis, the hippocampus and amygdala. High levels of immunoreactive AVP neurones have been demonstrated in these areas and also in the pituitary intermediate lobe, granular layers of the cerebellum and dentate gyrus. Lower levels are found in the medial habenula, adenohypophysis, area postrema, pineal, subfornical and subcommissural organs.

As with other peptide hormones, AVP exerts its effects through interaction with specific plasma membrane receptors of which 3 major subtypes have been identified [8]. V1a receptors are widely distributed on blood vessels, and have also been found in the central nervous system (CNS), including the PVN. V2 receptors are predominantly located in the principal cells of the renal collecting system, although there is some evidence for central V2 receptors as well. The ACTH-releasing properties occur via the V3 (V1b) receptor subtype. In situ hybridization studies reveal that V3 receptor mRNA is expressed in the majority of pituitary corticotropes, in multiple brain regions and a number of peripheral tissues including kidney, heart, lung, breast and adrenal medulla.

AVP has ACTH-releasing properties when administered alone in humans, a response which may be dependent on the ambient endogenous CRH level. Following the combination of AVP and CRH, a much greater ACTH response is seen and both peptides are required for maximal pituitary-adrenal stimulation. The precise nature of this synergism is incompletely understood with most information deriving from animal studies. It has been demonstrated that CRH, through cAMP, increases pro-opiomelanocortin gene transcription and peptide synthesis and storage. There may also be distinct corticotrope populations in the anterior pituitary some of which require both AVP and CRH for ACTH release.

While CRH and AVP are the major secretagogue peptides of the HPA axis/stress system, glucocorticoids play a pivotal feedback role in the regulation of the HPA axis. There are 2 receptors which bind cortisol [9]. The type 1 receptor (MR), which is indistinguishable from the peripheral mineralocorticoid receptor, is distributed principally in the septohippocampal region and mediates tonic influences of cortisol or corticosterone; the type 2 or glucocorticoid receptor (GR) has a wider distribution and mediates stress-related changes in cortisol level. By binding to the GR and the MR, endogenous glucocorticoids serve as potent negative regulators of HPA axis activity [10], in particular the synthesis and release of corticotropin-releasing factor (CRF) in the PVN and pro-opiomelanocortin/ACTH in the pituitary. These receptor systems also provide negative feedback loops at a limbic, hypothalamic and pituitary level.

The sensitivity of CRH and AVP transcription to glucocorticoid feedback is markedly different. CRH mRNA and $CRH_1$ receptor mRNA levels are reduced by elevated glucocorticoids, whereas V3 receptor mRNA levels and coupling of the receptor to

phospholipase C are stimulated by glucocorticoids, effects which may contribute to the refractoriness of AVP-stimulated ACTH secretion to glucocorticoid feedback. This suggests that vasopressinergic regulation of the HPA axis is critical for sustaining corticotrope responsiveness in the presence of high circulating glucocorticoid levels during chronic stress [5].

### Corticotropin-Releasing Hormone (Corticotropin-Releasing Factor) in Depression

CRF immunoreactivity is elevated in the CSF of patients with depression [11]. ACTH responses to exogenous CRH administration are often blunted in depressed patients compared to controls [12, 13]. Cortisol responses may not be similarly reduced, suggesting increased sensitivity of the adrenal cortex in major depression. Several studies have shown that ACTH and other pro-opiomelanocortin-derived peptide responses to CRH in depressives can be augmented by pretreatment with metyrapone, which, by inhibiting cortisol synthesis, reduces cortisol negative feedback to the pituitary and hippocampus, thus implicating increased cortisol as a significant factor influencing the reduced corticotrope response [14, 15]. The CRH test has also been administered following pretreatment with dexamethasone (DEX), which results in augmented, rather than reduced, ACTH and cortisol responses in depressed patients compared to normal controls [16, 17]. The combined DEX/CRH test has also been used to predict the possibility of relapse in remitted depressed patients [18]. In delusional depression, HPA axis overactivity is more distinct than in other subtypes of depression. HPA axis monitoring by DEX/CRH before and after active medication shows that cortisol and ACTH response normalizes with effective therapy [19].

Hennings et al. [20] reported on 842 inpatients admitted to a psychiatric hospital for treatment of a major depressive episode. The degree of HPA axis dysregulation was evaluated by means of the combined DEX/CRH test, and the predictive value assessed. 80.8% of patients responded to treatment (i.e., improvement in symptom severity of at least 50%) and 57.9% reached remission (i.e., near absence of residual depressive symptoms) at discharge after a mean treatment period of 11.8 weeks. Regression analysis identified early partial response (within 2 weeks) as the most important positive predictor for achieving remission. Previous ineffective treatment trials in the current episode and presence of a migration background are potent negative predictors for treatment outcome. In addition, remitters were characterized by a more pronounced normalization of an initially dysregulated HPA axis. The study demonstrates that a subgroup can be characterized by a set of demographic, clinical and neuroendocrine variables allowing to predict unfavourable outcome at an early stage of treatment.

Overall, the DEX/CRH test is probably the most reliable neuroendocrine test for assessing HPA axis dysregulation in depression [21]. It has a reasonable predictive value for the risk of depressive relapse. Persistent overdrive of HPA axis system

activity after successful antidepressant treatment predicts an enhanced risk for relapse of a depressive episode.

Variation in the $CRH_1$ receptor (CRHR1) gene has been shown to interact with early-life stress to predict adult depression [22]. CRHR1 polymorphisms interact with childhood maltreatment to predict HPA axis reactivity, which has been linked to both depression and early-life stress. DEX/CRH test and CRHR1 polymorphisms showed a significant interaction with maltreatment. CRHR1 moderates the effect of childhood maltreatment on cortisol responses to the DEX/CRH test. Excessive HPA axis activation could represent a mechanism of interactions of risk genes with stress in the development of mood and anxiety disorders.

## Vasopressin in Depression

A potential role for AVP in affective illness was forwarded in 1978 by Gold and Goodwin [23]. They postulated that animal studies showing (1) that AVP deficiency produces deficits of behaviour which are reversed when the peptide is replaced and (2) that well-developed systems exist for its distribution throughout the CNS, rendered AVP a suitable candidate for involvement in complex behavioural systems. They also described the symptom complexes in affective illness that AVP is known to influence, notably memory processes, pain sensitivity, synchronization of biological rhythms and the timing and quality of REM sleep.

A role for AVP was supported not only by the above spectrum of symptoms but also by dynamic tests of HPA axis activity, and in particular, the DEX/CRH test. The enhanced response to DEX/CRH seen in depression is thought to be due to enhanced AVP drive.

There are relatively few data on plasma AVP levels in depression. An early report found no change in plasma AVP levels in depression [24]. In contrast, van Londen et al. [25] reported basal plasma levels of AVP to be elevated. In this study, 52 major depressives and 37 healthy controls were compared; AVP concentrations were found to be higher in the depressed cohort, with greater elevation in inpatient compared to outpatient depressives and in those with melancholic features. A number of studies have shown a significant positive correlation between peripheral plasma levels of AVP and hypercortisolaemia in patients with unipolar depression.

A post-mortem study of depressed subjects reported an increased number of AVP-expressing neurones in paraventricular hypothalamic neurones [26, 27]. Dinan et al. [28] examined a cohort of depressed subjects on 2 separate occasions, with CRH alone, and with the combination of CRH and DDAVP. A significant blunting of ACTH output to CRH alone was noted. Following the combination of CRH and DDAVP, the release of ACTH in depressives and healthy volunteers was indistinguishable. It was concluded that whilst the CRHR1 is downregulated in depression, a concomitant upregulation of the V3 receptor takes place. This is consistent with the animal models

of chronic stress, described above, in which a switching from CRH to AVP regulation is observed. It is interesting that in CRHR1-deficient mice, basal plasma AVP levels are significantly elevated, AVP mRNA is increased in the PVN and there is increased AVP-like immunoreactivity in the median eminence [29].

In a further study, we have provided evidence for the upregulation of the anterior pituitary V3 receptor [30]. Fourteen patients with major depression and 14 age- and sex-matched healthy comparison subjects were recruited. Desmopressin 10 μg was given intravenously and ACTH and cortisol release was monitored for 120 min. The mean ± SEM ACTH response in the depressives was 28.4 ± 4.3 ng/l and in the healthy subjects it was 18.8 ± 4.9 ng/l ($p = 0.04$). The mean ± SEM cortisol response in the depressives was 261.8 ± 46.5 nmol/l and in the healthy subjects it was 107.3 ± 26.1 nmol/l ($p < 0.01$). This suggests that patients with major depression have augmented ACTH and cortisol responses to desmopressin, indicating enhanced V3 responsivity.

Van West et al. [31] identified 5 single nucleotide polymorphisms in the V1b receptor gene. In a study of patients with major depression, they identified what they describe as a protective haplotype. In a recent study, Dempster et al. [32] also implicated the gene in early-onset depression, especially in females.

### Secretion of Adrenocorticotropin and Cortisol in Depression

Both the peak and the nadir in circulating cortisol concentrations are elevated in depression, but overall there is little reduction in amplitude of the circadian rhythm and neither is its timing significantly shifted. Linkowski et al. [33] found increased 24-hour mean plasma cortisol, shorter nocturnal quiescence of cortisol secretion, decreased relative amplitude of the 24-hour cortisol rhythm, and advance of the cortisol nadir in patients with major depression. Posener et al. [34] did not find hypercortisolism or significant advance of the cortisol nadir, but they did report reduced amplitude of the 24-hour rhythm in the non-psychotic patients compared to the controls. Comparing 40 patients with research-diagnostic-criteria-defined endogenous depression to 40 matched controls, Rubin et al. [35] reported hypercortisolism throughout the 24 h in 15 of the patients, with no significant advance of the cortisol nadir. Overall the data indicate that HPA axis hyperactivity in depression is not limited to the early evening hours, but occurs throughout both the diurnal and the nocturnal periods.

Wedekind et al. [36] have found that basal HPA axis activity remains elevated even after remission of symptoms in patients with psychotic depression supporting the concept that a dysfunctional regulation of the HPA axis system in such patients is a trait- rather than a state-related marker.

The increased plasma cortisol is related to the adrenal hyperplasia reported in major depression [37].

### Dexamethasone Suppression Test

DEX, a potent synthetic glucocorticoid, binds primarily to GRs on anterior pituitary corticotropes and, by feedback inhibition, suppresses ACTH and cortisol secretion. The degree and duration of suppression depends on a balance between the amount of DEX administered, its pharmacokinetics in a given subject, and the degree of suprapituitary drive. While low-dose and high-dose dexamethasone suppression tests (DSTs) have been used for the differential diagnosis of Cushing's disease, a low-dose DST has been used as a marker of HPA axis hyperactivity in mood disorders [38].

The most widely used low-dose protocol in mood disorders has been the administration of DEX, 1 mg orally, at 11 p.m. or midnight, followed by serum cortisol determinations at intervals over the following 24 h. In normal individuals, cortisol remains suppressed to very low levels for the full 24 h. In contrast, up to 70% of patients with major depression, particularly those with melancholic features, show cortisol non-suppression or early escape from suppression during the 24 h following DEX administration. Studies in milder forms of depression indicate low levels of non-suppression similar to that seen in many other psychiatric disorders. Of note is the fact that high degrees of non-suppression are also found in mania.

The DST has also been used to follow the course of treatment in patients who had a positive DST while depressed. With successful treatment, the DST gradually becomes normal, and such patients tend to remain in remission longer than patients who show clinical improvement but still have an abnormal DST [39].

In summary, the DST is the most widely studied test in biological psychiatry. While it initially promised a lot, it has fallen into disrepute. The major legacy of the DST is the fact that it generated a vast amount of research into HPA axis function in major depression.

### Adrenocorticotropin Stimulation Test

Exogenous $ACTH_{1-24}$ administration has been used as a direct test of adrenal cortical responsiveness in depression. Two strategies have been employed using either pharmacological or physiological doses of ACTH. In general, exaggerated ACTH release has been reported with the standard supramaximal stimulation dose of 250 μg $ACTH_{1-24}$, thus testing maximal adrenal secretory capacity. In our study [40], subjects were then given an intravenous bolus dose (250 μg) of tetracosactrin. Plasma levels of cortisol were measured at 0, +30, +60, +90, +120 and +180 min. Patients were then randomized to receive either 50 mg of sertraline or 20 mg of paroxetine (both of which are selective serotonin reuptake inhibitors) and were retested while medication free. Treatment resulted in a significant decrease in delta (the difference between the baseline values and the maximum increase following ACTH administration) cortisol values. The data support the view that successful pharmacological treatment of major

depressive disorder is associated with a reduction in ACTH-induced cortisol release in drug-free patients.

Several studies using much lower, more physiological doses of $ACTH_{1–24}$ have also been conducted and many of these studies have failed to find differences between depressives and healthy controls.

### Serotonergic Stimulation

5-HT input to the hypothalamus is an important stimulus to CRH release. Of the many 5-HT receptors, the $5\text{-}HT_{1A}$ receptor appears dominant in this regard [41]. Stimulation of these receptors in humans activates the HPA axis and induces hypothermia. Lesch et al. [41] used ipsapirone, an azapirone that acts as a partial agonist at the $5\text{-}HT_{1A}$ receptor, as a challenge in 12 patients with unipolar depression and 12 matched healthy controls. Ipsapirone (0.3 mg/kg) or placebo were given in random order. High basal cortisol levels were present in the patients, and their ACTH/cortisol and hypothermic responses to ipsapirone were attenuated compared to the controls. The impaired HPA axis response in the depressed patients may have been due to a glucocorticoid-induced subsensitivity of post-synaptic $5\text{-}HT_{1A}$ receptors or defective post-receptor signalling pathways.

Lesch et al. [42] also examined the effect of amitriptyline treatment on $5\text{-}HT_{1A}$-induced hypothermia. Patients with major depression were chronically treated with amitriptyline, and their temperature responses to ipsapirone challenge were monitored. Amitriptyline caused further blunting in $5\text{-}HT_{1A}$-mediated hypothermia, supporting the view that effective antidepressant treatment downregulates $5\text{-}HT_{1A}$ receptors.

### Early-Life Stress and the Hypothalamic-Pituitary-Adrenal Axis

It is well established that early-life trauma can contribute to adult depression and the increased activity of the HPA axis might reflect a susceptibility that can be programmed through early life events. Animal studies have demonstrated that neonatal maternal separation in rodents elicits HPA axis changes that persist into adulthood. Maternal separation results in higher levels of corticosterone. The sensitivity of the glucocorticoid feedback is decreased due to downregulation of GR and MR gene expression in the CNS, particularly the hippocampal region CA1 and the PVN [43].

Clinical studies have also shown that women who were sexually or physically abused in childhood exhibit a markedly enhanced activation of the HPA axis [44]; if currently depressed, they exhibit the largest increase in ACTH secretion and heart rate, as well as a very large increase in cortisol secretion. Childhood trauma in humans is associated with sensitization of the neuroendocrine stress response, glucocorticoid

resistance, increased central CRF activity, immune activation, and reduced hippocampal volume, closely paralleling several of the neuroendocrine features of depression.

In a recent negative study controlling for childhood adversity, Carpenter et al. [45] assessed 34 patients with major depressive disorder and 34 age- and sex-matched control subjects who had no current or lifetime DSM-IV depressive disorder. Effect of diagnosis on cortisol response to the DEX/CRH test was examined in a repeated-measures general linear model. The matched groups were equivalent with regard to childhood adversity. Cortisol response to the DEX/CRH test among subjects with current major depressive disorder was not significantly different from that seen in matched healthy controls. Independent of diagnosis, an exploratory analysis showed a trend level association between maltreatment history and diminished cortisol response; no interactive effects with depression diagnosis were detected.

### Effects of Antidepressants on the Hypothalamic-Pituitary-Adrenal Axis

Antidepressants that block 5-HT and norepinephrine uptake increase HPA axis activity following acute administration [46]. Chronic administration of antidepressants to depressed patients, normalizes HPA axis activity when there is a remission of the depressive episode. Increased concentrations of hippocampal MR and GR occur between 2 and 6 weeks following the start of antidepressant treatment, which parallels the time course of clinical improvement of depressive symptoms. This suggests that increased corticosteroids may contribute to the depressive syndrome [47].

### Cortisol Synthesis Inhibitors in the Treatment of Depression

Results from open-label and double-blind studies have indicated that cortisol synthesis inhibitors may be efficacious or of adjunctive value in patients with depression, including those refractory to standard treatments. This strategy was initially proposed by Murphy [48]. The main drugs used to date include ketoconazole, metyrapone and aminoglutethimide [49, 50]. However, these studies are characterized by small sample sizes and there is a need for larger controlled studies.

Several reviews of this subject [51] suggest that cortisol synthesis inhibitors, when used alone, have a moderate antidepressant effect, but not necessarily sufficient to produce acceptable clinical remission. It also appears that hypercortisolaemic patients, rather than normocortisolaemic patients, are more likely to respond. On the other hand, a glucocorticoid synthesis inhibitor may be a useful adjunctive therapy in depressed patients with HPA axis hyperactivity who do not fully respond to conventional antidepressant treatment.

Kling et al. [52] argue that the mechanism of action of these agents may not be solely a function of inhibition of adrenal cortisol production.

## Conclusions

In recent years, an enormous amount has been learned regarding the role of the HPA axis in situations of stress and in major depression. It was hoped that the axis might yield diagnostic markers for depression, but this has yet to happen. It was also thought that the HPA axis might be an appropriate target for novel therapies. CRF, V3 and GR antagonists have been studied without resounding success. Perhaps studies have focused on the wrong patient subgroups. The most exciting data to emerge from the field indicate that early childhood trauma can permanently dysregulate the axis and lead to depression vulnerability.

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# Dysfunctional Circadian Rhythms and Mood Disorders: Opportunities for Novel Therapeutic Approaches

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**Abstract**

Discoveries in the molecular genetics of the circadian clock and the introduction of the antidepressant agent, agomelatine, with effects on circadian rhythms, have revived interest in rhythm disturbances as an aetiologically significant factor in the pathogenesis of mood disorders. There is also stimulated interest in new therapeutic modalities. Abnormal patterns of circadian timekeeping characterise a variety of psychiatric disorders, particularly major depressive disorder and bipolar disorder. The prevalence of circadian dysfunction in psychiatric disorders suggests that the human circadian system holds important clues regarding the aetiology of illnesses. Evidence for disturbances in circadian rhythms comprises the cyclic nature of the disorders themselves and their symptoms. Furthermore, studies of rhythmic physiological processes and of hormone secretion have provided additional evidence of the desynchrony of biological rhythms. Identification of the physiological basis for specific circadian dysfunctions may be helpful for designing selective drug treatments that target and normalise timekeeping disorders. While it remains debatable whether disturbances of circadian rhythms are causal or the consequence of mood disorders, there is, nonetheless, a compelling rationale that restoration of the circadian system may have potential beneficial therapeutic outcomes. The present review examines the evidence for circadian rhythm disturbances in major depression and bipolar disorder. It further examines the extent to which an interaction with the molecular clock can be utilised as an approach to the therapeutics of these disorders.



> For our body is like a clock, if one wheel be amiss, all the rest are disordered; the whole fabric suffers: with such admirable art and harmony is a man composed.
> Robert Burton, *Anatomy of Melancholy,* 1621

Circadian rhythms, i.e., rhythms with a period of about 24 h, are displayed by a diverse range of organisms. That these rhythms are endogenously generated by self-sustaining pacemakers is demonstrated by the ability of living systems to maintain

the 24-hour cycle even in the absence of environmental cues. Thus they are not merely responses to diurnal variation. Nevertheless many circadian rhythms match their function to daily cycles of the photoperiod thereby representing a major feature of adaptation to our environment. In mammals, circadian rhythms are generated and regulated by a circadian timing system. This system consists of entrainment pathways and pacemakers that are under circadian control. The primary entrainment pathway is the retinohypothalamic tract, which terminates in the circadian pacemakers, i.e., the suprachiasmatic nuclei (SCN) of the hypothalamus, the midline thalamus, and the basal forebrain. This provides a temporal organisation to the sleep-wake cycle, many physiological and endocrine functions and to psychomotor performance. Disorders of circadian timing primarily affect entrainment and pacemaker functions. Direct studies of the mechanisms underlying the endogenous circadian pacemaker are not possible but have been inferred from observations of overt rhythms. These measurements have associated methodological problems, as observed rhythms may reflect a combination of the endogenous component of the rhythm with exogenous or masking effects [1] or may passively follow the sleep-wake cycle and thus not be considered endogenous [2].

Burton's seventeenth-century statement is consistent with the description of rhythmic disturbances in mood disorders by many of the ancient, as well as more recent investigators. Burton goes so far as to imply that asynchrony of internal rhythms is an important aetiological determinant of melancholy. The extent to which such disturbances are causal, however, is not clear. What is clear is that we are all subject to circadian rhythms and that entrained or synchronised rhythms are necessary for good health.

## Circadian Rhythms

Abnormal patterns of circadian timekeeping characterise a variety of psychiatric disorders, such as major depressive disorder, bipolar disorder, mania, and seasonal affective disorder, melancholic versus non-melancholic depression, premenstrual dysphoric disorder, schizophrenia and panic disorder [3–6]. The relationships between circadian timekeeping and affective illness have been enumerated by many hypotheses, mainly desynchronisation, dysregulation, phase advance, amplitude, and rapid eye movement (REM) generator [4]. Distinct patterns of circadian rhythm abnormalities have been shown to distinguish diagnostic groups. For example, different patterns of sleep disturbances are associated with some psychiatric illnesses [7]. Delayed melatonin onset and hypersomnia are found in winter seasonal depression, diurnal mood variations are described in melancholic depression, and premenstrual dysphoric disorder, early timing and altered amplitude of hormones are present in both bipolar disorder and major depressive disorder patients [4]. The prevalence of circadian dysfunction in psychiatric disorders suggests that the human

**Table 1.** Evidence for a dysregulation of endogenous circadian clocks in major depression

| |
|---|
| Regular recurrence of mood disorders |
| Marked diurnal variation in mood (worse in mornings) |
| Phase advances in basal body temperature |
| Abnormalities of cortisol rhythms and secretion (melancholic patients) |
| Phase advance of melatonin circadian rhythms |
| Increased sensitivity of melatonin suppression by light in bipolar disorder |
| Sleep-wake abnormalities |
| Efficacy of light therapy and sleep deprivation |
| Association of polymorphisms in *clock* genes with depression |
| Antidepressant and mood-stabilising medications affect clock rhythmicity |

circadian system holds important clues regarding the aetiology of illnesses. It is possible that disturbances in physiological and endocrine processes are secondary to circadian rhythm abnormalities. Identifying the physiological basis for specific circadian dysfunctions (i.e. changes in central neurotransmitter systems) may be helpful for designing selective drug treatments that target and normalise timekeeping disorders.

## Circadian Rhythms and Major Depression

Alterations in circadian rhythms in major depression have been documented over the past 30 years or so. Dysregulation of circadian rhythms has been implied from the phenomenology of depression. On the one hand, regularly recurring episodes of the disorder hint at some cyclic phenomenon, which may or may not be regulated by internal circadian desynchrony. Diurnal variation in mood with a marked improvement towards the evening is a common presenting feature of the disorder [8]. Additionally there are symptoms of sleep disturbance, particularly early morning insomnia, which also point to disturbances of circadian rhythms [9]. These observations have led to a proposal that internal desynchronisation of circadian rhythms may result in a depressive state [10]. One of the earliest circadian hypotheses posited an abnormal advance of the pacemaker with timing of sleep [11]. This has not been a consistent finding with both advances and delays being reported [12]. Recent studies have found a relationship between the misalignment of circadian phase and severity of depression: the more delayed, the more severe the symptoms of illness [13]. These data suggest that in vulnerable patients the misalignment of the clock and sleep timing is 'depressogenic'. Table 1 summarises factors which suggest circadian disturbances in patients with major depressive disorder.

### *Sleep Disturbances*

> …that a melancholy man cannot sleep overmuch; Somnus supra modum prodest…
> Robert Burton, *Anatomy of Melancholy,* 1621

Sleep disturbances are the most frequently observed symptoms in depression. Up to 90% of patients report subjective complaints, such as difficulty falling asleep or staying asleep and early morning wakening [14]. On the other hand, hypersomnia is less frequently reported (6–29%) [15]. Patterns of electroencephalographic recordings of sleep in depressed individuals also show marked abnormalities [9]. For example, the latency between sleep onset and the first episode of REM sleep in depressed patients has generally been shown to be shortened compared to controls [16]. This appears to be a common marker of mood disorder [7]. There is an increased duration of REM sleep, a decrease in slow-wave sleep and an increased number of eye movements during REM sleep in depressed patients [9].

### *Temperature Dysregulation*

Basal body temperature is subject to circadian regulation. In healthy subjects, there is a diurnal pattern with a temperature rise in the morning, a gradual increase during the day and a decline at night (signalling sleep onset). Duncan [4] summarised an extensive number of studies examining temperature timing during major depression. The principal findings of these studies were that depressed patients often had abnormalities of the circadian phase or amplitude and elevated basal body temperature. The majority of studies reported an elevated nocturnal temperature. Blunted circadian amplitude and an alteration in phase were also noted. The trend is for a phase advance of the temperature rhythm but the evidence is not compelling [4].

### *Hormone Abnormalities*

#### *Cortisol*

In healthy individuals, cortisol secretion is characterised by a rise in the early morning period and with awakening. Studies of 24-hour cortisol secretion patterns in drug-free depressed patients have generally shown an elevation of concentrations through the diurnal and nocturnal phases of the circadian cycle [17]. Phase advances in the cortisol rhythm have also been reported [18]. However, the relationship is complicated by age: the 24-hour cortisol rhythm is advanced in elderly depressed subjects but not young subjects. On the other hand, the nocturnal rise of cortisol is reported to be advanced relative to sleep onset in both elderly and young patients [19]. Gender may also represent a further complicating factor in

examining cortisol circadian rhythms as male depressed patients appear to be more phase advanced than females [20]. Furthermore, the methodology used for the analysis ('cosinor' vs. 'periodogram method') of cortisol profiles can often obscure differences [4].

*Melatonin*

The natural light-dark cycle regulates the timing of circadian rhythms, as well as the secretion of the pineal hormone melatonin. Light plays an important role by providing entrainment of circadian rhythms and by suppressing melatonin secretion during the day [21]. The circadian rhythm of melatonin secretion is thought to be generated by pacemakers located in the SCN of the hypothalamus [22]. It has been demonstrated that melatonin itself is capable of entraining other circadian rhythms in laboratory animals [23] and probably man. Factors modulating the levels of melatonin, therefore, may be expected to have an effect on human circadian rhythms. While the melatonin rhythm persists in constant darkness, it does not persist in constant light [24]. It is now well recognised that light dose-dependently suppresses the nocturnal secretion of melatonin [25, 26]. This mechanism probably accounts for the ability of bright artificial light to induce phase shifts in melatonin rhythms. Compared to temperature or cortisol rhythms, melatonin rhythm as a circadian phase marker appears to be less influenced by direct masking effects due to the influence of sleep, stress or locomotor activity [2]. In the affective disorders, the study of the melatonin rhythm is of particular interest as it has been postulated that melatonin participates in the regulation of body temperature and cortisol rhythm, as well as the sleep-wake cycle [21].

Studies of nocturnal melatonin rhythm in major depression have been reported to be both normal [27, 28] and phase advanced [29]. Absolute concentrations of melatonin have been reported to be lower in depressed patients compared to healthy controls [30, 31], but this effect may be due to the influence of medications, especially benzodiazepines [32].

*Thyroid Hormones*

Circadian variation in thyroid hormone concentrations is not as well studied in mood disorders as that of other hormones. In subjects devoid of mood disorders, thyroid-stimulating hormone concentrations vary over a 24-hour period with peak levels near midnight [33]. Low concentrations and decreased circadian amplitude of thyroid-stimulating hormone have been observed in some studies of depressed patients [28]. This is not a universal finding, with normal rhythms and amplitude more often than not being observed [34, 35].

*Prolactin*

Profiles of 24-hour prolactin secretion tend to show a bimodal distribution with a major nocturnal elevation after sleep onset [18]. Few studies have examined the

24-hour pattern of prolactin secretion in depression but those that have suggest a phase advance of the rhythm [36, 37] with normal concentrations of the hormone [38, 39].

## Circadian Rhythms and Bipolar Disorder

Seasonal rhythms in bipolar disorder have been noted from the time of Hippocrates:

> …in the spring mania, melancholia and epilepsy are apt to occur… many diseases regarded as summer affectations may also occur in the autumn, such as epilepsy, mania and melancholia…

While not circadian, these early observations of seasonal variation have been repeated by many modern authors [40–42]. Such observations attest to a rhythmic variation in episode occurrence and suggest that other rhythmic processes may be of significance in mood disorders in general and bipolar disorder in particular.

### *Sleep and Daily Rhythms*

The most consistent circadian abnormality in bipolar disorder is an alteration to the sleep-wake pattern, both prior to the onset of and during an episode [41]. Kraepelin [43] in his description of bipolar disorder recognised sleep disturbance as a central feature of the disorder:

> The attacks of manic-depressive insanity are invariably accompanied by all kinds of bodily changes. By far the most striking are the disorders of sleep… In mania sleep is, in the more severe states of excitement, always considerably encroached upon; sometimes there is even almost complete sleeplessness, at most interrupted for a few hours…

By definition, mania/hypomania is characterised by a need for less sleep, with some patients functioning on 2–3 h of sleep per night but with maintenance of normal daytime functioning [44, 45]. However, there is still a need for sleep in these patients as Bell [46] documented several cases of nearly no sleep which resulted in death of the patient.

Not only are there abnormalities of sleep but circadian patterns of activity are disturbed in bipolar patients. Based on actigraphic monitoring phase advances of about 2 h were described in bipolar I patients compared to healthy controls [47]. On recovery, the phase advances persisted suggesting that this may be a trait marker of the disorder. In contrast, two studies evaluated circadian variation in bipolar I patients using a morningness-eveningness questionnaire and showed that bipolar I patients were evening types, suggesting that patients were phase delayed [48, 49]. These latter results are in substantial agreement with a study of social rhythms in patients with rapid-cycling bipolar disorder [50]. The social rhythm metric, a diary-based rating, was significantly lower in patients than healthy controls. Further the timing of some morning activities was phase delayed in patients.

*Hormones*

*Cortisol*

Few studies have examined the circadian rhythms of cortisol during the phases of bipolar disorder. Most studies are case reports. Thus Sachar [51] reported on 3 manic patients 2 of whom demonstrated elevated cortisol. Similarly Akesode et al. [52] found high cortisol concentrations in 1 of 3 manic patients. In a more detailed study, blood samples were collected at 15-min intervals across a 24-hour period in 8 manic subjects and compared to 14 healthy controls [53]. While the general pattern of secretion was not different between groups, manic patients had higher cortisol concentrations at night and the nadir occurred significantly earlier (by about 90 min). Significant cortisol hypersecretion was observed in bipolar patients examined in depressed, euthymic and hypomanic phases of the illness compared to controls [54]. Using a cosinor analysis, there did not appear to be any cortisol rhythm phase shifts at any phase of the illness. Given the relatively small number of patients examined this may be a type II error.

*Prolactin and Growth Hormone*

In the same study in which they measured cortisol, Linkowski et al. [53] also determined 24-hour prolactin and growth hormone profiles in manic patients and compared them to those of controls. There were no statistically significant differences between patients and controls for either prolactin or growth hormone.

*Melatonin*

Examination of the 24-hour rhythm of melatonin in patients with bipolar disorder would prove useful in determining the nature of any phase change in such patients, but has rarely been studied. Plasma melatonin concentrations have been reported to be higher in the manic than the depressive phase of bipolar disorder, suggesting a state-dependent change in adrenergic function [55]. However, a study of melatonin concentrations in bipolar disorder patients during manic, depressed and euthymic phases showed lower concentrations than controls in all 3 phases [56]. It was suggested that decreased serum melatonin might be a trait but not a state marker in bipolar affective disorder. Medications exert significant effects on plasma melatonin concentrations and failure to account for such effects may explain differences between different studies [57].

A more extensively studied phenomenon in bipolar disorder is the melatonin response to bright white light at night. Bipolar patients exposed to 500 lx of white light demonstrated a response about twice that of healthy controls [58]. Follow-up studies showed that the supersensitivity was present across all phases of the illness [59]. Confirmation of the supersensitivity has been demonstrated in some independent studies [60, 61] but not all [31, 62]. More recently supersensitivity across the dose-response curve has been demonstrated for melatonin response to light

[63]. While not a universal finding, it has been suggested that such light sensitivity may offer a mechanism for desynchronisation of circadian rhythms in bipolar disorder. Given the pivotal role of melatonin in setting circadian rhythms it could be envisaged that supersensitivity might play a role in producing a phase delay of the rhythms.

## The Molecular Clock and Bipolar Disorder

### *Genetics of the Mammalian Clock*

The genetic mechanisms controlling the function of the mammalian clock have been described in detail by Takahashi and colleagues [64, 65]. Although the mechanisms were largely elucidated from studies in the fruit fly, *drosophilia melanogaster,* and other organisms, subsequent research has shown that the circadian genes are remarkably conserved across species [64]. Circadian clock mechanisms involve a transcription-translation feedback loop of a core set of genes [66]. For mammals, a primary negative feedback loop comprising the core genes circadian locomotor output cycles kaput *(Clock* and its paralogue neuronal PAS domain protein 2, *Npas2), Bmal1* (aryl hydrocarbon receptor nuclear translocator-like, *Arnt1*), period homologue *(Per1* and *Per2),* cryptochrome *(Cry1* and *Cry2)* controls the circadian clock. In brief, during the light phase, the basic helix-loop-helix PAS domain containing the transcription factor CLOCK (or NAPS2) interacts with BMAL1 to activate the transcription of *Per* and *Cry* genes resulting in high concentrations of the corresponding protein products PER and CRY. In turn, these proteins heterodimerise, translocate to the nucleus of the cell and interact with the CLOCK-BMAL1 complex to turn off their own transcription [67]. In the dark phase, the PER-CRY repressor complex is degraded and the CLOCK-BMAL1 heterodimer can then begin the cycle again. In its entirety, the cycle takes about 24 h to complete. A secondary negative feedback loop has been identified which involves the nuclear hormone receptor *Reverbα,* which is a target of CLOCK-BMAL1. The protein product REV-ERBα feeds back to repress the transcription of *Bmal1*. A further layer of complexity is introduced into the system with the recognition that post-translational modification and degradation of circadian clock proteins can be important determinants of circadian periodicity. Thus casein kinase 1δ (CK1δ) and casein kinase 1ε (CK1ε) are important components of the system which phosporylate PER and CRY. In addition, other genes are potentially involved in the regulation of circadian rhythms and include the timeless *(tim), Dec1*, *Dec2* and *E4bp4* the roles of which are not yet clearly defined. Figure 1 represents a brief schematic overview of the molecular mechanism involved in the mammalian circadian system.

## Circadian Genes and Bipolar Disorder

### *Clinical Studies*

While there is a strong hereditary component to bipolar disorder, as determined for example from twin studies, the inheritance of no single gene has yet been identified as causative [68]. Rather it would seem likely that bipolar disorder can be explained by small contributions from many gene abnormalities. This 'polygenic' inheritance concurs with the diversity of symptoms that make up the disorder. If circadian disturbances are a significant contributor to the illness then a search for polymorphisms among genes which control circadian rhythms is logical. In line with this general idea, certain circadian sleep disorders have been found to be associated with mutations of *clock* genes. For example, delayed sleep phase syndrome has been associated with variants of the *Per3* gene [69] and a missense variation in the *CK1ε* gene [70]. Similarly familial advanced sleep phase syndrome, an autosomal dominant circadian disorder, is associated with a serine to glycine mutation in the *CK1ε* gene [71].

In major depressive disorder, associations between illness and *clock* genes have not usually been found, but there are a limited number of studies (for a review, see Mansour et al. [72]). However, vulnerability to depression has recently been linked to a variant in the *Per2* gene in a Swedish population study [73]. The situation is similar in bipolar disorder and findings are not often replicated. No case-control differences in the frequency of the S662G allele of the CK1ε-binding region of the *Per3* gene was found between bipolar patients and controls [74]. An analysis of the –50T/C single nucleotide polymorphism of the promoter region of the *GSK-3β* gene showed no association with bipolar I disorder [75]. On the other hand, T/T homozygotes had an earlier age of onset of the illness. An association of bipolar disorder with *Bmal1 (ARNTL)* has been noted in two independent studies [76, 77]. These groups also reported an association with *Tim* [77] and *Per3* [76]. In a Sardinian sample, there was an association between the *Rev-erbα* gene, a component of the circadian clock, and bipolar disorder [78], but this was not evident in a Japanese sample of unipolar and bipolar patients [79]. No evidence was found for linkage of 52 bipolar families to 2 *Cry1* flanking microsatellites [80]. A more recent study [81] found some suggestive, novel associations between circadian genes and bipolar disorder, but the data did not support associations with polymorphisms that conferred risk with odds ratios greater than 1.5.

An association study of 5 circadian genes was conducted in a family collection of 36 trios and 79 quads (sample 1), and of 10 circadian genes in an extended family collection of 70 trios and 237 quads (sample 2) [82]. There was a nominal significant association of bipolar disorder with 3 single nucleotide polymorphisms within or near the *Clock* gene. Suggestive evidence of an association between several circadian phenotypes in bipolar patients and other single nucleotide polymorphisms did not reach gene-wide or experiment-wide significance after correction for multiple testing.

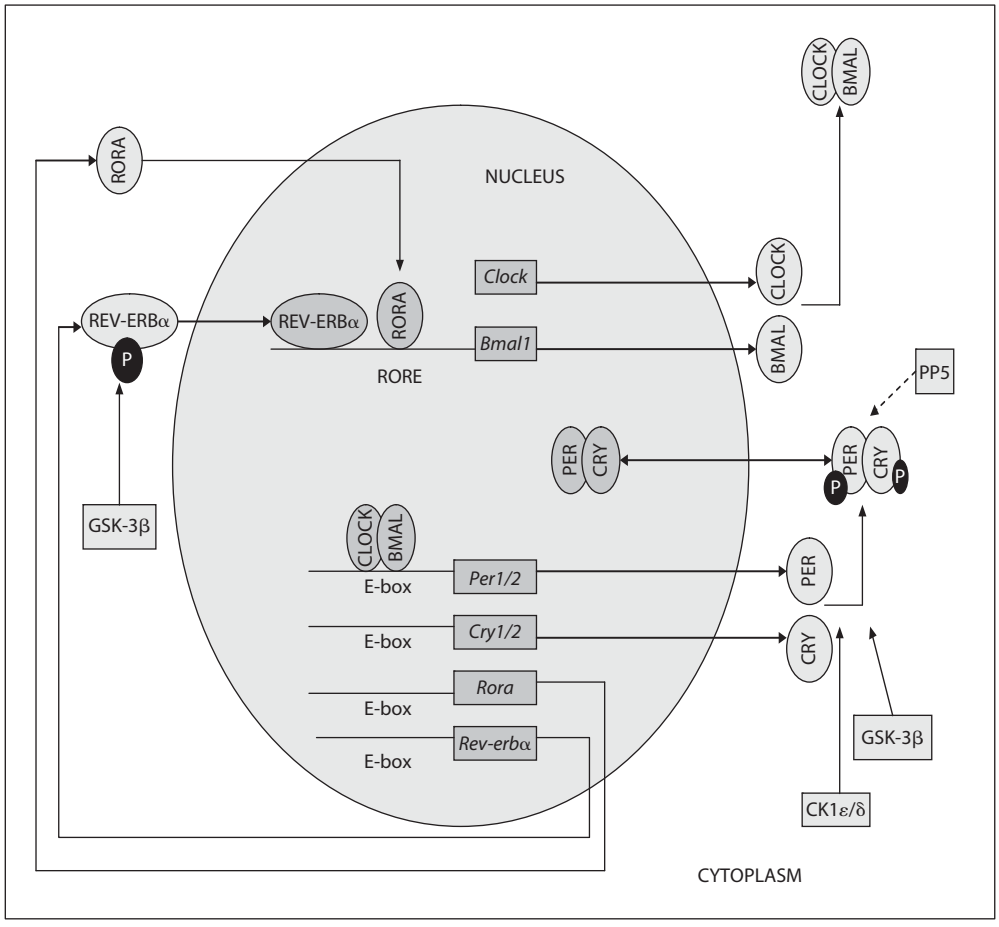


**Fig. 1.** A simplified schematic of the molecular mechanisms regulating the circadian clock. *Clock* genes are indicated in italics and their protein products in capital letters. The clock is regulated both positively and negatively by feedback loops. The proteins CLOCK and BLMAL1 dimerise and interact with E-box regions of the promoter region to activate the transcription of several genes but notably *per1, per2, cry1* and *cry2*. Transcription results in PER and CRY proteins which hetero-dimerise, translocate to the nucleus and interact with the BMAL1-CLOCK complex to inhibit their own transcription. In the cytoplasm the dimers are phosphorylated by kinases such as protein phosphatase-5 (PP5), casein kinase 1δ/ε and GSK-3β. In the SCN, *clock* genes are expressed in a rhythmic fashion, regulated by light and glutamate, and require phosphorylation of CREB. *Clock* genes can be mediated by extra-neuronal factors. There is a rhythmic expression of *clock* genes in other brain regions and peripheral tissues which do not always follow SCN rhythms. Furthermore, *clock* gene transcription factors can regulate non-*clock* genes that possess an E-box region. The core set of genes is highly conserved across animal species. Key genes are: *Clock; Npas2; Bmal1 (Arntl1); Per1* and *Per2; Cry1* and *Cry2*. Modified from: Schulz P, Steimer T: Neurobiology of circadian systems. CNS Drugs 2009;23(Suppl 2):3–13.

**Table 2.** Comparative behavioural signs in clock mutant mice and bipolar disorder

| Clock mutant mice | Mania symptomatology |
|---|---|
| Hyperactivity | Hyperactivity |
| Reduced total sleep time | Reduced total sleep time |
| Reduced helplessness/hyperhedonia | Increased euphoria |
| Reduction in anxiety | Increased risk taking |
| Increased cocaine preference | Comorbid substance use disorder |
| Disruption of circadian rhythms | Disruptions of circadian rhythms |

Adapted from Roybal et al. [83].

While the evidence is not strong for the involvement of circadian genes in mood disorder, the database is relatively sparse. There is no doubt about the association of some circadian genes and certain sleep disorders. This raises hope that further research in this area may find associations with at least some aspects of bipolar disorder. Replication of findings will be critical in accepting such associations. The findings will be useful in the characterisation of patients for aetiological and treatment outcome studies.

*Preclinical Studies*

While clinical observations have formed the bulk of the evidence for rhythmic dysfunction in bipolar disorder, recent preclinical studies have suggested a fundamental aetiological role for components of the molecular clock. Thus it has been reported that mice with a mutation in the *clock* gene demonstrate symptomatology akin to that of patients with bipolar disorder [83]. Using site-directed mutagenesis with N-ethyl-N-nitrosourea, a dominant-negative CLOCK protein mouse, which cannot activate transcription, was created. These animals demonstrate hyperactivity in novel situations across the entire light-dark cycle. Furthermore, they have an enhanced preference for psychostimulants and sucrose as well as a lower threshold for intracranial self-stimulation. Together these observations are similar to those used for the diagnosis of patients with bipolar disorder: increased goal-directed activities and excessive involvement in rewarding activities despite potential for self-harm (table 2). Using the Porsolt forced swimming test, the mice demonstrated increased swimming time, while in the learned helplessness test, there was a decrease in escape failures. Both tests suggest a diminution of 'helplessness' behaviour. At a neurochemical level, the animals demonstrate increased dopamine function in the ventral tegmental area of the brain. The behavioural effects observed in these mice could be reversed by the

clinically effective mood-stabilising agent lithium. Additionally viral-mediated gene transfer of the functional CLOCK protein into the ventral tegmental area could also rescue the behavioural abnormalities.

Central lesions of the SCN on measures of anxiety and depression in rodents have demonstrated the role of the central circadian pacemaker in these disorders. Bilateral lesions of the SCN in rats were found to have an antidepressant effect in the forced swimming test [84]. Thus animals showed decreased immobility time and greater swimming time, indicative of an antidepressant effect. A similar study found that SCN lesions did not offer protection against 'depression-like behaviour' but rather had no effect after a repeated social defeat paradigm [85]. On the other hand, the antidepressant effect of agomelatine, which is normally effective in this paradigm, was prevented. Thus the SCN plays some role in mediating the effects of this antidepressant agent and possibly in depression/depressogenic effects.

Studies in transgenic mice overexpressing certain circadian genes are beginning to elucidate the role of this system, if not in the entirety of the human syndrome, then at least as mediating certain symptoms of bipolar disorder. Thus mice overexpressing the circadian modulator *GSK-3β* (glycogen synthase kinase) demonstrate hyperactivity and decreased immobility in the Porsolt (forced swimming) test. The latter suggests less depression-like behaviour. Additionally the mice show an increased startle response reminiscent of behavioural responses in patients with bipolar disorder [86]. The inhibition of *GSK-3β* activity by lithium and valproate (an anticonvulsant with mood-stabilising properties) [87] would suggest that this finding is perhaps not surprising. Whether such mice have abnormalities of the circadian system is not clear. On the other hand, *GSK-3β* is linked to the expression of circadian genes (see above) and may well influence rhythm through this action. Nevertheless the behavioural phenotype is similar to that observed after manipulation of *clock* gene and is clearly an important preclinical avenue for further investigation.

Gene expression studies have suggested that both antidepressant medications and mood-stabilising agents can affect circadian genes. Thus the repeated administration of fluoxetine has been reported to have effects on the expression of *clock* genes in the hippocampus and striatum of mice [88]. Fluoxetine (10 mg/kg) increased *Clock, Bmal1* and *NPAS2* expression and suppressed *Per1* expression compared to controls in the hippocampus. In the caudate, fluoxetine decreased *Per1* and *Per2* expression. Given the putative critical role of the hippocampus in mood disorders [89], the changes observed in this region may be particularly relevant. Further the changes did not occur after single doses of fluoxetine, so may be relevant to the therapeutic effects of the drug.

Using a microarray study, valproate was shown to decrease the expression of *CK1δ* and *Cry2* in the amygdala, an area most often associated with anxiety and fear [90]. Mice were treated with a single dose of saline, methamphetamine (10 mg/kg), valproate (200 mg/kg), or a combination of methamphetamine and valproate. The responses induced by valproate were prevented by methamphetamine cotreatment,

which was given to induce behavioural hyperactivity (a putative model of mania). Clearly these findings demonstrate that circadian genes may be involved in the mechanism of action of the drugs. Furthermore, they show that psychoactive substances in turn can alter the expression of important circadian genes, which may also alter timing of the circadian clocks in patients taking them. An upshot of these effects is that the genes and their products may interact with the promoter regions of other genes with E-box motifs and modulate their expression. The consequences of such effects for psychiatric disorders are not clear, at present. However, further studies may potentially reveal novel therapeutic targets and lead to a better understanding of circadian gene function in bipolar disorder.

## Therapeutic Options Based on Circadian Manipulation

While it remains debatable whether disturbances of circadian rhythms are causal or the consequence of mood disorders, there is, nonetheless, a compelling rationale that restoration of the circadian system may have potential beneficial therapeutic outcomes. Both non-pharmacological and pharmacological strategies have been employed, with varying degrees of success, in unipolar depressed patients as well as in both poles of bipolar disorder.

### *Manipulation of the Circadian Cycle*

Total sleep deprivation has long been recognised as a rapid and effective antidepressant, although its effects, in the absence of other manoeuvres, are relatively short lived [91–93]. On recovery sleep, the majority of patients relapse into depression. Modifications of the technique (so-called 'wake therapy') have been developed subsequently to include partial sleep deprivation in the second half of the night, which appear to be as effective as total sleep deprivation [94]. This 'phase advance' treatment does not rely on the lack of sleep per se but attempts to advance the circadian phase by a few hours until response is achieved. Clearly such sleep manipulation techniques are intrinsically 'chronobiological' and attempt to manipulate circadian rhythms. From a mechanistic point of view, the efficacy of sleep deprivation is proposed to be due to an increase in process S (the homeostatic process in the two-process model of sleep) during waking [10].

Bright light therapy has found most application for the treatment of seasonal affective disorder, where it is clearly as effective as any other form of treatment [95]. On the other hand, the treatment is probably less effective for non-seasonal depressions and as an adjunctive therapy to antidepressants [95]. Light has been shown to be capable of shifting the circadian rhythm of melatonin; either advancing or delaying the rhythm depending on the time of day that it is administered. Therapeutic efficacy

in seasonal disorder has been postulated to arise from its phase shifting ability [96]. The relative lack of success in other forms of depression may require a more targeted use in patients with defined circadian abnormalities and a carefully considered application according to the phase position of individual patients. At the other end of the spectrum, 'dark therapy', i.e., extending the dark phase, has been shown in case studies to be effective in rapid-cycling bipolar patients [97, 98]. The mechanism of such an effect may be to induce an onset of melatonin release, which acts as a signal for the onset of sleep. Indeed melatonin has been shown to have chronobiotic properties in animals and men [99]. Despite this ability to shift circadian rhythms, its effect in depression has been to improve sleep but not to affect the core symptoms of the depressive illness [100].

*Interference with Clock Function*

The molecular clock itself would also seem to offer possibilities for therapeutic approaches in depression. As outlined above, the clock is a complex interaction of repressed and de-repressed genes as well as their protein products. This would seem to offer little possibility at present for drug design [101]. However, targeting the phosphorylation kinases would certainly be feasible but would it lead to therapeutic outcomes? As noted above, both lithium and valproate (and probably other anticonvulsants with mood-stabilising properties) inhibit GSK-3β activity [87]. Clearly development of agents with selective effects at GSK-3β may offer an avenue for new treatments of bipolar disorder. Valproate is also known to modulate *CK1δ* expression [90]. Inhibition of casein kinase might be expected to shorten the period length with the magnitude of the effect dependent on the duration of any such drug [101]. This has been demonstrated with the non-selective CK1δ/ε inhibitor 4-[3-cyclohexyl-5-(4-fluoro-phenyl)-3H-imidazol-4-yl]-pyrimidin-2-ylamine (PF-670462) which caused a phase delay in animal models of circadian rhythm [102]. On the other hand, a potent inhibitor of CK1ε, PF-4800567, with more than 20-fold selectivity over CK1δ had only a minimal effect on the circadian clock. The data indicate that CK1δ is the predominant mediator of circadian timing relative to CK1ε and therefore may be a preferred target of development for treatment of circadian rhythm disorders. The usefulness of such agents, even in animal models of disorders like major depression or bipolar disorders, is to be evaluated.

It is important to note, as Spouse [101] has pointed out, that CK1 δ/ε and GSK-3β are ubiquitous kinases and may be involved in the phosphorylation of numerous proteins both within and without the central nervous system. Some of these may activate oncogenes. Any putative therapeutic agents based on this approach will require careful evaluation during developmental phases, particularly as they are likely to be used long term.

Many existing antidepressants interfere with circadian rhythms of various hormones or the absolute concentrations of hormones as well as affecting the sleep-wake cycle [4, 103]. For example, fluoxetine has been shown to modulate circadian activity by phase advances of neuron firing within the SCN [104]. Furthermore, increases in the expression of *Clock* and *Bmal1* genes in the hippocampus have also been reported after chronic, but not acute, fluoxetine treatment [88]. Similarly lithium is well recognised to lengthen the circadian period in phylogenetically diverse organisms and men [105, 106]. This effect is probably through the action of lithium as an inhibitor of GSK-3β [107]. Thus existing pharmacological agents act to modify circadian rhythms through a diversity of mechanisms.

The recently introduced antidepressant agomelatine offers a new therapeutic strategy for the treatment of major depression. The compound combines potent agonist activity at melatonin receptors (MT1 and MT2 receptors) with antagonist actions at 5HT2C receptors [108, 109]. The compound is able to resynchronise circadian rhythms in animals [110]. The combination of alteration of circadian activity with increased dopamine and noradrenaline release in the prefrontal cortex [109] probably accounts for the clinical antidepressant activity [111]. Although circadian rhythms may normalise on recovery from depression, most existing antidepressants probably do not act as direct circadian modifiers. Agomelatine thus represents a unique therapeutic approach as it acts directly on the circadian system (through melatonin receptors) as well as targeting the more traditional monoamines. Whether this approach represents a significant advance in the treatment of depression will be determined by the development of other agents which mimic this mechanism of action. Clinical data to the present would suggest that, while agomelatine is an effective antidepressant, response and remission rates do not generally exceed those of the extant medications [111, 112].

## Conclusions

Discoveries in the molecular genetics of the circadian clock and the introduction of an antidepressant agent, agomelatine, with effects on circadian rhythms have revived interest in rhythm disturbances as an aetiologically significant factor in the pathogenesis of mood disorders. There is also stimulated interest in new therapeutic modalities. Like many of the existing hypotheses concerning the importance of biological factors in affective illnesses, such as the monoamine hypothesis, the question of cause and effect with respect to circadian rhythms has not been answered. Nevertheless provocative evidence is afforded by the 'social zeitgeber' theory of mood disorders and its successful application to treatment, particularly in bipolar disorder [113]. A key factor in the instability of rhythms would appear to be sleep disturbances, along with disruptions to other social rhythms which entrain the clock [114]. These observations

have opened the way for non-pharmacological approaches to treatment based on the restoration of circadian rhythms. Establishment of regular sleep and social patterns leads to the re-entrainment of circadian rhythms and management of bipolar disorder [115]. Furthermore, chronic insomnia is a risk factor for the development of depression as well as an antecedent of relapse in bipolar disorder [116, 117].

At present, pharmacological treatments either targeting components of the molecular clock or acting as entrainment signals are few. It would seem unlikely in the near future that direct gene effects would be feasible given the complexity of the molecular clock. On the other hand, some therapeutic agents in common clinical use (lithium, valproate, fluoxetine) have been shown to affect both circadian gene expression as well as kinases responsible for the phosphorylation of key circadian proteins. Perhaps an examination of the circadian effects of existing medications would disclose potentially new actions of these agents. However, it would seem unlikely that the disclosure of such information would lead to significant clinical gains. Based on the efficacy of the known 'chronobiotic' antidepressant, agomelatine, the long sought-after therapeutic goals, of fast onset of action and efficacy in a greater number of patients, have not been achieved. There does appear to be some advantages in terms of sleep, probably as a result of its circadian entrainment property. Further development of this aspect of treatment should prove to be useful in the clinic, particularly where an established circadian disturbance in a patient can be specifically targeted. In this respect, genetic studies of *clock* genes may ultimately identify patients with a high risk of circadian disturbance. This sits well with the circadian rhythm disruption hypothesis of mood disorders, which postulates that genetic variation of the circadian pacemaker underlies the biological and social rhythm disruptions which trigger mood episodes in patients with unipolar and bipolar disorders. Thus putative dysfunctions in circadian rhythms as an underlying basis for mood disorders continue to stimulate both fundamental research in the aetiology of such illnesses and the search for new therapeutic modalities.

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# The Concept of Depression as a Dysfunction of the Immune System

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**Abstract**

Chronic stress, by initiating changes in the hypothalamic-pituitary-adrenal axis and the immune system, acts as a trigger for anxiety and depression. Both experimental and clinical evidence shows that a rise in the concentrations of pro-inflammatory cytokines and glucocorticoids, as occurs in chronically stressful situations and in depression, contributes to the behavioural changes associated with depression. A defect in serotonergic function is associated with hypercortisolaemia and an increase in pro-inflammatory cytokines that accompany depression. Glucocorticoids and pro-inflammatory cytokines enhance the conversion of tryptophan to kynurenine. In addition to the resulting decrease in the synthesis of brain serotonin, this leads to the formation of neurotoxins such as the glutamate agonist quinolinic acid and contributes to the increase in apoptosis of astrocytes, oligodendroglia and neurons. The importance of the inflammation hypothesis of depression lies in raising the possibility that psychotropic drugs that have a central anti-inflammatory action might provide a new generation of antidepressants.



Major depression is a common and sometimes fatal disorder that has been identified by the World Health Organisation as a leading cause of disability worldwide [1]. While antidepressants are undoubtedly effective treatments in about 70% of cases, a substantial proportion of patients remain partially or totally unresponsive to treatment. There is no simple explanation for treatment resistance but there is a possibility that the current antidepressants do not effectively target all of the pathological processes that are responsible for the major symptoms of depression. Thus there is an urgent need to broaden the targets upon which antidepressants are considered to act.

All currently available antidepressants have been developed on the basis of the monoamine hypothesis of depression, a hypothesis which implicates a disorder of biogenic amines in the limbic and cortical circuits as the cause of the main symptoms of depression. Antidepressants are therefore postulated to act by correcting these abnormalities. However, in recent years greater attention has been directed to

the interrelationship between the brain and peripheral organs (the 'body-mind' connection) in which changes in the endocrine and immune systems play a major role in the pathological changes that occur in depression. Thus inflammation is beginning to emerge as a major contributing factor not only to depression and other major psychiatric disorders but also to the connection with medical disorders that are frequently associated with mental illness. For example, it is now apparent that, in major depression, there is a relationship between the severity and duration of the disorder and the increased frequency of heart disease, type 2 diabetes, various autoimmune diseases, arthritis and cancer [2].

The concept of a disordered immune system playing a major role in the mental state can be traced back to antiquity. However, it is only in the past 30 years or so that clinical and experimental evidence has been obtained clearly demonstrating that aspects of both cellular and humoral immunity were dysfunctional in major depression [3–5]. In the past 20 years, attention has been directed to the role of the immunomodulators and immunotransmitters, in particular the pro- and anti-inflammatory cytokines. Thus Maes et al. [3] reported that interleukin-6 (IL-6), a major pro-inflammatory cytokine, was increased in the blood of depressed patients. It was also apparent that about 45% of patients being therapeutically treated with the pro-inflammatory cytokine interferon (IFN)-α developed major symptoms of depression that terminated when the cytokine was withdrawn [6, 7]. Recently a meta-analysis of 9 cytokines in major depression, in which 24 studies were assessed [8], concluded that only the basal levels of IL-6 and tumour necrosis factor (TNF) were significantly raised. Such clinical observations suggest that pro-inflammatory cytokines contribute to the major symptoms of depression, which now forms the basis of the inflammation, cytokine or inflammatory response hypothesis.

## Interrelationship between Cytokines and Brain Function: Relevance to Depression?

Until recently, the brain was considered to be an immunologically privileged organ that was protected from the peripheral immune system by the blood-brain barrier. It is now apparent that this view is incorrect and that the brain is directly influenced by peripherally derived cytokines, chemokines, prostanoids and glucocorticoids, as well as some immune cells that can access the brain and thereby influence those neuronal networks that appear to be malfunctioning in depression [9]. The influence of large molecules from the periphery on the brain is somewhat surprising as specific transporters for peptides such as the interleukins do not appear to be present at the blood-brain barrier. Nevertheless, there is now experimental evidence to indicate that such molecules could access the brain (a) via a leaky blood-brain barrier that occurs in major depression, (b) by activation of endothelial cells that line the cerebral vasculature and produce inflammatory mediators inside the barrier, and (c) by binding

to cytokine receptors associated with the vagus nerve and thereby signalling inflammatory changes in the brain via the nucleus tractus solitarius and hypothalamus [10, 11]. Once in the brain, the pro-inflammatory cytokines activated both neuronal and non-neuronal (e.g. the microglia, astrocytes and oligodendroglia) cells via the nuclear factor κB (NF-κB) cascade in a similar manner to that occurring in the peripheral inflammatory response [11].

There is also evidence from clinical studies that peripherally administered cytokines can enter the brain. Thus the therapeutic administration of IFN to patients with hepatitis results in an increase in the cerebrospinal fluid not only of IFN but also IL-6 and monocyte chemoattractant protein 1 [12]. In experimental studies, it has been shown that monocyte chemoattractant protein 1 activates microglia to release IL-1 and TNF [13] and as the microglia are the primary source of pro-inflammmatory cytokines in the brain, this could be an important means whereby peripheral inflammatory mediators activate the inflammatory response in the brain. In addition, the pro-inflammatory cytokines modulate the release of biogenic amine neurotransmitters [14]. Recently much attention has been paid to the activation of the tryptophan-kynurenine pathway by these cytokines whereby tryptophan is shunted from the synthesis of serotonin to that of kynurenine. This pathway will be discussed in more detail later and clearly this is an important mechanism whereby serotonergic function is decreased in depression.

In addition to its role as a neurotransmitter, serotonin is an important neuromodulator involved in lymphocyte activation, delayed hypersensitivity responses, chemotaxis and macrophage function [106]. TNF-α and IFN-γ enhance serotonin transporter activity, thereby reducing serotonergic function in the periphery, and presumably also in the brain [107]. Conversely, the anti-inflammatory cytokine, IL-4, reduces the activity of the serotonin transporter in B-lymphocyte cultures [108]. This suggests that IL-4 could counteract the reduction in serotonergic activity caused by some pro-inflammatory cytokines at the level of the serotonin transporter.

The activity of the dopaminergic system is also reduced in response to inflammation. For example, IFN reduces the synthesis of dopamine by decreasing the concentration of the cofactor tetrahydrobiopterin, thereby reducing the synthesis of dihydroxyphenylalanine, the immediate precursor of dopamine, from tyrosine [15]. As IFN increases the synthesis of nitric oxide by activating the tetrahydrobiopterin-dependent enzyme nitric oxide synthase in the microglia, it seems likely that the reduction in dopaminergic function is linked to the increase in nitric oxide. This gaseous neurotransmitter is known to activate the glutamatergic system which, when this exceeds physiological limits, enhances apoptosis and neurodegeneration [14, 15].

Cytokines, and their signalling pathways, have been shown to enhance the reuptake of monoamine neurotransmitters and thereby reduce their functionally important intersynaptic concentrations in the brain [15, 16]. For example, IL-1 and TNF have been shown to activate the serotonin transporter on neurons by stimulating the p38 mitogen-activated protein kinase pathway [17].

In addition to the modulation of neurotransmitter function, pro-inflammatory cytokines contribute to the major symptoms of depression by activating the hypothalamic-pituitary-adrenal (HPA) axis by increasing the release of corticotrophin-releasing factor, thereby contributing to hypercortisolaemia, a feature of major depression [18–20]. The mechanism whereby the cytokines induce hypercortisolaemia involves a decreased sensitivity of the glucocorticoid receptors thereby leading to a reduction in the inhibitory feedback control and, as a consequence, glucocorticoid resistance; both the brain and the peripheral receptors become insensitive to glucocorticoid activation. The precise mechanism whereby the pro-inflammatory cytokines cause glucocorticoid receptor insensitivity is uncertain but it is known that the cytokines activate the inflammatory cascade. Thus the NF-κB, p38 mitogen-activated protein kinase and the STAT5 (signal transducer and activator of transcription 5) pathway is activated and lead to a disruption of the translocation of glucocorticoid receptors from the cytoplasm to the nucleus [21], thereby decreasing the active form of the receptor. While it would appear that glucocorticoid receptor resistance is correlated with the increase in the serum concentration of the pro-inflammatory cytokines, it seems unlikely that glucocorticoid resistance is directly related to the psychopathology of depression. Thus an increase in pro-inflammatory cytokines leading to glucocorticoid resistance also occurs in non-depressed individuals without any major change in the mood state [22]. However, such observations do throw light on the fact that many aspects of cellular and humoral immunity are not suppressed in patients with major depression despite the elevation of the plasma glucocorticoid concentration that is a common feature of the disorder.

## The Effect of Inflammatory Changes on Mood

The term 'sickness behaviour' has been applied to the cognitive and mood symptoms and the neurovegetative changes that frequently accompany systemic infections in otherwise healthy people [23]. Such symptoms show a marked similarity to those occurring in major depression (e.g. depressed mood, anhedonia, changes in sleep profile, anorexia, loss of libido). A high incidence of depression is also found in patients suffering from inflammatory disorders such as arthritis, psoriasis, diabetes and malignancies.

Sickness behaviour, when induced by systemic infections, or by the administration of IFN-α for the treatment of hepatitis for example, is usually expressed in terms of relatively mild neurovegetative symptoms (fatigue, psychomotor slowing, anorexia, and disturbed sleep profile) followed later by the core symptoms of depressed mood. These results suggest that inflammation is associated with two major phases of depression that activate different cellular mechanisms. Support for this view has been provided by O'Connor et al. [24] who showed that the depressive-like behaviour in mice following an immune challenge with Bacille Calmette-Guérin vaccine is associated with an activation of indoleamine 2,3-dioxygenase (IDO) [25]. Thus it was shown that sickness behaviour

occurred shortly after the vaccine was administered whereas the depressive-like symptoms only occurred later following the activation of IDO. Such depressive-like changes could be prevented by the administration of 1-methyltryptophan, an IDO antagonist.

The question arises how such cellular changes may be reflected in brain circuitry. Harrison et al. [26] investigated the effects of a typhoid vaccine on a group of 16 healthy male volunteers in a randomised, double-blind, crossover study. Mood questionnaires were completed at baseline and 2–3 h after administration of the vaccine; fMRI recordings were made while the subjects performed an implicit emotional face perception task. The results demonstrated that the inflammation induced by the vaccine produced a robust rise in IL-6 in the serum and a reduction in the mood state. These changes were not associated with those in the body temperature, salivary cortisol, or in such physical signs of sickness behaviour as nausea. The fMRI scans showed that the IL-6-induced mood changes were correlated with an increased activity of the subgenual anterior cingulate nucleus, an area recognised as important in mood regulation, and with the coordination of emotional processing [27], and are associated with similar neural circuits to those implicated in major depression (amygdala, nucleus accumbens, medial prefrontal cortex and superior temporal sulcus). To what extent are such changes associated with an induction of IDO, that by increasing the activity of the tryptophan-kynurenine pathway contributes to a reduction in brain serotonin? From the experimental studies of O'Connor et al. [24], it would be anticipated that the initial inflammatory changes associated with the rise in IL-6 would cause symptoms of sickness behaviour whereas the depressive mood symptoms would occur days later after the induction of IDO. Clearly, it is necessary to study vaccine-induced changes further with respect to those in IDO and their connection to the cardinal symptoms of depression.

### The Role of Stress and Pro-Inflammatory Cytokines

An important conceptual shift in the possible cause of depression has occurred recently with the discovery that inflammation plays a crucial role in the psychopathology of the disorder. However, as major depression is often accompanied by inflammatory diseases (such as irritable bowel syndrome, type 2 diabetes, arthritis and autoimmune disorders) that can activate the peripheral and central inflammatory response, it is possible that such inflammatory disorders initiate the inflammatory changes that precipitate depression. Although this is plausible, it is evident that inflammation also occurs in depressed patients who are not suffering from concurrent inflammatory disorders. Thus the increased vulnerability of depressed patients to psychosocial stress is probably the key factor that leads to the activation of the immune and endocrine axes in depression. It is known, for example, that even the relatively mild acute stress of public speaking causes an increase in NF-κB activity, a key element in the induction of the inflammatory cascade [28]. In this regard, it is also known that patients with major depression frequently show an enhanced responsiveness of IL-6 and NF-κB

to an antigen challenge [29]. However, chronic stress, as experienced by caregivers or individuals subject to marital discord, but who are not suffering from depression, also induces an increase in plasma C-reactive protein, IL-6 and other inflammatory mediators [29, 30]. Whatever the cause, such changes appear to be associated with activation of the microglia thereby suggesting that the inflammatory changes are also occurring in the brain [31].

The mechanism whereby psychological stress influences both the peripheral and central inflammatory cascade is co-ordinated by the autonomic nervous system. Thus the release of noradrenaline and adrenaline following the activation of the sympathetic system results in the activation of both α- and β-adrenoceptors on immune cells thereby initiating the release of pro-inflammatory cytokines, via the activation of the NF-κB cascade, particularly on macrophages and monocytes in peripheral blood; antagonists of these adrenoceptors block the stress-induced rise in these cytokines [32]. Conversely stimulation of the parasympathetic system has the opposite effect on the stress-induced inflammatory response. Thus stimulation of the vagus nerve results in release of acetylcholine that activates the $\alpha_7$ subunit on nicotinic receptors thereby reducing the activation of NF-κB [33]. It is possible that the antidepressant-like action of vagal nerve stimulation, occasionally used to treat resistant depression, is associated with such an anti-inflammatory action.

The question arises: why should inflammation occur in depressed patients despite the frequently observed increase in glucocorticoids? The most parsimonious explanation is that glucocorticoid receptor resistance in the brain and periphery contributes to the lack of suppression of most types of immune cells with the possible exception of the natural killer cells. One possible explanation is that the stress-induced increase in the sympathetic nervous system, combined with steroid resistance, leads to the activation of the microglia in the brain, and macrophages and monocytes in the periphery, thereby leading to the inflammatory state (fig. 1).

However, despite the clinical and experimental evidence that, in depression, pro-inflammatory cytokine concentrations are raised in the presence of high glucocorticoid concentrations, there is clinical evidence that the potent synthetic glucocorticoid, dexamethasone, has an antidepressant action. Thus in a small, placebo-controlled trial, dexamethasone treatment resulted in 37% of depressed patients showing a positive response compared to only 6% of the controls; this antidepressant effect was apparent after 14 days of treatment [34]. McQuade and Young [35] subsequently suggested that drugs modulating the function of the glucocorticoid receptor may be future targets for antidepressant development.

The precise mechanism of antidepressant action of dexamethasone is complex. It is possible that the differences in the kinetic properties of the natural glucocorticoids (cortisol, hydrocortisone) and potent synthetic glucocorticoids (dexamethasone) could contribute to the unexpected pharmacological effect. Alternatively, the antidepressant effect may be due to dexamethasone inhibiting the release of IL-12 from monocytes and macrophages thereby reducing the ability of these cells to initiate

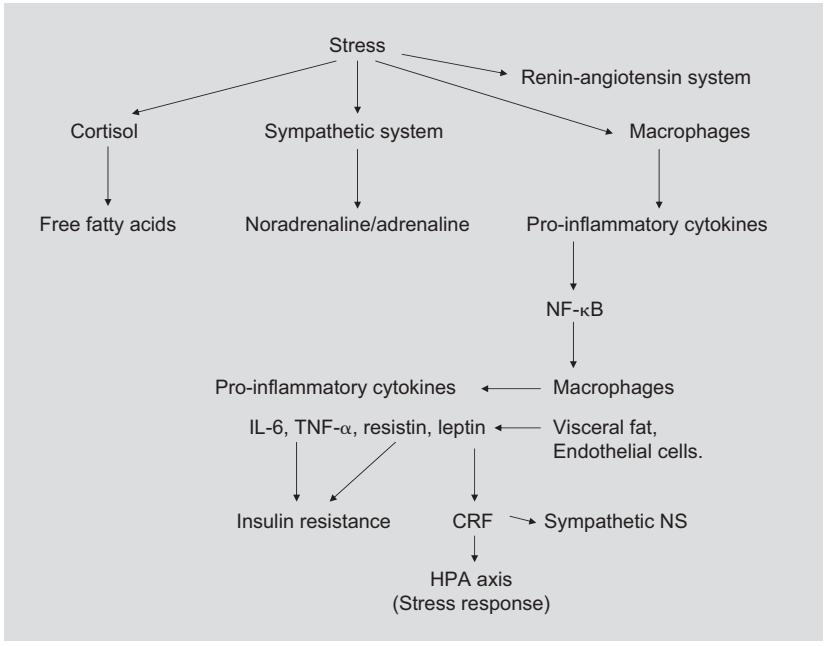


**Fig. 1.** Stress and the metabolic syndrome: the role of inflammation. Stress causes the activation of the corticotrophin-releasing factor (CRF) in the corticolimbic regions of the brain. CRF activates the HPA axis resulting in an elevation in circulating glucocorticoids. In addition, CRF activates the locus coeruleus, which results in an increase in central and peripheral sympathetic activity. Stress increases the release of pro-inflammatory cytokines from microglia in the brain and from macrophages in the blood. In addition, noradrenaline and adrenaline, from the sympathetic system, also activate the macrophages and microglia thereby contributing to the inflammatory response. Chronic hypercortisolaemia that results from the lowered stress threshold in depression, combined with the activation of the anterior pituitary by the pro-inflammatory cytokine IL-6, desensitises the glucocorticoid type 2 receptors on immune cells, and in the pituitary, hypothalamus and on neurons. This causes glucocorticoid resistance. The increase in the mobilisation of fat by the glucocorticoids contributes to the increased deposition of visceral fat. Visceral fat acts as an extra-endocrine organ and liberates pro-inflammatory cytokines together with the peptides leptin and resistin. Leptin contributes to the activation of the HPA axis while resistin, together with the increased glucocorticoids, contributes to insulin resistance.

an inflammatory (Th1-mediated) response. Under these conditions, the Th2 anti-inflammatory response would be increased thereby producing an effect similar to that occurring after antidepressant treatments [36].

### Serotonin, Stress and Depression

Of the numerous neurotransmitters that have been postulated to be dysfunctional in major depression, serotonin has been widely implicated for its contributory role in

the symptoms of the disorder (sleep disturbance, depressed mood, anorexia, loss of libido and anxiety).

Serotonin modulates the stress axis by activating the corticotrophin-releasing factor pathways in the paraventricular nucleus thereby increasing the release of adrenocorticotrophic hormone from the anterior pituitary gland [37]. There is a close relationship between the plasma cortisol concentration and the serotonergic system. Thus the stress-induced rise in cortisol is associated with an increased turnover of serotonin, a change that is linked to the stimulation of the rate-limiting enzyme, tryptophan hydroxylase, in the pathway leading to the synthesis of serotonin from tryptophan [38]. Chronic stress that results in a sustained rise in cortisol has the opposite effect and serotonin release is decreased. This is associated with the glucocorticoid activation of tryptophan 2,3-dioxygenase (TDO) in the liver whereby tryptophan is diverted from serotonin synthesis down the tryptophan-kynurenine pathway [39].

The increase in anxiety, and the impairment if adapted to chronic stress, that has been observed in both animals and men, can be explained by the changes in the functional activity of the somatodendritic serotonin 1A receptors located on the median raphe nucleus and the hippocampus [40]. Experimental studies have shown that rats raised in a stressful, overcrowded environment show an increase in anxiety which is correlated with a decrease in the functional activity of the serotonin 1A receptors. These receptors are influenced by the mineralocorticoid receptors that inhibit the serotonin 1A receptor activity under conditions of chronic stress [41]. In contrast to the serotonin 1A receptors, the serotonin 2 receptors are activated by chronic stress [42] and the serotonin 1B receptors, that act as autoreceptors and thereby control the release of the transmitter, are also activated [43]. Thus the stress-induced changes in the circulating glucocorticoids can help to explain the decrease in the functional activity of the serotonergic system in depression.

However, this evidence has mainly been provided from experimental studies. What is the evidence from clinical studies? In some depressed patients during remission, the administration of a tryptophan-deficient amino acid drink triggers an acute depressive response; this change is associated with the hypersecretion of cortisol [44]. The elevation in plasma prolactin and cortisol in depressed patients following the acute administration of the serotonin-releasing agent fenfluramine is also reduced in depressed patients [45], as is the secretion of growth hormone, in response to a tryptophan challenge [46]. These observations add additional evidence to the importance of serotonin in depression not only for its mood-modulating role but also for its direct effect on the endocrine axis.

Thus the results of experimental and clinical studies clearly demonstrate that chronic stress, as a result of hypercortisolaemia, initiates changes in the serotonergic system that appear to play a critical role in the onset of anxiety and depression.

The emphasis in this review is on the adverse effects of the pro-inflammatory cytokines that, in pathological concentrations in the brain and periphery, are likely to cause cellular injury. However, it must be remembered that at physiological concentrations, these same cytokines provide trophic support for neurons, enhance neurogenesis and contribute to normal cognitive function [47]. Such effects are severely compromised when the cytokines are present in pathological concentrations and result in changes that are important in the psychopathology of depression. Thus in major depression, the prolonged activation of the inflammatory network in the brain results in a decrease in neurotrophins, leading to reduced neuronal repair, a decrease in neurogenesis, and an increased activation of the glutamatergic pathway that contribute to neuronal apoptosis, oxidative stress and the induction of apoptosis in astrocytes and oligodendrocytes [48–51].

The consequences of inflammation on the structure and function of the hippocampus and the prefrontal cortex, key regions implicated in the pathology of depression, were considered by the European College of Neuropsychopharmacology targeted expert group in its meeting of 2007 [52]. It was concluded that a lasting reduction in neurogenesis following severe or chronic stress may reflect impaired hippocampal plasticity and contribute to the cognitive symptoms of depression but that such changes are unlikely to produce all the symptoms of the disorder. The ability of antidepressants to reverse the reduction in neurogenesis caused by stress or inflammation is at least partly implicit in their mode of action.

In addition to the pro-inflammatory cytokines, nitric oxide and the glucocorticoids, glutamate plays a crucial role in the pathological processes that are associated with depression. The pro-inflammatory cytokines, and inflammatory mediators such as nitric oxide, increase glutamate release and decrease the expression of glutamate transporters on astrocytes and oligodendroglia thereby decreasing glutamate reuptake and enhancing the intersynaptic concentration [53].

Stimulation of the extrasynaptic N-methyl-D-aspartate (NMDA) glutamate receptor not only causes excitotoxic damage to the neurons and astrocytes but also results in a decrease in synthesis of brain-derived neurotrophic factor, a key neurotrophic factor governing neuronal repair [53]. To add to the potential neurotoxic changes, IL-1 and TNF, that are generally raised in depression, trigger the release of reactive oxygen and nitrogen species from activated microglia and astrocytes; these are toxic to both neurons and oligodendroglia [53, 54]. The net result of these changes is a loss of astrocytes and oligodendroglia, and neuronal apoptosis particularly in the subgenual prefrontal cortex, the amygdala and the hippocampus, brain regions that are thought to be crucially involved in the genesis of the symptoms of depression [55].

The question now arises regarding the possible link between the neurotoxic effects of the pro-inflammatory cytokines, excess glutamate and the tryptophan-kynurenine

pathway which, in depression, produces neurotoxic end products that contribute to neurodegeneration in depression.

Tryptophan is metabolised through two main pathways, one of which leads to the synthesis of serotonin and the other to kynurenine and kynurenic acid. In the latter pathway, tryptophan is metabolised by IDO, an enzyme that is quite widely distributed in peripheral tissues and the brain, and by TDO, that is primarily located in the liver [56]. IDO is activated by pro-inflammatory cytokines, while TDO is activated by glucocorticoids. As both the cytokines and cortisol are raised in major depression, it is not surprising to find that the tryptophan-kynurenine pathway is increased [56, 57]. Anti-inflammatory cytokines reduce the activity of this pathway [58].

There are two main pathways that lead to the metabolism of tryptophan following the formation of kynurenine. Kynurenine hydroxylase metabolises kynurenine first to 3-hydroxykynurenine and then to 3-hydroxyanthranilic acid and quinolinic acid. This pathway is increased in depression and dementia [56, 57]. In glia and neurons, 3-hydroxykynurenine increases the formation of reactive oxygen species while quinolinic acid activates NMDA glutamate receptors and thereby enhances apoptosis. By contrast, kynurenine can be metabolised by kynurenine aminotransferase to the neuroprotective end product, kynurenic acid, an antagonist of NMDA receptors [58].

In the brain, the metabolism of tryptophan by IDO occurs both in the microglia and astrocytes [59]. The microglia synthesise both 3-hydroxyanthranilic acid and quinolinic acid, while the astrocytes produce mainly kynurenic acid. Astrocytes also metabolise quinolinic acid and therefore under physiological conditions can reduce the impact of the neurotoxins [60]. In chronic depression, however, the activated microglia produce an excess of the neurotoxin that cannot be adequately metabolised by the astrocytes. Furthermore, quinolinic acid can cause apoptosis of the astrocytes. This results in a reduction in the metabolic and physical buffer to the neurons that is usually provided by the astrocytes and thereby further exposes the neurons to the neurodegenerative actions of quinolinic acid [61] (fig. 2).

The clinical evidence supporting the hypothesis that the tryptophan-kynurenine pathway is activated comes from two major studies. Wichers et al. [62] showed that the concentration of kynurenic acid was reduced in patients with hepatitis being treated with IFN, while Myint and colleagues [63] reported evidence that components of the neurodegenerative pathway were increased in the blood of depressed patients before antidepressant treatment. Effective treatment for 8 weeks only partially reversed these changes in patients being treated for their first major episode of depression but had no effect in those patients who had suffered several episodes. This suggests that more permanent changes may occur in the brain of those with a chronic depression.

The structural changes observed in the brain of patients with chronic depression lends support to the neurodegenerative hypothesis of depression [64]. It is known that there is a shrinkage of the hippocampus in patients with major depression [65] and a decrease in the number of astrocytes and a neuronal loss in the prefrontal cortex [66] and in the striatum. Such changes could be the consequence of chronic low-grade

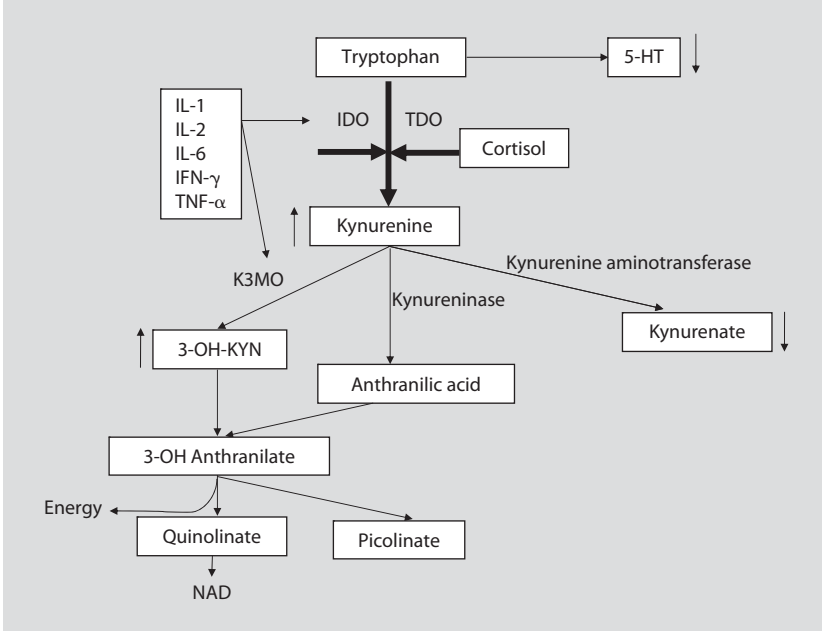


**Fig. 2.** Kynurenine pathway – beyond IDO. In depression, tryptophan is preferentially metabolised through the kynurenine pathway. The two key enzymes that convert tryptophan to kynurenine are IDO and TDO. IDO, that is quite widely distributed in the peripheral tissues and the brain, is induced by pro-inflammatory cytokines, while TDO, which is confined to the liver, is induced by glucocorticoids. In depression, kynurenine is further metabolised to 3-hydroxykynurenine by kynurenine 3-monooxygenase (K3MO), an enzyme that is also induced by pro-inflammatory cytokines. The most important end product of this inflammatory pathway is the neurotoxin quinolinic acid which activates NMDA glutamate receptor on neurons leading to neuronal apoptosis. Activated microglia synthesise quinolinic acid in the brain while astrocytes can metabolise the toxin to nicotinamide adenine dinucleotide (NAD) when present in low concentrations. High concentrations of quinolinic acid, thought to occur in chronic depression, cause apoptosis of astrocytes thereby increasing the vulnerability of the neurons to neurotoxic damage [50, 57].

inflammation in which the pro-inflammatory cytokines, nitric oxide, prostaglandin $E_2$(PGE2) and other inflammatory mediators play key roles; the cytokines are known to induce the cyclooxygenase (COX) and nitric oxide synthase pathways in the brain and thereby increase the inflammatory insult [67]. The inhibition of neurotrophin synthesis in the brain by glucocorticoids [68] and the neurotoxic action of quinolinic acid add further to the impact of the inflammatory changes.

### Antidepressants and Immune Regulation

Over the past decade, several investigators have reported that antidepressants attenuate inflammatory changes in depressed patients following effective response

to treatment [69–71]. More recently, it has been shown that effective treatment of a group of mainly hospitalised depressed patients with moderate to severe depression resulted in a reduction in the Th1 inflammatory cytokine IFN-γ and an increase in the Th2 anti-inflammatory cytokine IL-10; these changes were associated with an increase in the Th3 cytokine transforming growth factor β, a cytokine now known to play a key role in modulating the balance between the Th1 and Th2 arms of cellular immunity [72]. The precise mechanisms whereby the antidepressants produce their anti-inflammatory effects remain a matter of speculation. However, it is known that, in vitro, antidepressants from different classes reduce the release of pro-inflammatory cytokines from activated macrophages [73], possibly by a direct action on monoamine receptors located on the surface of immune cells [73, 74]. In addition, and as discussed above, antidepressants modulate the activity of the HPA axis and thereby reduce the impact of stress. As stress is a major trigger for the inflammatory cascade in depressed patients, it has been postulated that antidepressants reduce the impact of stress on the activation of peripheral macrophages and central microglia. At the cellular level, Taler et al. [75, 76] have demonstrated that serotonin reuptake inhibitor antidepressants reduce the activity of COX-2 that is induced by inflammation and thereby reduce the synthesis of PGE2 that is known to be increased in the cerebrospinal fluid of depressed patients [77, 78]. However, evidence for the anti-inflammatory effects of antidepressants in the brain has been limited to experimental studies in which antidepressants from different classes have been shown to attenuate the acute effects of a lipopolysaccharide challenge [79]. Recently, Myint et al. [80] have also shown that the COX-2 inhibitor celecoxib, administered chronically to the olfactory bulbectomised rat model of depression, not only attenuated the depressive-like behaviour but also reduced the pro-inflammatory, and increased the anti-inflammatory cytokines in the brain and blood.

Confirmation of the anti-inflammatory effects of antidepressants has also been demonstrated in cell cultures in which tricyclic antidepressants have been shown to decrease the concentrations of TNF-α and nitric oxide in mixed microglia and astrocyte cultures [81]. In vivo studies have also demonstrated that clomipramine, administered chronically to rats in the chronic mild stress paradigm of depression, reduced microglia activity and reversed the depressive-like behaviour of the animals [82].

The effects of antidepressants on the activity of the thymus gland have largely been ignored. While it is well known that the thymus plays an important role in the maturation of the T cells during the initial stages of growth and development of the mammal, it is often overlooked that the thymus also plays a role in cell-mediated immunity in the adult mammal [83]. Over a decade ago, Song et al. [84] showed that thymectomy resulted in a decrease in the brain dopamine and an increase in the turnover of serotonin in the hippocampus and striatum. Subsequently, it was shown that thymectomy also affected memory by enhancing the activity of IL-1 in the hippocampus [85]. Thus the thymus appears to play a vital role not only in regulating the balance between cellular and humoral aspects of the immune system but also by

affecting the functional activity of some key monoamine neurotransmitters thought to play a central role in the psychopathology of depression. Chronic antidepressant treatment of rats subject to chronic mild stress has also been found to increase the serotonin content of the thymus [86]; evidently the changes in the noradrenaline and dopamine content of the thymus are gender dependent whereas those in serotonin are not. As the frequency of depression is considerably higher in women than men, perhaps it is now time to consider the role of a dysfunctional thymus as a causal factor in the greater frequency of depression in women compared to men [86].

## Could the Inflammation Hypothesis of Depression Contribute to the Development of Novel Antidepressants?

If inflammation plays a significant role in the pathogenesis of depression, it would be anticipated that effective antidepressant treatments would attenuate the inflammatory changes. Indeed, there is now substantial evidence that antidepressants not only inhibit the release of pro-inflammatory cytokines from whole-blood cultures in vitro but also stimulate the release of anti-inflammatory cytokines such as IL-10 [87]. In addition, some antidepressants act as anti-inflammatory agents indirectly by increasing the availability of noradrenaline, a neurotransmitter with known immunosuppressive properties in the brain [88]. Thus the immunosuppressive effects of the noradrenaline reuptake inhibitors desipramine and atomoxetine have recently been shown to be an indirect consequence of the activation of adrenoceptors on the microglia by noradrenaline [89]. Clinically, different strategies for treating depression, that include electroconvulsive therapy and psychotherapy in addition to antidepressants, have been shown to attenuate the inflammatory changes that correlate with the improvement in the mood state [90, 91]. Such findings suggest that a reduction in inflammation is causally related to the treatment response.

Further evidence for the relationship between inflammation and depression is provided by the observation that depressed patients with a history of partial or a lack of response to antidepressant treatments have elevated plasma concentrations of IL-6 and acute-phase proteins that persist despite antidepressant treatment [91]. It has been suggested that patients who are resistant to conventional antidepressant treatment possess abnormal alleles of the IL-1 and TNF genes, and possibly for T-cell function [92].

If effective treatment with conventional antidepressants is associated with the attenuation of the inflammatory response, it may be argued that there is little urgency to develop new types of antidepressants that act by mechanisms other than modulating the brain monoamines. However, there is abundant clinical evidence that the available antidepressants, though largely effective in attenuating the depressed mood state and associated symptoms of anxiety, are far less effective in treating the memory and cognitive dysfunction (fatigue, psychomotor retardation) that commonly affect

middle-aged and elderly depressed patients [93]. This not only emphasises the need to develop better antidepressants but also to consider anti-inflammatory drugs as possible candidates.

There are already indications from the clinical literature that TNF antagonists, such as etanercept and infliximab, reduce the symptoms of depression in a variety of patients with autoimmune diseases (e.g. rheumatoid arthritis and psoriasis), the mood state of the patients improving before the signs of improvement of the autoimmune disorder [94, 95].

Anti-inflammatory cytokines have also been shown to block the depressive-like state in rodents that was induced by TNF or following the mitogen activation of macrophages by lipopolysaccharide. Thus IL-10, and insulin-like growth factor that has prominent anti-inflammatory activity, have been shown to attenuate the depressive-like behaviour in rodents induced by an inflammatory challenge [96].

Another novel method for targeting inflammation lies in reducing the activity of the glutamatergic system that is activated by the neurotoxic end products of the tryptophan-kynurenine pathway (e.g. quinolinic acid) and by nitric oxide whose synthesis is enhanced by pro-inflammatory cytokines. Riluzole, used in the treatment of the symptoms of amyotrophic lateral sclerosis (motor neuron disease), acts by enhancing the uptake of glutamate into astrocytes via the excitatory amino acid transporter and thereby reduces the activation of neurons by glutamate. Clinical observations also show that riluzole has antidepressant-like activity and could therefore act as a prototype for glutamate-modulating antidepressants [97].

The pro-inflammatory cytokines that activate IDO and kynurenine monooxygenase activate the synthesis of the neurodegenerative components of the tryptophan-kynurenine pathway that contribute to a reduction in the mood state [56, 57]. Thus specific inhibitors of IDO may have a role in the treatment of depression. There is experimental evidence that the IDO inhibitor, 1-methyltryptophan, attenuates the depressive-like symptoms in mice following the challenge with the Bacille Calmette-Guérin vaccine [24]. Thus a novel approach would be to develop brain-selective IDO inhibitors. However, it is now apparent that there are two IDO enzymes in the mammalian brain that are differently distributed and regulated so the precise importance of these enzymes with respect to changes in the mood state must be further determined before such antidepressants are developed.

Perhaps the most obvious step to the reduction of inflammation both centrally and peripherally is to reduce the activity of the prostanoid pathway and thereby reduce the synthesis of inflammatory prostaglandins such as PGE2. There is already some clinical evidence that a reduction in the activity of COX-2 by celecoxib has a beneficial effect in depressed patient who were only partial responders to reboxetine [98]. Even aspirin has been reported to have similar effects to celecoxib in a pilot study of depression [99]. These are important 'proof of concept' studies. However, it must be cautioned that several COX-2 inhibitors have already been withdrawn because of cardiac complications. Furthermore, some prostanoids and eicosanoids can have

opposing roles in different tissues and may even change from pro- to anti-inflammatory substances during the course of an inflammatory disease [100]. It must also be remembered that pro-inflammatory prostanoids such as PGE2 have physiological actions in the brain and periphery that are not directly connected with the inflammatory response [101]. Thus it is important to be cautious in developing anti-inflammatory drugs such as antidepressants particularly when they only target one aspect of the inflammatory cascade.

One hundred years after the discovery of aspirin it is amazing to find that anti-inflammatory drugs could be considered as antidepressants. By inhibiting COX enzymes, the synthesis of the inflammatory mediator PGE2 is reduced. However, in the brain, the two COX enzymes, 1 and 2, differ in their distribution, regulation and connections to other intracellular pathways [102]. Of the two COX enzymes, COX-2 is mainly involved in the response to inflammatory stimuli thereby underlining the importance of COX-2 inhibitors as anti-inflammatory agents that do not inhibit important physiological functions of COX-1 (e.g. by inhibiting COX-1 in the gastric mucosa). However, in the brain, COX-2 is mainly expressed in the hippocampal and cortical glutamatergic neurons where it plays an implicit role in modulating synaptic activity and plasticity, and in the neurovascular coupling during functional hyperaemia [103, 104]. Further, studies of the potential importance of COX-1 and -2 inhibitors as potential antidepressants have led Choi et al. [105] to conclude that COX-1, rather than COX-2, inhibitors are more likely to reduce neuroinflammation as COX-1 is located primarily in the microglia and therefore involved in the synthesis of PGE2 in response to microglia activation following stress and in depression. By contrast, COX-2 is located in pyramidal neurons where it responds to insults that directly challenge the integrity of neurons (e.g. ischaemia). Thus COX-2 inhibitors are unlikely to be effective in reducing the release of inflammatory cytokines from activated microglia. Clearly careful consideration needs to be given to the balance between COX-1 and -2 activity when considering such inhibitors as potential, novel antidepressants.

In conclusion, it must be emphasised that inflammation is also a protective mechanism against invading bacteria, viruses, oncogenes and an important component of the stress response. Inflammation starts as a time- and site-specific defence mechanism aimed not only at protecting the organism from pathogenic microorganisms but also at removing damaged neurons and, under physiological conditions, repairing damaged neuronal networks. It is only in situations where the inflammatory mediators occur in concentrations above the physiologically relevant range that they play a pathological role. While it is now evident that this situation applies to patients with major depression, it must be remembered that inflammation plays a vital role in protection against infection. Caution must therefore be exercised in developing immunomodulators that, if they are to become clinically effective antidepressants, will have to be administered for months, or even years, to patients in order to maintain remission from depression.

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# The Role of Polyunsaturated Fatty Acids in the Pathology and Treatment of Depression

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**Abstract**

Several epidemiologic investigations have reported that the consumption of fish is associated with a lower onset of depression. In the blood and postmortem brain of patients with major depression, decreased contents of total n–3 fatty acids and eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), and an increased ratio of n–6:n–3 were reported. In other types of depression, similar changes in omega fatty acids were also found in the blood of depressed patients. Most clinical trials showed that EPA as a monotherapy or adding EPA to other treatments could effectively improve depressive symptoms. With regard to the efficacy of fish oil (containing DHA and EPA) treatment, results are somehow inconsistent. In addition, most trials with pure DHA treatments failed. In this chapter, experimental studies demonstrating that n–3 fatty acids, especially EPA, could reduce glucocorticoid secretion, inhibit inflammatory responses, normalize serotonergic and noradrenergic neurotransmission, upregulate the expression of neurotrophins and downregulate the expression of apoptosis and reactive-oxygen-species-related genes are reviewed. Furthermore, the limitations and future research directions are raised and discussed.


n–3 fatty acids have been used to treat depression and depression-related symptoms. Clinical trials have demonstrated that the efficacy of n–3 fatty acids in the treatment of depression is similar to effective antidepressants [1]. Compared to most antidepressants, one of the most important findings in the eicosapentaenoic acid (EPA) clinical trials is few side effects. Nemets et al. [2] reported no clinically relevant side effects; Peet and Horrobin [3] reported only mild stomach disturbance with the group receiving 4 g/day of oils, but this has little relevance given that the optimal EPA amount is 1 g/day. In addition, EPA seems to have a beneficial side effect in that it lowers levels of triglycerides and helps prevent heart disease [3], which is often comorbid with major depression [2, 4]. Experimental studies have demonstrated that n–3 fatty acids may be of benefit to depression patients by reducing inflammation, modulating the hypothalamic-pituitary-adrenal (HPA) axis, and normalizing the neurotransmitter system

and neurotrophic system. This chapter is concerned with (1) the reason why n–3 fatty acids are important for brain function; (2) abnormalities in essential fatty acid contents in patients with different types of depression; (3) n–3 fatty acids in treatments of depression in clinical trials, and (4) therapeutic mechanisms revealed by experimental studies in patients and animal models of depression. Finally, the limitations of current studies of n–3 fatty acid treatments and future research directions are discussed.

## n–3 and n–6 Fatty Acids and Their Functions in the Brain and Immune System

n–3 and n–6 fatty acids are long-chain polyunsaturated fatty acids (PUFAs) that are synthesized from dietary precursors such as α-linolenic (n–3) and linoleic (n–6) fatty acids. n–3 fatty acids include EPA and docosahexaenoic acid (DHA), while the n–6 fatty acids include dihomo-γ-linolenic acid and arachidonic acid (AA). These fatty acids cannot be synthesized by the human body, and only come from dietary sources. Therefore, they are also called essential fatty acids. n–3 and n–6 fatty acids are important components of membrane phospholipids in neurons, glial and immune cells. Because the quaternary structures and the final modeling and folding of proteins often depend on the precise nature of the lipid environment, membrane components of fatty acids can influence the fluidity and structure of the membrane [5, 6]. Therefore, the functions of receptors, enzymes and peptides in the brain and in the immune system are influenced by these fatty acids. n–3 and n–6 fatty acids can also influence cell signal transduction. For example, many neurotransmitters, hormones and immune system-produced cytokines bind to specific targets and induce functional changes by activating phospholipases, which then generate a wide range of cell signaling or signal transduction pathways. In addition, fatty acids and other lipids can switch on and off many different genes. In particular, by binding to peroxisome proliferator-activated receptors, fatty acids can switch on and off whole genetic programs. It is known that peroxisomal enzymes are essential for the synthesis of plasmalogen, which is used for myelin formation [7]. Furthermore, n–3 fatty acids can reduce, while n–6 fatty acids can redistribute cholesterols in the brain [6]. Cholesterols, similar to inflammation, can reduce membrane fluidity and be metabolized into glucocorticoids (GCs), a group of stress hormones. Dysfunction of the HPA axis and oversecretion of GCs have been found in depressed patients [8, 9], which may be a cause of mood disorders [8–10]. In addition, n–6 fatty acid AA is a precursor of eicosanoids that can produce prostaglandin $E_2$ ($PGE_2$), leukotrienes, thromboxanes, and related compounds, which may activate macrophages to produce proinflammatory cytokines, and shift the response of T-helper 1 (Th1) and Th2 cells. Excessive inflammatory response has been consistently reported in depressed patients [11, 12]. In contrast to AA, high intake of long-chain n–3 fatty acids, such as EPA (in fish oils), inhibits certain immune functions, for example, antigen presentation, adhesion molecule expression, Th1 responses, and the production of eicosanoids and proinflammatory cytokines [13]. Thus, these fatty

acids play an important role in the brain and immune system, and proper intake of n–3 and n–6 fatty acids is important to maintain brain and immune health.

The mechanism by which n–3 fatty acids are beneficial to depression patients may be related to their direct effects on the membrane of brain and immune cells. Changes in the phospholipid content of neuronal membranes directly affect membrane viscosity and fluidity, which may cause abnormalities in basic physiological functions, such as neuronal function, synaptic growth and plasticity, neurotransmitter binding, release and reuptake, membrane enzyme binding, receptor density and affinity, ion channels (i.e. $Na^+$ and $Ca^{2+}$), and hormone secretion. In the central nervous system and the immune system, n–3 and n–6 fatty acids fulfill different roles [5]. n–6 fatty acid AA is a precursor of inflammatory mediators and may trigger microglial activity [14]. As mentioned above, increased inflammatory response has been reported in patients with depression or schizophrenia. Therefore, AA may contribute to inflammation in psychiatric diseases. Indeed, significant increases in the concentration of AA and proinflammatory cytokines [interleukin-6 (IL-6) and tumor necrosis factor α] and a decrease in EPA concentration were found in the blood of patients with major depression [15]. In contrast, n–3 fatty acids have been found to compete with n–6 fatty acids. Both EPA and DHA have been found to protect neurons from inflammatory and oxidative toxicities [14].

## Changes in n–3 and n–6 Fatty Acid Contents in Different Types of Depressed Patients

Several epidemiologic investigations have reported that the consumption of fish (rich in EPA and DHA) is associated with a lower onset of psychiatric diseases, including depression. Many studies have shown a negative correlation between fish consumption and rates of depression [2, 4, 16, 17]. Some epidemiological reports also supported the assumption that dietary fish intake could protect against bipolar disorder [18] and seasonal depression [19]. More important evidence comes from postmortem investigations, which found that DHA was significantly lower in the orbitofrontal cortex of the brains of people who had suffered major depressive disorder [20] and bipolar disorder [21]. In the red blood cells, depletion of EPA has been related to suicide attempts [22].

Changes in n–3 and n–6 fatty acid blood contents occur in different types of depression. In patients with major depression, lower levels of EPA, DHA, total n–3 fatty acids and higher AA:EPA and higher AA:DHA ratios were found in erythrocytes and serum, and a lower level of EPA and a higher AA:EPA ratio were positively correlated with the severity of depression [23, 24]. In pregnant women, high levels of depressive symptoms were found to correlate with low n–3 fatty acid intake from fish [25]. Compared with women consuming more than 1.5 g n–3 fatty acid from seafood per week, those consuming none were more likely to have high levels of depressive

symptoms at 32 weeks' gestation. In a case report, fatty acid contents in plasma samples from pregnant women with and without depression were analyzed. The higher levels of DHA and total n–3 fatty acids, and lower n–6:n–3 ratio were associated with low symptoms of depression [26, 27]. Furthermore, since inflammation has been reported to contribute to the etiology of depression, a study by Dinan et al. [15] showed that decreased levels of n–3 fatty acids and increased AA:EPA ratio were correlated with enhanced releases of the proinflammatory cytokine IL-6 in depressed patients [28]. The total fatty acid content in mania patients did not differ from that in controls, and manic symptoms were correlated negatively with levels of free AA and free EPA, and positively with the free AA:EPA ratio [29]. Moreover, increased levels of total n–6 fatty acids were seen in phospholipids of healthy first-degree relatives of patients with bipolar disorders and lower circulating levels of n–3 fatty acids were found in patients compared with controls [30]. More interestingly, the seasonal depression or violent suicide deaths, which usually appear in winter, were significantly correlated with the changes in the long-chain n–3 fatty acids EPA and DHA, and the n–6 fatty acid AA. Significantly lower concentrations of EPA, DHA and total n–3 fatty acids and higher concentrations of AA were found in winter compared to summer [31]. The changes in EPA and AA were significantly correlated to violent suicide deaths [31]. The results suggest that the seasonality in PUFAs may be related to the incidence of violent suicide and the expression of the serotonin transporter complex. In addition, a 10-year epidemiologic study reported that increased intake of n–6 fatty acids was associated with increased risk of severe depressed mood among men [32]. Most of these studies were conducted on cell membranes from red blood cells (RBC) or plasma. RBC membrane or blood levels of PUFA are regarded as a reflection of brain levels since these fatty acids can cross the blood-brain barrier from blood. These studies strongly suggest that decreased levels of n–3 fatty acids and increased n–6:n–3 ratio are common changes in different types of depression.

## n–3 Fatty Acids in the Treatment of Depression: Evidence from Clinical Trials

Increasing evidence suggests that certain n–3 fatty acids are an effective treatment for different types of depression. The following section presents the results from clinical trials.

### *Treating Major Depression*

Several double-blind, placebo-controlled clinical trials have tested the efficacy of the n–3 fatty acid EPA in combination with existing approved treatments. Generally, the studies indicate that EPA improves almost all of the symptoms of depression. Peet and Horrobin [3] conducted a 12-week study testing 1, 2, or 4 g/day of EPA

in 70 patients with major depression currently taking prescribed antidepressants. The patients were assessed with the Hamilton Depression Rating Scale (HDRS), the Montgomery-Asberg Depression Rating Scale (MADRS), and the Beck Depression Inventory. One gram per day of EPA, as the optimal dose, significantly improved the scores of depression, sleep, and anxiety in the fourth week. Nemets et al. [2] found similar results regarding HDRS score reduction when using ethyl EPA as a supplement to treat remittent depression. Twenty participants were assessed over a period of 4 weeks based on scores on the HDRS and compared with a placebo control group. Results from the EPA treatment group differed from the placebo group in the second week and EPA had an ongoing positive interaction with time. Participants all had HDRS scores above 18 before the treatment; at the end of the fourth week, the placebo group had a mean score of 20.0, whereas the group treated with EPA had a mean score of 11.6. Su et al. [4] also found that the improvement occurred after treatment for 4 weeks. They administered a combined dose of roughly 1 g/day of EPA with DHA to 28 patients. To reduce the possibility of placebo effects, the researchers excluded patients who had a reduction of 20% or more in HDRS scores in a preliminary placebo study. Although the treatment group showed significant reductions in HDRS scores, there was no significant rise in EPA levels in RBCs. However, there was a rise in DHA, and the researchers believe that this may be due to DHA being more a component part of cell membranes than EPA, and also that it may impact serotonin more than EPA. However, the researchers realized that all except 1 of their participants had been successfully treated with standard antidepressants. To assess whether EPA also works with patients who cannot be treated with standard antidepressants, Puri et al. [33] used 4 g/day of EPA on a patient with remittent, treatment-resistant depression. The patient's suicidal ideation disappeared at 1 month and all other symptoms declined in the subsequent 9 months. His MADRS score decreased from a baseline score of 32 to 0; social phobia disappeared; the niacin skin flush test results improved; the lateral ventricular volume in the brain was reduced, and cerebral proton magnetic resonance spectroscopy showed that EPA reduced phospholipid turnover and breakdown and augmented phospholipid biosynthesis. Even though studies demonstrated that DHA reduction commonly occurs in depressed patients, most clinical trials so far supported that EPA is an effective treatment for major depression but not DHA or fish oil (mixture of both EPA and DHA). For example, 35 depressed patients were randomly assigned to receive DHA, 2 g/day, or placebo for 6 weeks. Response was defined a priori as a ≥50% reduction in the score on the MADRS. This trial failed to show a significant effect of DHA monotherapy in subjects with major depression [34]. In a randomized double-blind placebo-controlled trial, the fish oil dose was 3 g, which also failed to significantly improve depressive scores [35]. Moreover, in a randomized controlled trial, 109 patients and 109 controls were treated for 12 weeks, using 1.5 g/day fatty acids (630 mg EPA and 830 mg DHA) or 1.5 g olive oil (as control). The blood concentration of n–3 fatty acids was significantly increased, but there were no significant difference between patients and controls in the primary outcome

as measured by the depression subscale of the Depression Anxiety and Stress Scales at 12 weeks [36]. However, in a double-blind dose-finding pilot study, DHA at doses of 1, 2 and 4 g/day was used to treat patients with major depressive disorder for 12 weeks. It was found that the 17-item HDRS score was significantly reduced in 83% of patients in the lowest dose group (1 g/day) and in 45% of patients in the 2 g/day group and no significant improvement was observed in the 4 g/day group [37]. n–3 fatty acids have also been applied to treat children with major depression. The patients took n–3 fatty acids (1 g/day with 400 mg EPA and 200 mg DHA) for 16 weeks. Six weeks after the treatment, the Childhood Depression Rating Scale, the Childhood Depression Inventory, and the Clinical Global Impression Scale (CGI) scores were significantly improved when compared to controls who took linoleic acid (an n–6 fatty acid) [38]. These evidences suggest that n–3 fatty acids, especially EPA, are an effective treatment for major depression.

### *Treating Bipolar Depression*

A major problem with standard treatment of bipolar depression is that many patients do not respond to treatments, and that treatments such as lithium carbonate have many side effects [39]. Although there have been concerns that EPA would bring about mania [40], recent experiments did not find this effect. A clinical trial by Keck et al. [41] tested EPA on 57 bipolar and 59 rapid cycling bipolar disorder patients. The researchers found no significant changes on the CGI for bipolar disorder, the Young Mania Rating Scale (YMRS), or the Inventory of Depressive Symptomatology, nor were there any significant differences on the Bleeding Time Test, despite the hypothesis that n–3 fatty acids would prolong bleeding time. In addition, another of the researchers' measurements was the number of instances the patients had 'switches' to mania, and EPA did not cause significant differences either. However, the researchers recognized that the dose of EPA of 6 g/day, based on older test amounts, may be too high, and there might also have been interference from mood stabilizers. In other studies such as that of Frangou and Lewis [42], where 30 bipolar patients were administered 1 or 2 g of EPA, the results show that the depression scores on the HRDS and the CGI were greatly improved, with little difference between 1 and 2 g. However, mania, as scored by the YMRS, did not change significantly. Subsequently, Osher et al. [39] conducted an open-label study and used 2 g/day of EPA over 6 months rating the patients every month with the 24-item HDRS. At 1 month, 7 of 10 patients already had a reduction in the 24-item HDRS score. Two patients who completed the study had an end score of 0. Although the study sample was small, it established that EPA is an effective treatment and does not induce mania.

Frangou et al. [43, 44] also examined EPA treatment of bipolar disorder at the neurobiological level. Previous research revealed that EPA could fortify cell membranes and thus help with second messenger systems, and induce brain regeneration.

In addition to HDRS ratings, researchers used magnetic resonance spectroscopy to look at levels of chemicals that are associated with neuroprotective function in the brain, such as N-acetylaspartate. Due to the small number of patients, the researchers limited the study to females and selected moderately depressed cases. Although the researchers did not find any changes in gray or white matter after 12 weeks of 2 g/day ethyl EPA treatment, the levels of N-acetylaspartate increased significantly. However, there was no improvement in HDRS scores or depressive symptoms, which may have been due to the small sample size, or because changes in N-acetylaspartate impact symptoms in a belated fashion or indirectly. Similar to the finding of DHA treatment for major depression, Marangell et al. [45] also reported that bipolar women who use DHA as monotherapy could not significantly improve disease symptoms.

Since 2006, there seemed not to have been any further clinical study on n–3 fatty acid treatments in adult bipolar patients, while several clinical trials explored the n–3 fatty acids in the treatment of pediatric patients or adolescents with bipolar depression. Eighteen children and adolescents with bipolar depression received supplements containing 360 mg/day EPA and 1,560 mg/day DHA for 6 weeks in an open-label study. Intake and fasting RBC n–3 PUFA level, mania, depression and global function were assessed before and after supplementation. RBC EPA and DHA levels were significantly higher following supplementation. Clinician ratings of mania and depression were significantly lower and global functioning significantly higher after supplementation. Parent ratings of internalizing and externalizing behaviors were also significantly lower following supplementation [46]. Another study reported that 20 outpatients (6–17 years) with a DSM-IV diagnosis of bipolar depression and YMRS score of >15 were treated over an 8-week period in an open-label trial with n–3 fatty acids 1,290–4,300 mg combined with 375 mg EPA and 55 mg DHA in each capsule. Subjects experienced a statistically significant but modest 8.9 ± 2.9 point reduction in the YMRS scores (baseline YMRS score = 28.9 ± 10.1; end point YMRS score = 19.1 ± 2.6, $p < 0.001$). RBC membrane levels of EPA and DHA increased in treated subjects. As only 35% of these subjects had a response according to the usual accepted criterion of a >50% decrease on the YMRS, n–3 fatty acid treatment was associated with a very modest improvement in manic symptoms in children with bipolar depression [47].

### *Treating Other Types of Depression*

Perinatal depression is common, and treatment remains challenging. A profound decrease in n–3 fatty acids in the mother during pregnancy is associated with the higher demand of fetal development and might precipitate the occurrence of depression. Su et al. [48] examined the efficacy of n–3 fatty acid monotherapy (2.2 g EPA and 1.2 g DHA) for the treatment of depression during pregnancy. This was an 8-week, double-blind, placebo-controlled trial. As compared to the placebo group, subjects in the n–3 fatty acid group had significantly lower HDRS scores at weeks 6 and 8. At

the study end point, subjects in the n–3 fatty acid group also had significantly lower depressive symptom ratings on the Edinburgh Postnatal Depression Scale (EPDS) and Beck Depression Inventory. However, another double-blind randomized placebo-controlled trial failed, which investigated women with major depression during the perinatal period receiving either fish oil or placebo for 6 weeks. A total of 26 subjects were recruited and changes in depression scores were recorded weekly. The investigators found no significant difference in depression scores between those receiving fish oil and those receiving the placebo. The reason could be the small sample size and recruitment difficulties as indicated by the researchers [49]. Freeman's group [50] also reported a study on n–3 fatty acids for perinatal depression in addition to supportive psychotherapy. Perinatal women with major depression were randomized to EPA and DHA, 1.9 g/day, or placebo for 8 weeks. A manualized supportive psychotherapy was provided to all subjects. Symptoms were assessed with the HDRS and EPDS biweekly. Fifty-one women had 2 data collection points that allowed for evaluation of efficacy. Participants in both groups experienced significant decreases in EPDS and HDRS scores from baseline. However, a benefit of n–3 fatty acids over placebo was not found [50]. The limitations of this study were that dietary n–3 fatty acid intake was low among participants and the benefits of supportive psychotherapy might limit the ability to detect an effect of n–3 fatty acids. Similar results have also been observed in postpartum depressed patients. Llorente et al. [51] assessed 138 healthy mothers who received either DHA (200 mg/day) or placebo for 4 months starting after delivery. They found no difference in depression scores between groups after the supplementation period. However, the dose of DHA might be too low, because decreased DHA was found in perinatal depression, which has been associated with postpartum depression, especially in women with a low intake of DHA. A clinical trial investigated whether supplementation of low doses of DHA or DHA plus AA during pregnancy and lactation could prevent depressive symptoms and sleep disturbances in this period. One hundred and nineteen women were supplemented daily with placebo, DHA (220 mg) or DHA + AA (220 mg each) from week 16 of pregnancy till 3 months postpartum. Fatty acid analyses were performed in the available plasma samples at 16 and 36 weeks of pregnancy. Depressive symptoms were measured in weeks 16 and 36 of pregnancy and 6 weeks postpartum using the EPDS and within 1 week postpartum using a blues questionnaire. The supplementation groups did not differ in mean EPDS scores or changes in EPDS scores, nor in incidence or severity of postpartum blues. RBC DHA and AA levels and DHA/AA ratio did not correlate with EPDS or blues scores. Indices of sleep quality did not differ between the groups. Thus, supplementation of 220 mg/day of DHA or DHA + AA (220 mg/day each) does not prevent peripartum depressive symptoms, in a population-based sample with low background DHA intake [52].

It is well known that psychological distress (PD) and depressive symptoms are commonly observed during menopausal transition. An enriched ethyl EPA supplementation was compared with placebo for the treatment of PD and depressive symptoms

in middle-aged women. Women with moderate to severe PD (n = 120) were randomly assigned to receive 1.05 g ethyl EPA/day plus 0.15 g ethyl DHA/day (n = 59) or placebo (n = 61) for 8 weeks. The clinical measurements of PD (Psychological General Well-Being Schedule) and depression (20-item Hopkins Symptom Checklist Depression Scale), and the 21-item HDRS were applied. At baseline, women with PD were mildly to moderately depressed, and 24% met the major depressive episode criteria of the DSM-IV. These women were separated from the subjects with depression. Eight weeks after EPA treatment, improvements in PD and depressive symptoms only appeared in women without depression when compared to matched women treated with placebo [53].

Furthermore, 29 patients with Parkinson's disease-associated depression were divided into 2 groups in a double-blind manner receiving fish oil or mineral oil capsules for 3 months. Each group was divided into 2 further groups: one taking antidepressant medication and one taking placebo. Patients supplemented with fish oil showed a significant decrease in MADRS and CGI scores. High-pressure liquid chromatography analysis of the fatty acid profile showed an increase in the n–3 fatty acid level in the erythrocyte membrane of patients taking fish oil [54]. These results reveal that Parkinson's disease patients taking fish oil, with or without antidepressants, presented an improvement in depressive symptoms indicating that n–3 fatty acids can be used with an antidepressant or as adjuvant therapy with some other medication.

Recently, a series of studies have linked 'sadness' and 'heart break' [55–57]. In patients with cardiovascular diseases, the prevalence rate of depression is higher, and depression observed following cardiovascular diseases is common and associated with increased risk of mortality [55]. Low-grade inflammation is one possible common mechanism responsible for the relationship between cardiovascular diseases and depression, such as increased levels of proinflammatory cytokines and $PGE_2$ [58]. The hyperactivity of the HPA axis could be another possible mechanism that links major depression and cardiovascular diseases [59]. Studies have shown that elevated morning plasma cortisol concentrations have been significantly correlated with moderate-to-severe coronary atherosclerosis in young and middle-aged men [60]. The third correlation between depression and cardiovascular diseases is the changes in membrane n–3 and n–6 fatty acids. In epidemiological studies, it has been observed that societies with a high consumption of n–3 fatty acids appear to have a lower prevalence of cardiovascular diseases and depression [61]. In case-control studies, lower levels of n–3 fatty acids have been found in patients with depression [62] or cardiovascular diseases [63]. As mentioned above, n–3 fatty acids are an effective treatment for depression, while many clinical studies also demonstrated that diet of EPA and/or DHA could decrease the risk of cardiovascular diseases [64]. However, Carney et al. [57] in a randomized controlled trial showed that sertraline with mixed EPA and DHA did not result in superior depression outcomes at 10 weeks, compared with sertraline and placebo. The investigators pointed out that higher doses of n–3 fatty acid or sertraline, a different ratio of EPA:DHA, longer treatment, or n–3 fatty

acid monotherapy might be considered in future studies to improve depression in patients with coronary heart disease. As reviewed previously, several clinical trials showed that EPA and DHA mixed together failed to improve depressive symptoms. In Carney's study, the dose and duration of the treatment should be right. However, since the ratio of EPA:DHA was near 1:1, the effects of DHA may dilute EPA effects on depression.

## The Pharmacological and Therapeutic Mechanism of n–3 Fatty Acids for Treating Depression

In the past decade, many experimental studies demonstrated that n–3 fatty acids exert significant effects on inflammation, apoptosis gene expressions, oxidative stress, neurotrophic functions and neurotransmitter systems.

### *Anti-Inflammation*

Increased inflammatory response and proinflammatory cytokine release or productions have been consistently reported in different types of depression. Proinflammatory cytokines, such as IL-1β, IL-6 and tumor necrosis factor (TNF)-α, can stimulate the hypothalamus to release corticotrophin-releasing factor that, via adrenocorticotropic hormone, induces GC secretion [65, 66]. Excessive secretion of GC can cause stress and depressive symptoms, and downregulate GC receptors in the hippocampus, which impairs the GC feedback system. A similar neuroendocrine abnormality also occurs in depressed patients.

Epidemiological studies have revealed that overintake of n–6 fatty acids in most developed countries may contribute to the increase in psychiatric and autoimmune diseases [67]. AA, an n–6 fatty acid, is the precursor of eicosanoids which produce $PGE_2$, leukotrienes, thromboxanes, and related compounds that activate macrophages to produce proinflammatory cytokines, and shift the response of Th1 and Th2. Th1 cells trigger proinflammatory responses, while Th2 cells suppress Th1 responses. In contrast to AA, a high intake of long-chain n–3 fatty acids, such as EPA and DHA (in fish oils), inhibits certain immune functions, for example, antigen presentation, adhesion molecule expression, Th1 responses, and the production of eicosanoids and proinflammatory cytokines [13]. DHA has been reported to reduce inflammation, such as reducing antigen-related activities, reducing productions of proinflammatory cytokines IL-1, IL-6 and TNF-α, and suppresses microglia activity in the periphery and the central nervous system [68, 69]. Thus, DHA may protect neurons by anti-inflammatory effects. With regard to the immune effects of EPA, rodent studies from Lynch's group [70] reported that EPA increases anti-inflammatory cytokine IL-4, and my team reported that EPA could reduce IL-1 but increase IL-10 and reverse anxiety-

like behavior induced by IL-1β in the rats [71, 72]. However, as mentioned above, EPA seems to play a more significant role in the antidepressant effect than does DHA or the combination of DHA and EPA. Maes et al. [73], from an immune point of view, addressed the question as to why EPA is more effective in the treatment of depression than DHA. The study examined the immunoregulatory effects of the n–3 fatty acids EPA and DHA, and the n–6 fatty acid AA on the production of interferon-γ, IL-10 and TNF-α. They found that EPA did not have any significant effects on the above cytokines. DHA significantly increased the interferon-γ:IL-10 production ratio, caused by a greater reduction in IL-10 than in interferon-γ. AA significantly decreased TNF-α production. The results show that DHA induces a Th1-like inflammatory response. Thus, using pure DHA or fish oils (contain both DHA and EPA) may not be effective enough for depression treatment, while highly concentrated and pure EPA seems to be a better choice.

*Antioxidants and Antiapoptosis*

As a consequence of inflammation, an overactivated HPA axis and increased oxidative stress can induce neuronal apoptosis [74, 75]. Due to the presence of double bonds and unsaturated long chain, n–3 fatty acids have antioxidant effects. n–3 fatty acids affect the oxidant/antioxidant status of the brain by stabilizing the membrane structure [76]. The n–3 fatty acids DHA and EPA can modulate oxidative stress and nitric oxide production, and may have a regulator role in the synthesis of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase [77]. The mechanism by which n–3 fatty acids DHA and EPA reduce oxidative stress is due to the fact that they form more hydrophobic interfaces between the phospholipid bilayers which might prevent the entry of the hydrophilic $H_2O_2$ molecule. n–3 fatty acids also appear to operate generally against endogenously produced reactive oxygen species and can protect the membrane proteins (proteins inside and outside organelles in eukaryotes) from being damaged oxidatively [78]. Several studies reported that EPA increases the antioxidant enzyme SOD and prevents $H_2O_2$ from entering the cells in the plasma membrane [79, 80]. It was also found that EPA attenuates inducible nitric oxide synthase (iNOS) and nitric oxide (NO) levels in lipopolysaccharide (LPS)-stimulated BV2 microglia [81]. Indeed, in a study of patients with first-episode psychosis, proton magnetic resonance spectroscopy showed that ethyl EPA administration bilaterally increased glutathione, an antioxidant that can protect dopaminergic neurons from oxidative and excitatory damage, in the hemispheres [82]. Moreover, the improvement in negative symptoms correlated with metabolic brain changes, particularly glutathione.

In a perinatal hypothyroid model, mixed DHA and EPA prevented increases in the level of proapoptotic basal cell lymphoma protein 2 (Bcl-2)-associated X protein in the cerebellum during thyroid hormone deficiency, and increased the levels

of antiapoptotic proteins like Bcl-2 and Bcl-extra large [83]. In stroke and ischemia models of rodents, fish oil also markedly reduced antioxidants SOD and catalase, and attenuated neuron apoptosis in the frontal cortex [84]. In addition, DHA has been reported to promote neurogenesis and inhibit apoptosis in neuronal stem cells [85]. My team has recently found that EPA alone does not change oxidant and anti-oxidant expressions, but upregulates the antiapoptosis gene Bcl-2. However, EPA largely reduces oxidant production, and reduces apoptosis gene expressions in a neurotoxin-induced model of PD [unpubl. data].

### *n–3 Fatty Acids Upregulate Neurotrophic and Normalize Neurotransmitter Functions*

Depression theories of serotonin or noradrenaline deficiency have been accepted for 50 years. We have previously reported that EPA can markedly normalize serotonergic and noradrenergic neurotransmitters and their metabolites in the limbic brain regions of a depression model, olfactory bulbectomized rats [86]. A new hypothesis of depression has postulated that deficiency or dysfunction of neurotrophins or their receptors may contribute to the etiology of depression [87, 88]. Neurotrophins play an important role in maintaining, repair and genesis of neurons, including serotonergic and noradrenergic neurons. Decreased blood or brain brain-derived neurotrophic factor (BDNF) content was reported in patients with major depression, bipolar depression or in animal models of depression [88, 89]. Effective antidepressant treatments significantly enhance neurotrophic function in the blood and limbic brain regions, while neurotrophins have been found to act as antidepressants in the treatment of depression [88, 90]. In an n–3 fatty acid-deficient model of rats, decreased brain DHA was associated with decreased hippocampal BDNF gene expression and increased relative corticosterone response to an intense stressor. In virgin females with decreased brain DHA, serotonin content and turnover in the frontal cortex were decreased compared to virgin females with normal brain DHA. In parous dams with decreased brain DHA, the density of 5-$HT_{1A}$ receptors in the hippocampus was increased, corticosterone response to an intense stressor was increased, and the latency to immobility in the forced swimming test was decreased compared to parous dams with normal DHA [91]. Furthermore, n–3 PUFA deprivation-induced decreases in the frontal cortex BDNF was demonstrated via a p38 MAPK-dependent mechanism [92]. We have previously reported that nerve growth factor was downregulated in the olfactory bulbectomized rat, a depression model, with memory impairment in Morris water maze. EPA treatment for 7 weeks significantly reversed nerve growth factor reduction and improved the memory. Moreover, EPA effects on memory was blocked by anti-nerve growth factor administration [93].

These findings strongly indicate that n–3 fatty acids may normalize neurotransmitter systems in depression by modulating neurotrophins.

## Limitations and Future Research Directions

In clinical trials, there are several limitations, which may mislead our understanding of some results and findings. First, some trials used n–3 fatty acids as monotherapy and some added n–3 fatty acids to other drug treatments. Side effects of drug treatments and the interaction between drugs and fatty acids were not considered. Second, different doses of n–3 fatty acids and treatment duration were used to treat depression. The variation is from 1 to 6 g/day, and treatment durations were from several weeks to months. It is possible that the different severity and term of depressive illness may need different doses and durations of treatments. Third, different sources of n–3 fatty acids and different ratios between n–3 fatty acids have been utilized, such as pure EPA or DHA, fish oil or n–3 fatty acids from plant seeds, which may cause variations. Fourth, different depression measurements were used. Fifth, diet information is either unclear or no diet control is used. Thus, patients' bodies contain different n–3 and n–6 fatty acids before and during n–3 fatty acid treatments. These limitations are important research avenues for the future. Furthermore, to better understand the mechanism by which n–3 fatty acids treat depression, other depression markers, such as neurotransmitters and metabolites, neurotransmitter receptors, GCs and receptors, immune cellular and cytokine functions, brain functions by MRI and other parameters should be co-investigated. Moreover, the link between depression and cardiovascular diseases should be further studied at multiple mechanism levels.

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# Overcoming Antidepressant Treatment Resistance: Focus on Glutamate

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**Abstract**

Current antidepressant treatments have a delayed onset of action and limitations in efficacy, particularly for patients who fail to respond to 2 or more antidepressant trials. Translational neuroscience offers the potential for the development of a new generation of antidepressants with novel mechanisms of action. Antidepressants with targets outside the monoamine system may offer the potential for more rapid onset of action and improved efficacy in treatment-resistant patients. There is now compelling evidence for glutamatergic dysfunction in the pathophysiology of major depression. These findings have prompted vigorous investigation of the glutamate system as a central target for antidepressant drug discovery. In particular, there is now substantial initial evidence for rapid-onset antidepressant properties of the N-methyl-D-aspartate glutamate receptor antagonist ketamine. Ongoing research aims to characterize the safety, feasibility, and efficacy of single-dose and repeated-dose intravenous ketamine, and will explore optimal delivery methods, means to minimize neuropsychiatric side effects, and relapse prevention. Strategies understanding the mechanism of therapeutic benefit of ketamine is an important research goal, with functional neuroimaging investigations implicating the anterior cingulate cortex. More broadly, neuroimaging techniques may help identify depression biomarkers associated with response, and may thereby facilitate rational drug discovery.



An important finding from the STAR*D program recently completed in the US was that patients who fail to remit to more than 2 optimally delivered consecutive antidepressant trials have low rates of remission (<20%). Further, even when remission is achieved, these patients are more likely to relapse earlier than less treatment-resistant patients [1]. These algorithmic series of studies performed in both primary care and mental health specialty settings enrolled patients with unipolar major depressive disorder (MDD) with common comorbidities, including axis III disorders, substance use, and anxiety disorders. The sobering conclusion from these studies was that standard antidepressant monotherapy and augmentation strategies in real-world practice settings infrequently result in sustained, durable remission. From a translational

neuroscience perspective, STAR*D offered a compelling mandate for the field to discover novel antidepressant targets beyond monoaminergic receptors and transporters. Indeed, multiple novel targets for mood disorders have been investigated over the past decade, including the neurokinin 1 receptor, the corticotropin-releasing factor 1 receptor, neuronal nicotinic receptors, and multiple receptor subtypes within the glutamate system [2].

In this chapter, we first briefly summarize the neuroscientific basis for the development of antidepressant medications impacting the glutamate system. We then focus on the N-methyl-D-aspartate (NMDA) glutamate receptor antagonist ketamine as a proof-of-concept glutamatergic antidepressant in patients with treatment-resistant major depression (TRD). We describe clinical research suggesting the safety, tolerability and efficacy of ketamine, as well as findings related to clinical, neuroimaging, and neurochemical predictors of treatment response. We conclude with a brief discussion of current limitations in antidepressant drug discovery and the potential role of neuroimaging biomarkers to aid in rational drug discovery [3].

## Evidence of Glutamatergic Dysfunction in Major Depressive Disorder

Converging evidence from in vivo brain imaging studies, postmortem investigations, and microarray gene expression studies suggests that glutamate [and γ-aminobutyric acid (GABA)] neurotransmitter systems play an important role in the pathophysiology of MDD [4–7]. Microarray gene expression studies have found downregulation of genes coding for glutamine synthetase and for glial excitatory amino acid transporters, whose function includes the regulation of glutamate clearance and metabolism. In postmortem studies, reduced glial cell numbers and density have been found in MDD patients, consistent with a pathogenic mechanism whereby disrupted glial glutamate clearance and cycling are associated with the illness. Finally, brain imaging studies using high-field proton magnetic resonance spectroscopy techniques have characterized steady-state abnormalities in regional concentrations of glutamate, glutamine, and GABA, as well as abnormalities in glutamate cycling, using dynamic spectroscopy ($^{13}C$) techniques.

The ubiquity and complexity of the glutamate system posed significant obstacles for drug discovery efforts for neuropsychiatric conditions, largely due to the potential for adverse neurocognitive effects and seizure induction. However, over the past decade, a number of early-stage clinical programs in neuropsychiatric disorders have been initiated, with drugs impacting multiple components of the glutamate system, including ionotropic receptors (NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), metabotropic receptors, and glycine site receptors. These investigations were motivated by the recognition that glutamate and its specific receptor subtypes subserve fundamental roles in the regulation of synaptic plasticity and impact basic human processes of mood, cognition, and reward.

*Clinical Experience: Single-Dose Studies*

Ketamine is a noncompetitive NMDA receptor antagonist used for many years as an anesthetic agent in children and adults (dose approximately 2 mg/kg). Anesthetic uses of ketamine have included premedication, sedation, and induction and maintenance of general anesthesia. The first report in patients with MDD described a rapid-onset antidepressant effect following a single intravenous subanesthetic dose of ketamine [8]. In this study, 8 patients were administered ketamine hydrochloride (0.5 mg/kg infusion over 40 min) or saline under randomized double-blind conditions. Ketamine significantly reduced the 25-item Hamilton Rating Scale for Depression (HRSD-25) scores within 4 h. Further reductions in HRSD-25 scores were noted at 24, 48, and 72 h after infusion, and persisted in some patients beyond 72 h. HRSD-25 scores were significantly reduced (mean ± SD 14 ± 10) in the ketamine arm compared to the saline arm, with 4 of 8 patients demonstrating a 50% or greater reduction in HRSD-25 scores at 72 h after infusion. Ketamine produced significant but transient increases in psychotomimetic symptoms, as reflected in the Brief Psychiatric Rating Scale (BPRS), particularly the positive symptoms subscale. Mood improvement associated with ketamine returned to baseline levels several days or a week after infusion.

These initial findings were striking and unanticipated, particularly the robustness of the treatment effect, rapidity of benefit (within 24 h), and persistence of symptom improvement. In this initial study [8], ketamine appeared to directly target core depressive symptoms, including depressed mood, suicidality, helplessness, and worthlessness, rather than inducing a nonspecific mood-elevating effect. Importantly, the mood improvement associated with ketamine was temporally distinct from any ketamine-induced neuropsychiatric effects, including psychotomimetic and dissociative effects, or feeling 'high'. Investigators measured transient positive feelings associated with ketamine using the Visual Analog Scale (VAS)-high item. VAS-high scores returned to baseline by 110 min after infusion, while improvement in core depressive symptoms persisted days after infusion. Of note, neither BPRS nor VAS-high scores correlated with decreases observed in HRSD-25 scores.

A larger replication study was subsequently conducted in the Intramural Research Program at the NIMH in patients with TRD [9]. This study demonstrated a rapid-onset antidepressant effect of ketamine in a similar randomized, placebo-controlled, double-blind crossover design. Patients receiving ketamine showed significant improvement in depressive symptoms compared to placebo within 110 min. The effect size for the drug difference was very large (d = 1.46, 95% confidence interval = 0.91–2.01) after 24 h and moderate to large (d = 0.68, 95% confidence interval, 0.13–1.23) after 1 week. Of the 17 subjects receiving ketamine, 12 (71%) met response criteria and 5 (29%) met remission criteria the day following ketamine infusion (24 h after ketamine infusion). The response was durable in that approximately 50% of patients receiving

intravenous ketamine maintained the antidepressant response at 72 h, defined by a 50% reduction in baseline HRSD-21 scores. Six subjects (35%) maintained response for at least 1 week, and 2 patients maintained response for at least 2 weeks.

Taken together, these 2 proof-of-concept inpatient studies suggested that a single dose of an NMDA antagonist could result in a rapid and somewhat sustained antidepressant response, even in patients with medication resistance. However, challenges to the implementation of this approach included the acute neuropsychiatric side effects of ketamine and uncertainties regarding the optimal strategies for continuation therapy. To address these questions, our research group conducted a pilot study aimed at optimizing the safe delivery of intravenous ketamine with lamotrigine pretreatment, and enhancing time to relapse following ketamine with the glutamate release inhibitor riluzole [10]. Based on a report in healthy volunteers [11], we hypothesized that a single oral dose of lamotrigine, an anticonvulsant and mood-stabilizing medication with anti-glutamatergic activity, would blunt the acute neuropsychiatric effects of ketamine in TRD patients. Based on preliminary data for the efficacy of riluzole in mood disorders [12–15], and potential mechanistic synergy between riluzole and ketamine, we hypothesized that riluzole would increase the time to relapse in TRD patients who demonstrated an initial antidepressant response to ketamine.

In our pilot study [10], 26 patients were randomized to a single dose of lamotrigine (300 mg) or placebo 2 h prior to receiving open-label intravenous ketamine (0.5 mg/kg over 40 min). Seventeen patients (65%) met response criteria [≥50% reduction from baseline on the Montgomery-Asberg Depression Rating Scale (MADRS)] 24 h following ketamine. Fourteen patients (54%) met response criteria 72 h following ketamine and proceeded to participate in a 4-week, randomized, double-blind, placebo-controlled, flexible-dose continuation trial of riluzole (100–200 mg/day). There was no significant difference in time to relapse between riluzole and placebo groups. Lamotrigine did not significantly impact the mild, transient psychotomimetic side effects associated with ketamine. Although the 2 primary hypotheses of the study were not supported, the response rate in our outpatient TRD sample corroborated previous reports [8, 9]. Overall, ketamine was well tolerated with relatively few acute neuropsychiatric effects, potentially contributing to the negative result for lamotrigine pretreatment. The failure of riluzole to separate from placebo for relapse prevention following ketamine could have been attributed to type II error. Larger studies testing riluzole for relapse prevention following ketamine are in progress at several centers in the US.

Given that the rationale for a continuation trial of riluzole following ketamine is based on potential mechanistic overlap or synergy between the 2 drugs, this strategy suggests several other glutamate-modulating candidate drugs for continuation of antidepressant benefit following ketamine, including memantine or lamotrigine. However, while riluzole has demonstrated preliminary efficacy in mood disorders [12–15], studies of memantine in depression have been disappointing [16]. Lamotrigine may remain a candidate for continuation following ketamine, although

the long titration period required to minimize risk of rash may limit its feasibility in a continuation trial.

*Repeated-Dose Studies*

Repeated-dose ketamine represents a potential antidepressant continuation strategy in patients who respond to an initial ketamine infusion. To begin to test this strategy, our group recently explored repeated-dose intravenous ketamine over 2 weeks in 10 patients with TRD [17]. On day 1, patients received a 40-min intravenous infusion of ketamine (0.5 mg/kg) in an inpatient setting with continuous vital sign monitoring. The primary efficacy measure was change from baseline in the MADRS score. If patients showed at least a 50% reduction in MADRS scores on day 2, they received 5 additional infusions on an outpatient basis on a Monday-Wednesday-Friday schedule, modeled after electroconvulsive therapy. Following the sixth infusion, follow-up visits were conducted twice weekly for 4 weeks or until relapse.

Overall, repeated subanesthetic-dose ketamine elicited minimal psychotic symptoms, as detected by the BPRS positive subscale [17]. Three patients experienced clinically significant but transient dissociative symptoms. Side effects during and following each ketamine infusion were generally mild. Nine patients met the response criterion after the first infusion, and all 9 continued their response throughout the 2-week experimental period. The mean reduction in MADRS score after the sixth infusion was 85%. Relapse occurred, on average, 19 days after the final infusion while patients remained off antidepressant medication. Of note, this is significantly longer than what is seen following a single ketamine infusion, where relapse typically occurs after just a few days or a week. There was a very wide range in the number of days until relapse between individual patients, from 6 to 45 days. Overall, the safety of repeated-dose intravenous ketamine for TRD was demonstrated, at least for up to 6 infusions over 2 weeks. No patients demonstrated worsening on neurocognitive measures or engaged in drug-seeking behavior. Controlled investigations with assessments of acute and longer-term neurocognitive outcome are in progress.

A particularly promising new lead following these initial single-dose and repeated-dose investigations was the impact of ketamine on suicidal ideation. A post hoc analysis of our 2 trials in TRD [10, 17] found that intravenous ketamine was associated with rapid reductions in explicit and implicit suicidal cognitions within the first 24 h after infusion, which persisted in those who received additional infusions [18]. To measure implicit cognitions, a computerized test (the Implicit Association Test) was used to track implicit cognitions related to suicide over the course of treatment. Although promising, these pilot findings were limited in that the TRD group was not markedly suicidal. Prospective, controlled investigations of the anti-suicidal properties of ketamine are underway.

*Age*

In the study by Mathew et al. [10], exploratory analyses revealed that ketamine responders were significantly older (52.4 ± 10.9 years) than non-responders (40.3 ± 9.6 years). In addition, age was positively correlated with percent improvement in MADRS score 24 h following infusion. The positive association of age with outcome is intriguing given the similar findings reported with electroconvulsive therapy [19].

*Family History of Alcohol Dependence*

In a separate study at the NIMH, a positive association was found between the acute antidepressant response to ketamine and a family history of alcohol dependence [20]. Twenty-six subjects with TRD received open-label intravenous ketamine (0.5 mg/kg) and were rated using MADRS at baseline, and 40, 80, 120, and 230 min after infusion, in a manner similar to this group's initial report [9]. A post hoc analysis revealed that TRD patients with a positive family history of alcohol dependence (defined as at least 1 affected first-degree relative or 2 second-degree relatives) showed significantly greater early (230 min) improvements in MADRS score compared to patients without a family history of alcohol dependence. Of note, a personal history of alcohol use disorder or a family history of mood disorder did not predict response.

This report is of interest given the significant comorbidity between major depression and alcohol use disorders, and evidence for NMDA receptor dysfunction in both disorders. Alcohol-dependent individuals demonstrated marked reductions in the subjective intoxicating effects of ketamine compared to healthy controls [21]. Healthy individuals with a positive family history of alcohol dependence had fewer perceptual alterations and lower dysphoric mood after receiving ketamine than those without a family history [22]. A potential limitation of these laboratory-based challenge studies was the failure to distinguish between type I and type II alcoholism, or known intermediate characteristics that interact with environmental factors to produce risk for alcoholism [23]. The implications of the association between antidepressant response to ketamine and family history of alcohol dependence are unclear. A recent study found evidence of an association between the NMDA NR2A subunit and alcohol dependence [24]. Genetic variations in NMDA subunits may increase vulnerability to alcohol dependence by altering the sensitivity of subunits of the NMDA receptor. Because ketamine acts as a partial NR2A antagonist, it is possible that difference in NR2A sensitivity may impart a greater response to the antidepressant effects of ketamine. Future studies examining potential heritability of depression responsivity to ketamine would be of significant interest. Further, given the influence of genetics on expression of intermediate phenotypes related to alcoholism, including the flushing response to alcohol, an attenuated subjective response to alcohol, and personality characteristics (impulsivity, sensation seeking, and behavioral disinhibition) [24], research scrutinizing these variables in relation to outcome is necessary.

Many questions regarding clinical factors that influence the efficacy of ketamine on depression remain unanswered. With the exception of age, demographic or clinical factors that predict antidepressant response to ketamine remain to be discovered, including the degree of treatment resistance and symptom characteristics such as subsyndromal bipolarity, melancholia, and anxiety. Research groups are now investigating intravenous ketamine for bipolar depression, with initial encouraging results.

*Biomarkers: Anterior Cingulate Cortex Activity*

The identification of neural biomarkers of depression with predictive validity for therapeutic response represents an important research goal [25, 26]. Towards this end, rostral and subgenual regions of the anterior cingulate cortex (ACC) have increasingly been implicated in response to antidepressant treatment across multiple modalities [27–29]. Salvadore et al. [30] described an association between rapid antidepressant response to ketamine and ACC activity. Using magnetoencephalography (MEG), ACC activity was recorded in 11 patients with MDD prior to receiving a single ketamine infusion, and in 11 healthy control subjects. Magnetoencephalography recordings were performed during rapid presentation of fearful faces, a paradigm that reliably activates rostral ACC regions. At baseline, healthy subjects showed decreased neuromagnetic activity in rostral ACC across repeated stimulus exposures, while patients with MDD showed the opposite pattern (e.g. increases in ACC activity). In patients, increased rostral ACC activity was positively correlated with subsequent antidepressant response to ketamine 4 h after infusion, suggesting a biomarker predictive of acute response to intravenous ketamine.

The identification of a relationship between ACC activity and antidepressant response to ketamine is of particular interest given the emerging role of the ACC, and related medial prefrontal structures, in the etiopathophysiology of depression [27, 31, 32]. Ventral and medial regions of the prefrontal cortex (PFC), including ACC, are envisioned as part of a mood regulation network (or, more likely, multiple overlapping networks), dysfunction in which putatively underlies the symptoms characteristic of depression or other mood disorders [31, 33, 34]. The study by Salvadore et al. [30] supports the hypothesis that ketamine exerts its antidepressant effects by impacting this circuitry. In line with this hypothesis, a pharmaco-fMRI study by Deakin et al. [35] revealed acute reductions in ventromedial PFC activity associated with ketamine administration in healthy volunteers. Prospective neuroimaging studies conducted in the context of a controlled clinical trial of ketamine will be necessary to elucidate the neural mechanisms of the antidepressant effects of ketamine.

*Biomarkers: Brain-Derived Neurotrophic Factor*

The neurotrophin brain-derived neurotrophic factor (BDNF) has been a major research focus in the pathophysiology of depression [36, 37]. The neurotrophic hypothesis posits that depression is associated with decrements in neurotrophic factors, which are neurodevelopmentally expressed growth factors that regulate plasticity

within the adult brain [37]. By extension, antidepressants may work by potentiating BDNF or otherwise restoring neurotrophic function in the central nervous system [37]. The largest meta-analysis to date in humans (748 patients across 11 studies) showed a significant decrease in serum BDNF levels in depressed subjects compared to healthy controls [38]. In addition, this report found higher BDNF levels after chronic treatment with antidepressants (220 patients across 8 studies).

Synaptic and extrasynaptic NMDA receptors may have regionally selective and inverse effects on neuroplasticity. Subanesthetic-dose ketamine may simultaneously decrease activation of extrasynaptic NMDA receptors and enhance glutamatergic tone at synaptic NMDA receptors (via preferential blockade of NMDA receptors on GABAergic interneurons), resulting in enhancement of neuroplasticity [4]. The only published investigation to our knowledge, which examined the relationship between ketamine and BDNF in TRD patients, failed to detect an association between peripheral levels of BDNF and acute response [39]. There were no significant changes in peripheral BDNF levels following ketamine infusion and for up to 230 min after infusion. A limitation of this study was that only very early (<4 h) antidepressant effects of ketamine were measured.

Thus, while substantial data support an association between BDNF and depressive illness, the utility of peripheral BDNF levels as a biomarker for response in depression is uncertain. For glutamate-modulating agents such as riluzole, preclinical models have shown an impact of chronic drug administration on expression of several neurotrophic factors, including BDNF, VEGF, and glial-derived neurotrophic factor [40]. Our group explored the impact of cysteamine, a transglutaminase inhibitor approved for the treatment of cystinosis, on the expression of BDNF in patients with TRD. Cysteamine has been shown to increase BNDF in neuronal tissue in preclinical models of Huntington's disease [41]. In a series of 3 patients with TRD chronically treated with cysteamine bitartrate, no significant changes in serum BDNF levels were observed (fig. 1).

## Conclusions and Future Directions

Drugs with varied effects on glutamatergic neurotransmission (i.e. modulation of glutamate release, direct and indirect effects on glutamate receptor activation, effects on extracellular glutamate clearance, and glutamate metabolism) are under investigation for TRD. Glutamate modulation with NMDA receptor antagonists such as ketamine may represent a particularly intriguing avenue for drug discovery for patients who have failed to respond to conventional approaches. However, studies to date have occurred at only a few academic medical centers, and despite enthusiasm generated from the first phase of studies, the worldwide controlled experience with intravenous ketamine is fewer than 100 TRD patients.

An improved understanding of the heterogeneity inherent to TRD is essential to characterize the type of individual who would benefit from glutamate-based

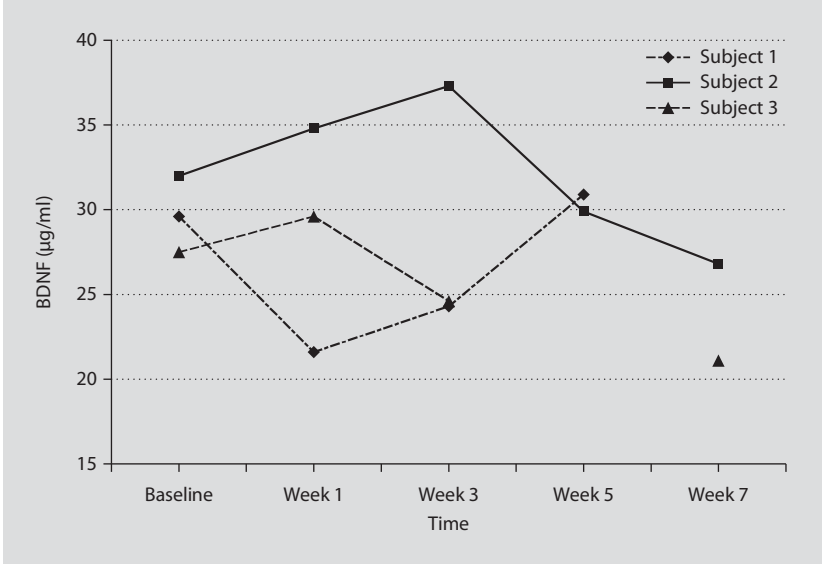


**Fig. 1.** Change in BDNF levels during an 8-week trial of cysteamine bitartrate in patients with TRD. Subject 1 exited the study at week 5; subject 3 missed the week 5 BDNF measurement.

therapies. Industry-sponsored regulatory studies in TRD of pharmacotherapy (e.g. olanzapine/fluoxetine combination) and neurostimulation (e.g. repetitive transcranial magnetic stimulation, vagus nerve stimulation, deep brain stimulation) have tended to exclude patient subgroups typically seen in clinical practice with marked chronicity, axis II and III disorders, anxiety, suicidality, and substance abuse. Further, the generalizability and validity of TRD trials is threatened by the tendency to cap the number of previous failed antidepressant trials in the current episode (generally between 2 and 4 trials). We thus lack sufficient information to guide pharmacotherapy approaches for truly severe medication-resistant forms of depression.

Future research in the identification of biomarkers for depression and treatment response is critical to progress. Through neuroimaging research in particular, we envision the establishment of 'neural biosignatures' for both the depression disease state, and for treatment response. On the clinical side, these biomarkers would enhance diagnostic specificity, and guide treatment decisions. On the research side, they would provide a more reliable measure of disease state and treatment response in the context of clinical trials, potentially enhancing rational drug discovery. For example, if a signal within the ventromedial PFC could be developed into a reliable biomarker of antidepressant effect, then new candidate antidepressants could be evaluated in a relatively small number of participants using neuroimaging. This strategy may serve to screen out ineffective antidepressants, prior to conducting an expensive large-scale, randomized, controlled trial.

At the molecular level, the specific role of glutamate and its receptors in the etiopathology of depression remains to be elucidated. It will be important to link glutamate dysfunction with the putative impairments in neural plasticity and resilience hypothesized in depression. Given that severe, recurrent mood disorders have been conceptualized as involving neuronal corticolimbic circuits that are in a hyperexcitatory state (for a review, see Drevets et al. [31]), increased excitatory glutamate transmission and/or reduced inhibitory GABA transmission could result in the observed impairments in neural trophic function through excitotoxic mechanisms.

Future drug discovery in major depression will continue efforts to identify and develop agents with glutamatergic and other novel mechanisms of action in the hope of achieving safe, effective, and rapid-acting pharmacotherapies for patients suffering from this disabling disorder.

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# Neuroimaging and the Pathophysiology and Treatment of Depression: Recent Advances and Future Needs

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**Abstract**

Imaging the human brain still cannot be considered a clinical tool in the field of psychiatry and currently (early 2010) does not contribute directly to the alleviation of patient suffering. However, recent years have witnessed a rapid increase in the number of studies moving us closer to this goal through incorporating neuroimaging techniques into studies of treatment mechanism and prediction of treatment response and those studies taking genotype into account. In addition, the range of neuroimaging modalities and outcome parameters in use, to index abnormalities of the brain, continues to expand and technical developments in the quality of derived parameters and images confer ever increasing levels of sensitivity. Disease models of depression currently include early- and later-life stress including immigration, genetic loading or liability, neurotransmitter system abnormalities, and structural and physiological perturbations that interfere with brain function. It is now indisputable that imaging of the brain has contributed profoundly to the development of the latter 3 models. However, despite these advances in our understanding of depression, imaging has not yet affected clinical monitoring or treatment practices, presaged treatment response, or ultimately affected outcome for patients today. This chapter endeavours to highlight the latest imaging findings that demonstrate the potential to achieve such goals in the near future.



## The Pathophysiology of Depression: Imaging Studies

### *The Molecular and Neurochemical Pathophysiology of Depression*

Molecular and neurochemical studies have used single photon emission tomography (SPECT), positron emission tomography (PET), and magnetic resonance spectroscopy (MRS) techniques in depressed populations to examine receptors, transporters,

enzymes, neurotransmitter concentrations and storage capacities, markers of neuronal membrane turnover and integrity, metabolic turnover, pharmacokinetic characteristics of drugs such as lithium and drug occupancy rates. To date these studies have revealed the dysfunctional involvement of several neurotransmitter systems in depression. In addition to assessing target binding, studies in depression have assessed the rate of uptake of serotonin (5-HT, L-[$^{11}$C]5-HTP) and dopamine ([$^{11}$C] L-dopa), and 5-HT synthesis (α-[$^{11}$C]MTrp), estimated dopamine storage capacity ([$^{11}$C]raclopride), and have assessed 5-HT and dopamine involvement using depletion studies. Norepinephrine lacks such radioligand availability but has been investigated using less direct methods including pharmacological challenge during assessment of cerebral blood flow. Based on evidence for disruption in each of these 3 monoamine neurotransmitter systems, it will be important to assess the involvement of vesicular monoamine transporters in depression ([$^{11}$C]dihydrotetrabenazine). We are currently unable to assess numerous further targets of interest such as the norepinephrine transporter as efforts are still underway to develop suitable radioligands. The latter step is a significant challenge to the field and the quality of the PET images and data produced for a given target molecule are vitally dependent on the selectivity and specificity of the radioligand developed among other factors [1].

In major depressive disorder (MDD), brain 5-HT uptake and synthesis in frontal, temporal and cingulate cortices are reduced, while its metabolism by monoamine oxidase A is elevated. In addition, the levels of the 5-HT transporter, which removes 5-HT from the intrasynaptic space, reducing signalling, has also been reported to be elevated [2–5], though not consistently [6, 7]. Reduced somatodendritic and postsynaptic 5-$HT_{1A}$ and 5-$HT_2$ receptors have been reported in orbitofrontal and anterior insular cortices, and in the hippocampus in older depressed subjects. However, elevated levels of 5-$HT_2$ receptors have been associated with MDD involving severely pessimistic or dysfunctional attitudes [8] and were positively related to measures of these characteristics among patients. On the whole, these data support the depressed mood state being associated either with (1) abnormalities that are compensatory in nature and in response to acutely elevated 5-HT concentrations, perhaps as a result of stressful events, or (2) abnormalities that are etiological in nature, that result in reduced 5-HT neurotransmission thereby causing depressive signs and symptoms. However, discerning which or what combination of these abnormalities of the 5-HT system is playing a causative role is probably the greatest challenge currently to progressing our understanding of the pathophysiology of the serotonergic system in depression.

The dopamine system has been explored using PET to examine its synthesis ([$^{18}$F] fluoro-L-dopa), release ([$^{11}$C]raclopride, [$^{18}$F]fallypride), the effects of dopamine depletion (α-methyl-*p*-tyrosine), $D_1$ receptor ([$^{11}$C]SCH-23390, [$^{11}$C]NNC) and $D_2$ receptor ([$^{11}$C]FLB-457, [$^{18}$F]fallypride) binding, and clearance capacity ([$^{11}$C] RTI-32, [$^{123}$I]βCIT). Dopamine synthesis or [$^{18}$F]dopa uptake rate ($K_i$) was reduced across the blood-brain barrier as well as in the striatum in depression with affective

flattening and psychomotor retardation and in the anterior cingulum and hypothalamus in those who were impulsive. These data implicate distinct dopaminergic signalling systems underlying different depressive phenotypes. [$^{11}$C]Raclopride binds to striatal $D_{2/3}$ receptors and is sensitive to competition from the release of endogenous dopamine. [$^{11}$C]Raclopride binding was higher in the putamen during depression associated with psychomotor retardation in medication-free subjects relative to healthy controls suggesting lower dopamine concentrations but not in a study involving MDD in which just 36% of subjects presented with psychomotor retardation. Extrastriatal $D_{2/3}$ receptor binding ([$^{11}$C]FLB-457), on the other hand, did not differ in a small sample of individuals with MDD (n = 7) and 7 healthy controls. Consistent with the possibility of reduced dopamine levels during depression, dopamine depletion studies using α-methyl-*p*-tyrosine induce depressive symptoms and several antidepressants raise dopamine concentrations. Dopamine transporter levels assessed using PET and [$^{11}$C]RTI-32 in the striatum were reduced during depression potentially indicating a reduced capacity for dopamine clearance in select brain regions. Finally, $D_1$ and $D_2$ receptors are reduced in the striatum in MDD subjects exhibiting motor retardation, while no difference was detected in $D_2$ receptor levels in extrastriatal regions. Clearly, perturbation of striatal dopaminergic neurotransmission is more pronounced in those depressed subjects also experiencing psychomotor symptoms than other subpopulations.

Other neurotransmitter systems have been less extensively investigated in MDD using imaging techniques. Widespread reduction in cholinergic muscarinic 2 autoreceptor levels, most pronounced in the cingulate cortices in depression, is associated with bipolar disorder but not MDD [9]. Decreased levels of histamine 1 receptor have additionally been detected and related negatively to the severity of depressive symptoms [10]. Finally, in individuals who did not meet criteria for a depressive or anxiety disorder or mild cognitive impairment, subsyndromal depression and anxiety scores were related to amyloid senile plaques and tau neurofibrillary tangle binding in the medial temporal cortex, and in the medial temporal and frontal cortices, respectively [11].

Several recent advances in molecular imaging in particular should help progress our understanding of the pathophysiology of depression. The resolution available to PET studies has been superseded by the development of high-resolution research tomography (HRRT) [12]. The HRRT can achieve a resolution of 2.5–3 mm compared to a PET scanner which gives 6–7 mm. This will permit more accurate quantification of molecular target binding in smaller brain regions than previously possible. The increased resolution also confers more sensitivity in the form of greater statistical quality of PET data and the detection of smaller biological signals, and increased power when comparing a control and patient population thus reducing the number of participants necessary.

Perhaps most frequently investigated in MDD using [$^{1}$H]MRS (for reviews, see Ende et al. [13] and Dager et al. [14]) are the concentrations of glutamate and γ-aminobutyric acid (GABA) or the Glx peak comprising glutamate, glutamine and

GABA. In depression, the concentration of glutamate assessed using MRS has been reported to be reduced in the cingulate and prefrontal cortices and temporal lobe. However, this signal is contributed to by the total pool of glutamate in the human brain (approx. 9 mM) and only a small proportion of that signal will represent intrasynaptic glutamate. Interpretation of the impact of such a deficit in depression is therefore multifaceted and non-specific. Lower GABA concentrations have been detected in the occipital cortex in both depressed and unmedicated remitted MDD subjects, and more recently with the advent of technical advances in MRS in dorsomedial/dorsoanterolateral areas of the prefrontal cortex in depressed subjects with MDD. The latter is consistent with glial cell abnormalities detected in these prefrontal regions using post-mortem based techniques. $GABA_A$ receptors have additionally been reported to be reduced in bilateral parahippocampal and lateral temporal regions in depressed MDD outpatients using PET and [$^{11}$C]flumazenil with an inverse relationship to the hypothalamic-pituitary-adrenal axis activity (dexamethasone suppression corticotrophin-releasing hormone test) suggesting that a contribution of reduced GABAergic signalling in hypothalamic-pituitary-adrenal axis hyperactivity is associated with MDD. Early studies detected no difference in $GABA_A$ receptor binding between MDD and healthy groups; however, these were preformed at a reduced resolution using SPECT and [$^{123}$I]iomezenil.

Reduced neuronal integrity (N-acetyl aspartate, [$^{1}$H]MRS) has been detected in some but not all studies examining depressed subjects. No change in N-acetyl aspartate/creatine ratio, inositol/creatine ratio or choline/creatine ratio was observed, for instance, in the dorsolateral prefrontal cortex in drug-naïve females during their first episode of depression. Reports on choline levels are mixed with elevations, reductions and no differences reported. [$^{31}$P]MRS studies in depression have identified reduced brain energy metabolism and pH in depressed, manic and euthymic states in bipolar disorder potentially implicating mitochondrial dysfunction, and state-specific abnormalities in phospholipid membranes including reduced phosphocreatine levels during depression.

MRS, first used clinically in the early 1980s, in general has a trade-off between spatial and temporal resolution that has been rapidly advancing through the increased field strengths, coil sensitivity and spectral analysis methods including proton editing methods to decipher peaks such as the Glx peak into its glutamate, glutamine and GABA components. Hyperpolarizing carbon-13 confers a 10,000-fold increase in the signal over conventional nuclear magnetic resonance and is currently used to label pyruvate to study metabolism. Efforts are currently focusing on extending this to other suitable agents such as bicarbonate, which permits measurement of pH distribution, and hyperpolarized carbon-13 may in the future permit measurement of metabolic flux rates relevant to psychiatric disorders. Ultimately, these advances should permit further enhancement of the signal/noise ratio and permit more spatially refined voxels to be investigated and should provide more anatomically localized information regarding the metabolic abnormalities associated with depression to date.

## *The Structural Abnormalities Associated with Depression*

Structural anatomical abnormalities associated with depression have been examined in vivo for several more decades than molecular aspects, using computerized tomography (CT) and magnetic resonance imaging (MRI) with a continuing linear trend in the improvement in resolution capabilities. Grey and white matter concentrations are examined most commonly and using structural MR images such as the $T_1$-weighted image. Computational neuroanatomy techniques employed for these purposes include the region-of-interest approach and voxel-based morphometry. While region-of-interest-based studies are confined to regions that can be reliably delineated visually, voxel-based approaches have enabled whole-brain analyses albeit with some limitations such as the sensitivity to registration accuracy. Less widely used techniques include cortical or surface mapping to examine curvature and thickness, and deformation-based and tensor-based morphometry. In the case of depression, the structural pathology has proved subtle in magnitude relative to neurological disorders, and in the case of several structures has proved elusive to consistent replication.

Of the many studies comparing grey and white matter concentration between depressive disorder and healthy control groups, the most consistent findings are those reporting enlarged ventricle size mostly of the third and lateral ventricles. However, the latter has largely been derived from studies in elderly and chronically depressed late-onset populations and has not been replicated unanimously. The aetiology of ventriculomegaly is poorly understood but has been suggested to be related to tissue loss in adjacent structures, cerebrovascular disease, and other structural anomalies that may lead to accumulation of cerebrospinal fluid. White matter hyperintensities additionally have commonly been associated with MDD [15] and bipolar disorder [16]. A variety of types of evidence have since emerged that suggest that depression is associated with white matter pathology, including post-mortem, genetic association, structural magnetic resonance, and most recently diffusion-weighted imaging (DWI) studies. However, white matter hyperintensities are a particularly non-specific phenomenon, and are present in almost all individuals by the age of 85 [17]. The incidence of deep frontal and basal ganglia white matter hyperintensities is increased in MDD and bipolar disorder with a late age of onset of depression. On an MR image, a white matter hyperintensity represents an encapsulated area of water, which can result from demyelination, astrogliosis, neuropil atrophy, or ischaemia due to local vesicular pathology among other cerebrovascular risk factors. The clinical implications of white matter hyperintensities are currently unclear, but will vary with the spatial extent of the lesion and the functional specialization of the affected fibres. Functionally, poorer performance on the Stroop test has been linked to an increased frequency of white matter lesions [18]. White matter hyperintensities have regularly been reported in the medial temporal [19] and deep frontal white matter, areas that connect the hippocampus and amygdala to the frontal cortex. White matter abnormalities are now more widely examined in depression using DWI. Sulcal widening

has also been associated with depression and total cerebral volume appears to be conserved, unlike in schizophrenia. On the other hand, numerous reports of local grey matter concentration abnormalities have been associated with depressive disorders with varying degrees of consistency.

In MDD, morphometric studies examining the hippocampus have generally converged to support the degenerative model of depression showing reduced volume in adults. However, these studies include mostly elderly, middle-aged or chronically ill populations and several studies failed to replicate this finding (for a review, see Savitz and Drevets [20]). Overall, the literature suggests that the volume of the basal ganglia structures (caudate, putamen, globus pallidus, subthalamic nuclei and substantia nigra) may not be altered in MDD or bipolar disorder, although a reduced volume has been associated with late-onset MDD and chronic or severe illness. Subgenual anterior cingulate cortex involvement in depression has recently been reviewed [21]. Recent reviews [20, 22] and meta-analyses [23] are available in the extensive body of literature studying morphometric changes associated with depression. Most recently, Savitz and Drevets [20] provided an up-to-date and comprehensive review of grey and white matter morphometric studies in MDD and bipolar disorder. Across this large body of literature, there is an indication of a divide between the abnormalities observed in the elderly, adults and children with depression, and between early- and late-onset subgroups. Further factors that currently inhibit comparison across these studies include medication status, mood state, disease heterogeneity, methodology employed and genotype (rarely explicitly examined; see section 'Combining neuroimaging and genotyping in the study of depression'). Evidence is mounting for the role of each of these factors in complicating the interpretation of structural MR studies.

While grey matter structural abnormalities are most commonly studied using $T_1$- or $T_2$-weighted MR acquisition sequences that produce greyscale images with good grey-white matter contrast, the contrast within the white matter in these images is poor. In order to study the microstructural organization of white matter, MR images weighted to be sensitive to diffusion (Brownian motion, DWI) of water molecules have proven highly informative [24]. In particular, DWI data obtained across multiple gradient directions capture the rate of diffusion (eigenvalues, $\lambda 1$, $\lambda 2$ and $\lambda 3$) along 3 principal directions (eigenvectors, $\nu 1$, $\nu 2$ and $\nu 3$) in the case of diffusion tensor imaging (DTI). Images of the apparent diffusivity coefficient (or mean diffusivity) represent the average of the values of 3 eigenvalues $(\lambda 1 + \lambda 2 + \lambda 3)/3$ per voxel. Fractional anisotropy maps, on the other hand, are weighted towards $\lambda 1$, the principal diffusion direction, therefore providing more information about the organization or coherence in the direction in which diffusion is greatest. Diffusion in white matter fibres is greatest parallel to the fibre ($\lambda 1$) and impeded by the axonal wall and myelin sheath in the directions perpendicular to the fibre ($\lambda 2$ and $\lambda 3$). As a result, fractional anisotropy is expected to be impaired in regions where the microstructural organization of bundles of white matter fibres is altered either through increased radial diffusivity or reduced axial or longitudinal diffusivity. Increased radial diffusivity has been suggested to

result from a loss of myelin sheath integrity along the axon, consistent with evidence of macromolecule loss (thought to primarily reflect loss of myelin) detected in depression using magnetization transfer imaging. Fractional anisotropy maps have been analysed using a voxel-based approach, region-of-interest analyses, tract-based spatial statistics and most recently by comparing fractional anisotropy for tracts derived following a range of tractography procedures under rapid development.

In MDD during depression, reduced fractional anisotropy has been detected in the sagittal stratum, cingulate cortex and the posterior body of the corpus callosum, the anterior limb of the internal capsule, and the superior longitudinal fasciculus, and no difference has been reported in the brainstem relative to non-depressed controls. Reduced fractional anisotropy was additionally associated with depression in an older population (mean age 70) relative to remitted age-matched MDD subjects, in the anterior and posterior cingulate cortices, genu of the corpus callosum, parahippocampal gyrus, insula, neostriatum, temporal and parietal regions, dorsolateral prefrontal cortex and midbrain. During depression associated with bipolar disorder, the white matter tract that mediates signalling between the subgenual cingulate and amygdala-hippocampal complex has been implicated as have the frontal cortex, the internal capsule, cingulum, longitudinal fasciculi, uncinate fasciculi and optic and anterior-thalamic radiations and the genu of the corpus callosum. The latter is consistent with the increase in white matter hyperintensities detected in the corpus callosum in bipolar disorder. Some of the inconsistencies observed in this literature to date are likely to be due to the widely varying analysis methods employed and to variation in the medications involved at the time of scanning across studies. For instance, fractional anisotropy in the genu of the corpus callosum has been reported to be 6–7% greater in two studies [25, 26] but reduced in a separate study [27]. Deciphering which abnormalities are specific to the pathophysiology of disease versus medication effects will significantly advance our understanding of the white matter abnormalities that underlie the neurobiological basis of affective illnesses.

White matter fibre bundles contain myelinated neuronal axons and glial cells. The myelinated axons connect grey matter regions and are integral to neuronal communication between grey matter regions. Post-mortem studies in mood disorders may inform these in vivo findings, which may be associated with reductions in markers of astrocytes reported in the frontal cortex, for example, or the observed reductions in the density of oligodendroglia in BA9 and apoptotic and necrotic changes in satellite oligodendroglia in BA10 in the caudate nucleus. Glial cells and neurons have a well-established reciprocal relationship that underlies their mutual development, proliferation and functioning. This is achieved via neurotrophic factors and cytokines synthesized and released by astrocytes and microglia which include brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor, and nerve growth factor. Glial-derived neurotrophic factor functions to promote synaptic plasticity and BDNF plays a role in differentiation and growth of new neurons and synapses. This role of glial cells in providing energy and trophic factors to neurons suggests that

altered white matter could feasibly contribute to the consistently reported changes in activation in response to emotional stimuli and abnormal volume of grey matter structures detected in depression. With the advent of in vivo neuroimaging-based evidence for glial dysfunction in depression using DWI, such PET radiotracers as those developed to measure glial metabolism may become more relevant for application in depressive disorders [28].

It is clear that MDD-associated morphometric abnormalities *alone* are not and may never be diagnostically useful. Rather their potential may more readily be realized in prediction of treatment response and monitoring of illness course (see section 'Neuroimaging and the treatment of depression') and/or upon re-examination taking sample genetic homogeneity into account (see section 'Combining neuroimaging and genotyping in the study of depression').

### *The Functional Pathophysiology of Depression*

Functional imaging has provided a myriad of information on the pathophysiology and regional localization of functional disturbances both at rest and in emotion-related tasks during various mood states. Imaging outcome measures contributing to these data range from cerebral blood flow ($[^{15}O]H_2O$) and glucose metabolism ($[^{18}F]$FDG), to MRI to assess blood flow (perfusion or arterial spin labelling MRI), and activation and deactivation [blood oxygen level-dependent (BOLD) signal]. Additionally, MRI has been combined with pharmacological challenge to probe the role of neurotransmitter systems in cognition and/or drug actions. Less commonly used methods employed to study the functional pathophysiology of depression that are spatially restricted to the surface but provide excellent temporal resolution include assessing electrical activity directly (electroencephalography, EEG), the magnetic fields produced by electrical brain activity (magnetoencephalography, MEG), and surface haemoglobin levels as an indicator of activation (near-infrared spectroscopy).

By far, the most commonly applied of these to date is BOLD functional MRI (for reviews, see Sava and Yurgelun-Todd [29] and Bandettini [30]). In general, elevated activity in the amygdala has been associated with depression in response to negatively valenced stimuli [20] and is most likely to relate to altered mood or affect regulation, in particular in response to aversive stimuli or stressors during depression associated with MDD. Considerably fewer studies exist and they disagree on a functional role for the hippocampus in depression with recovery being associated with increased and reduced metabolism. Thus, it is currently difficult to understand exactly how hippocampal pathophysiology relates to the signs and symptoms in MDD; however, they are likely to play a role in the deficits identified in MDD in emotion-related memory and executive function [31]. Reduced blood flow and metabolism in the caudate nuclei and putamen during depression associated with MDD is well replicated

in the literature and hypothesized to relate to disruption of the underlying dopaminergic system and related behaviours including motivation. Depression has also been associated with elevated blood flow and metabolism in lateral, medial, orbitofrontal and ventromedial or subgenual cortices, although not all studies agree. Reduced metabolism in the orbitofrontal cortex has been associated with successful treatment in unmedicated depressed subjects responding to cognitive behavioural therapy and in response to deep brain stimulation(see section 'Neuroimaging and the treatment of depression'). Orbital gyri are connected to the temporal lobe structures including the amygdala and hippocampus via the uncinate fasciculus, disrupted in MDD (see previous section) and they mediate reward prediction and behavioural choice. Medial aspects are involved in memory processes including emotion-related autobiographical memory retrieval, and functional disruption has been associated with overgeneralization of autobiographical recall MDD [32]. Analysis of regional activity relationships has suggested altered interhemispheric amygdalar (increased) and frontal lobe (reduced) connectivity in MDD relative to non-depressed subjects. Frontal lobe connectivity normalized upon successful treatment and frontal-temporal connectivity has been shown to potentially be under the influence of genotype in the case of the 5-HT transporter insertion/deletion polymorphism (5-HT transporter-linked promoter region, 5-HTTLPR; see section 'Combining neuroimaging and genotyping in the study of depression').

Most recently, the interpretation of BOLD signal changes has benefited from the use of some alternate haemodynamic contrast outcome parameters [30] and investigation of altered default mode network function. In addition, the examination of perturbations of activation by pharmacological challenge to test the involvement of specific receptor subtypes or other molecular elements in brain function during depression should prove highly informative. For example, pharmacological MRI has shown the involvement of 5-HT in inhibiting low-level amygdala reactivity to aversive stimuli [33].

Additional examples of neuroimaging methods studying brain function in MDD that are less commonly employed include perfusion MRI (arterial spin labelling and dynamic susceptibility contrast-enhanced MRI; for a review, see Theberge [34]), optical imaging (near-infrared spectroscopy), EEG, and MEG. Combined EEG/PET studies have suggested disconnection between the thalamic metabolic rate and average electrical activity during depression, and associated reduced metabolism in the subgenual anterior cingulate cortex with increased inhibitory delta activity during melancholic depression. Few studies have used MEG in MDD and the analysis methods vary widely. The low-dose antidepressant response to ketamine in depressed subjects has been associated with increased activity in the anterior cingulate cortex in response to fearful faces using MEG [35]. MEG appears to hold promise as a potential diagnostic tool for post-traumatic stress disorder having correctly identified 97% of post-traumatic stress disorder sufferers. However, 12% of healthy participants additionally presented with abnormal activity in the same study.

**Table 1.** Synopsis of imaging techniques reviewed and their primary attributes

| | | Advantages | Disadvantages |
|---|---|---|---|
| CT | Computed tomography | Widely available | Poor resolution relative to SPECT, PET or HRRT |
| DTI | Diffusion tensor imaging | Measures directional organization of white matter | Can be misleading where fibres cross or are interdigitated |
| DWI | Diffusion-weighted imaging | See DTI | |
| fMRI | Functional MRI | Speed of acquisition | |
| HRRT | High-resolution research tomography | Resolution relative to PET or SPECT | Not widely available |
| MRI | Magnetic resonance imaging | See fMRI, MRS, DTI | |
| MRS | Magnetic resonance spectroscopy | Measures levels of neurochemicals | Single large voxel of interest |
| PET | Positron emission tomography | Receptor mapping | Expensive and sometimes requires arterial blood sampling |
| SPECT | Single photon emission computed tomography | More widely available than PET | Poorer resolution than PET |

In short, few other techniques have the potential that functional MRI provides to link functional brain activity and connectivity impairment in depression with disturbances in cognition and behaviour. The methods employed now go beyond localizing areas involved in pathological illness features, to probe predictors of outcome and the involvement of neurotransmitter systems. In particular, pharmacological challenge of functional MRI may present a useful tool in studying depression and recovery in the future (table 1).

## Combining Neuroimaging and Genotyping in the Study of Depression

Functional, structural and molecular imaging fields have all recently benefited from the advent of rapid and widely available genotyping methods, in explaining a degree of the variance in imaging outcome measures among depressed populations. More importantly, this combined approach is expected to lead to the identification of

intermediate phenotypes [36] with the potential for clinical application in diagnosis or prediction of treatment response. Genetic-based approaches that have been exploited in the imaging field to date include genotyping single nucleotide, and more extensive polymorphisms, genome-wide association studies, recruiting a genetically liable or at-risk group, twin and adoption studies, and yet to be exploited in combination with neuroimaging approaches is copy number variation.

The short allele of the 5-HTTLPR is reported to confer an increased risk of developing depression when combined with stressful life events, and to be associated with reduced cingulate and amygdala volume, increased amygdala reactivity, and increased anxiety-related personality traits. In addition, functional MR-derived changes in regional brain activity in response to fearful faces show uncoupling of communication between amygdala and cingulate regions in healthy people carrying the short allele of the 5-HTTLPR. Tight coupling of activity in these regions is implicated in the appropriate extinction of negative emotional responses. Genetic variation in genes coding for further elements of the serotonergic system have been implicated in MDD and provide evidence to support further investigation. 5-HT transporter availability does not appear to be tightly related to the 5-HTTLPR genotype by either post-mortem or in vivo based investigations despite earlier in vitro evidence to that effect. In a large-scale genome-wide association study, the 5-HT transporter binding levels were strongly associated with variation in the gene coding for the $5\text{-HT}_{2A}$ receptor [37, 38], however, not differentially so in depressed individuals relative to healthy controls. In addition, the $5\text{-HT}_{1A}$ receptor polymorphism, C1019G (rs6295) has been shown to be overrepresented among MDD subjects and to influence $5\text{-HT}_{1A}$ receptor expression levels in vitro. However, this has not been explicitly examined in vivo in MDD to date.

In the dopaminergic system, researchers did not detect an influence of variation in the functional catechol-O-methyltransferase val158met polymorphism, known to modulate dopaminergic neurotransmission, on $D_2$ receptor binding using PET and [$^{11}$C]raclopride in healthy controls. The val/val alleles, on the other hand, have been associated with higher $D_1$ receptor binding levels in the cortex but not the striatum compared to met carriers. However, neither of these relationships have been examined in MDD despite evidence of perturbed $D_1$ and $D_2$ receptor binding during depression [39]. Similarly, while striatal $D_2$ receptor affinity is under the influence of the $D_2$ receptor gene polymorphism C957T in healthy controls, the relationship has not been examined in MDD. The genetic contributions to altered dopaminergic neurotransmission in MDD have yet to be elucidated. Similarly, GABA receptor subunit genes associated with mood disorders and MRS-based evidence of reduced GABA levels during depression provide a sound basis for examining the contribution of these genotypes in MDD toward in vivo GABA levels.

Our understanding of the aetiology of *morphometric* abnormalities associated with depression is additionally benefiting from combined genotyping imaging studies. These data perhaps most pertinently inform some of the sources of variation in

structural imaging findings in MDD to date. In MDD, reduced hippocampal volume may be associated with several genes including the BDNF met allele of the val66met polymorphism, the short allele of the 5-HTTLPR, and has been related to a reduced likelihood of remission at the 1-year follow-up. In psychosis, on the other hand, variation in BDNF, 5-HTTLPR, or in catechol-O-methyltransferase, NRG1 (neuregulin 1) and DTNBP1 (dysbindin) did not affect hippocampal or lateral ventricle volume in a recent study. The right hippocampus and bilateral temporal gyri in MDD appear additionally to be under the influence of the glycogen synthase kinase 3β polymorphism at rs6438552 (and rs12630592). How this array of genes may interact to influence the hippocampus will ultimately be vital to understand. While white matter hyperintensities are less likely to be a useful or specific marker in MDD, regional deficits in white matter microstructural organization in MDD have been identified (see section 'The functional pathophysiology of depression') and these may be under the influence of a number of genes according to preliminary evidence. In healthy controls, NRG1 genotype (rs6994992, SNP8NRG243177) was associated with increased risk for developing psychosis (TT genotype) and has been shown to influence white matter density (structural MRI) in the anterior limb of the internal capsule and a DWI-derived measure of white matter microstructural organization or integrity (fractional anisotropy) in the same region. Extending these approaches to depressed populations is likely to reveal specific genetic contributors to identify white matter abnormalities associated with MDD. A promising alternative approach has involved the recruitment of unaffected individuals with a first-degree relative with MDD, as a manner of examining the possible biological factors associated with having a genetic liability for developing the depression. This design recently revealed an association between the genetic liability for developing bipolar disorder and reduced white matter concentration in the left frontal and temporoparietal regions.

Examining imaging phenotypes against genotype alone is limited by not accounting for epigenetic factors such as environmental factors and in vivo stimuli that result in chromatin methylation and histone acetylation. These control the silencing and activation of genes and the search for knowledge of their possible role in the pathophysiology and therapeutic potential [40] in MDD is in its infancy. For instance, valproic acid activates a number of genes but the exact mechanism by which it exerts its mood-regulating effects is not clear. This emerging work will contribute significantly to our understanding of the specialization and involvement of brain regions in depression and potentially yield new reversible treatments. Furthermore, the potential pathological silencing of genes in MDD, which may vary regionally in the brain, is likely to make redundant several gene elements studied against regional brain imaging parameters today.

The challenges to genotyping-imaging studies in mood-disordered populations are numerous [41]. Certainly, the sample size necessary for examining an effect of genotype in a population far exceeds the usual size of imaging studies, especially the more expensive molecular imaging studies. While this can be addressed to

some extent via an a priori hypothesis-based approach, ultimately confirmation and replication in larger samples will be called for. However, significantly more challenging will be disentangling gene-gene and gene-environment/behaviour/aging-related interactions as well as deciphering individual variation versus that derived from disease-related abnormalities. Nonetheless, the potential of combining genotyping and imaging ultimately to contribute to developing clinical tools for diagnostic classification or identification of intermediate phenotypes will be invaluable and inform the prediction of illness course, outcome and treatment response.

## Neuroimaging and the Treatment of Depression

Neuroimaging studies have provided insight into both pharmacological- and non-pharmacological-based treatments of depression and range in design from those informing mechanism, those attempting to identify predictors of response or non-response, designs measuring target occupancy requirements to achieve efficacy, to those designs examining the efficacy of early intervention [42]. The potential of such studies to reduce the amount of time patients spend unwell cannot be overstated and the field is likely to witness a significant growth in the number of such studies over the coming decade.

### *Pharmacological Treatment*

Metabolism, blood flow and activity at baseline in responders has been studied most plentifully to date as a means to identifying potential predictors of response to medication-based treatment. In particular, selective serotonin reuptake inhibitor (SSRI) efficacy has been linked to altered baseline anterior cingulate and midbrain metabolism. However, studies disagree on whether lower or higher baseline cingulate metabolism is associated with response. For example, response to paroxetine was associated with lower baseline anterior cingulate metabolism [43], while responders to a single SSRI (mixed) were differentiated from non-responders by higher baseline rostral anterior cingulate (area 24a/b) metabolism. Remission in response to inhibition of monoaminergic uptake has been associated with reduced baseline midbrain metabolism but not in the anterior cingulate [44] and response to other weak or mixed monoaminergic inhibitors such as bupropion and venlafaxine has been associated with hypometabolism at baseline in frontal and temporal regions [45].

Changes in metabolism following treatment in responders have been examined as a means to investigating the regional and functional mechanism of action of antidepressants [46]. Sertraline response is associated with normalization of amygdala

and subgenual cingulate cortex (BA24/25) metabolism, while response to paroxetine is associated with normalized (increased) metabolism in the ventrolateral prefrontal cortex and orbitofrontal cortex but not other regions abnormal at baseline (dorsolateral prefrontal cortex or in the inferior frontal gyrus) [47, 48]. These studies implicate a ventral prefrontal and subcortical circuit in mediation of the response to chronic inhibition of 5-HT uptake in MDD. These SSRI-induced changes appear to be specific to MDD as response to the same dose and duration of treatment with paroxetine in non-depressed subjects with obsessive-compulsive disorder resulted in reduced metabolism in a different set of regions compared to an MDD group, including the right caudate nucleus, thalamus, right ventrolateral prefrontal cortex and bilateral orbitofrontal cortex [49]. Thus, metabolic changes in response to SSRIs are likely to vary, not only with degree of response to SSRI treatment, but also with baseline pathophysiology prior to treatment. It is unclear whether these SSRI-induced effects on metabolism are specific to inhibition of 5-HT reuptake without studies involving a range of antidepressants from multiple pharmacological classes with adequate power to compare effects between classes. However, changes observed in responders to other mixed monoaminergic inhibitors appear to present a somewhat overlapping regional profile of metabolic changes. For example, response to bupropion is associated with reduced anterior cingulate, medial prefrontal cortex and right anterior insula metabolism [50]. Interpretation of these findings is complicated by a number of factors (see below) but nonetheless they contribute to and corroborate the circuitry involved in mood regulation during depression and recovery.

In addition to metabolism and blood flow, quantitative EEG and MRS have been used to study treatment-related brain changes in responders.

Quantitative EEG has been reported to have 72–88% accuracy in predicting response to antidepressant treatment (for a review, see Hunter et al. [51]). Response to SSRIs has been examined using [$^{1}$H]MRS and GABA concentrations, which are reduced at baseline in depression and normalized following effective treatment with SSRIs [52, 53]. The N-acetyl aspartate/creatine, myoinositol/creatine and choline/creatine ratios did not differ between controls and drug-naïve first-episode MDD subjects but the myoinositol/creatine ratio was elevated following 8 weeks of antidepressant treatment suggesting a possible role for non-neuronal cells such as glial cells in the action of antidepressants [54]. Reduced white matter energy metabolism has also been reported at baseline during depression by measuring high energy phosphate compounds including nucleotide triphosphates and phosphocreatine using [$^{31}$P]MRS. In grey matter, 12 weeks of sertraline administration to geriatric subjects who were depressed is reported to result in normalization of tissue pH ([$^{31}$P]MRS) [55].

Molecular imaging employed in the study of treatment response provides a practical example of in vivo imaging informing preclinical drug development. The level of occupancy of 5-HT transporters necessary to achieve a response to a range of SSRIs

is estimated to be 80% in vivo in the human using PET imaging [56]. Thus, SSRIs under development with a significant side effect profile in rats, at doses that do not achieve this level of occupancy, are less likely to be effective at tolerable doses in vivo in humans. These findings have ultimately reduced time and expense involved in preclinical SSRI development by the early elimination of poor candidates. In addition, the 5-$HT_{1A}$ antagonist pindolol was shown to achieve minimal occupancy at doses of 5 mg or lower in vivo and subsequent trials at 7.5 mg show increased antidepressant efficacy relative to earlier lower-dose trials [57, 58].

In addition to occupancy studies, molecular imaging has been employed to examine the relationship between the levels of various receptors and response to antidepressant treatment. However, too few of such studies have been carried out to gauge their potential impact at present. Response to nefazodone, a 5-HT and norepinephrine uptake inhibitor with antagonist activity at 5-$HT_2$ and $\alpha_1$-adrenergic receptors, has been associated with reduced 5-$HT_2$ receptor but not $D_1$ receptor availability in right mesial frontal and left parietal regions following 6 weeks of treatment in 3 subjects with depression and anger attacks. More recently, [$^{11}$C]verapamil and PET have been used to examine the function of the blood-brain barrier molecular efflux pump, p-glycoprotein in MDD [59]. p-glycoprotein function was increased in a group of MDD subjects exposed to antidepressant medication relative to healthy controls. However, it is yet to be elucidated whether this is an effect of the medications involved or a component of the aetiology of depression.

Structural imaging studies have suggested that lithium may be neuroprotective and may promote neurogenesis. In depressed bipolar subjects, increased prefrontal and left subgenual prefrontal cortex grey matter was detected following 4 weeks of lithium treatment in responders versus non-responders [60]. Another study showed lithium exposure prevented volume loss in the subgenual prefrontal cortex and finally, another group showed prevention of volume loss in the dorsolateral prefrontal cortex. MRS using lithium-7 [61] has shown that cerebral lithium concentrations correlated with antimanic responses and [$^1$H]MRS has suggested lithium levels may be related to changes in the combined glutamate-glutamine-GABA peak magnitude [14]. However, clinical rather than imaging predictors of response to lithium have been much more extensively studied [62]. Indeed, many of the molecular effects of lithium cannot be studied directly in vivo currently due to the lack of radioligands for such targets. The efficacy of the mood stabilizer, valproic acid could potentially be related to epigenetic effects such as histone acetylation and DNA methylation [40]; however, examining such modification is also beyond the scope of currently available in vivo neuroimaging methods. Finally, response to anticonvulsants in depressed bipolar subjects has been associated with baseline metabolism. For example, carbamazepine and nimodipine responses correlate with baseline metabolism in the left insula [63]. Clinical predictors implicated including lithium unresponsiveness should perhaps be combined with future imaging studies aimed at identifying predictors of response to carbamazepine.

A number of studies have attempted to identify imaging predictors of response to non-pharmacological, non-psychotherapy-based somatic treatments for depression such as electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS), vagal nerve stimulation (VNS) and deep brain stimulation (DBS). The most commonly employed of these, ECT, results in a bilateral reduction in anterior and posterior frontal metabolism in most, but not all studies [64]. However, just 2 of these studies examined, and 1 detected, a relationship between the reduction in metabolism and improvement in symptoms. Adverse effects of ECT including anterograde amnesia have been associated with hippocampal atrophy possibly associated with increased membrane turnover in the hippocampus suggested by bilaterally increased choline-containing compounds measured using [$^{1}$H]MRS. In addition to glucose metabolism, a 2-fold increase in GABA concentration was associated with response to ECT by comparing pre- and post-treatment levels, implicating GABAergic involvement in the therapeutic effect of ECT [65]. ECT additionally increases PET-[$^{123}$I]iomazenil binding to $GABA_A$ receptors [66] and may reduce anterior cingulate $D_2$ receptor, assessed using PET and [$^{11}$C]FLB-457. Many of the elements of signalling cascades activated and potentially involved in antidepressant response to ECT are not targets we can currently measure using in vivo imaging. Replication and great sample sizes would aid in elucidating whether metabolism or GABA levels may represent a reliable predictor of response to ECT.

TMS may induce similar signalling cascade activity without the necessity for the seizure induced by ECT [67]. However, it remains unclear whether TMS may have effects similar to those observed following ECT on the GABAergic system. Low versus high TMS has been suggested to differentially alter metabolism and potentially mood [68, 69]. Dorsolateral prefrontal TMS in healthy subjects increases blood flow in anterior cingulate, medial and ventrolateral prefrontal cortices and ventral striatal regions. However, lower doses than those used in the latter studies applied in a similar region were observed to reduce metabolism in frontal brain regions. Thus, baseline metabolism or blood flow may act as a predictor of the frequency of TMS that will be effective in depression. A single study has demonstrated altered limbic 5-HT synthesis in healthy volunteers following left dorsolateral prefrontal cortex stimulation relative to stimulation over the left occipital cortex with reductions in the left parahippocampal gyrus and right insula and increases detected in the right cingulate gyrus and cuneus. Acute effects, and an absence of effect of TMS on dopaminergic signalling, have been reported with left but not right dorsolateral prefrontal cortex stimulation using [$^{11}$C]raclopride-PET. Interpretation of these studies is significantly hampered by heterogeneity in the location of stimulation applied, frequency (Hz), duration and regimen of treatments investigated.

The bilateral vagus nerves (cranial X) provide afferent input to the central nervous system that modulates mood and behaviour in general. VNS, approved for the

treatment of medication-resistant depression, appears to alter blood flow in a dose/intensity-dependent manner [70] in limbic regions, orbitofrontal and medial frontal cortices [71, 72], reduce ventromedial prefrontal cortex glucose metabolism [73], increase cerebrospinal fluid GABA and 5-hydroxyindoleacetic acid concentrations and reduce glutamate levels [74]. The neuroimaging studies conducted to date provide the first pieces of evidence for altered limbic brain activity and the involvement of GABA, glutamate and 5-HT signalling in the mechanism of action of VNS. Target regions for DBS implants in refractory depression have included the nucleus accumbens, shown to alleviate anhedonia in 3 patients while stimulation is turned on [75], and the subgenual cingulate cortex, resulting in a sustained remission in 4 of 6 patients again using chronic stimulation [76]. In treatment-resistant depression, metabolism prior to surgery was elevated in the ventral anterior cingulate cortex (BA25) and reduced in the dorsal anterior cingulate cortex and antidepressant response was associated months later with normalization of these metabolic rate abnormalities in both the dorsal and ventral anterior cingulate cortex. In the case of VNS and DBS, patient samples are medication-resistant and potential predictors of response implicated by imaging studies may not generalize to all depressed populations.

*Neurosurgery*

Neurosurgery in the past has targeted the cingulate cortex using anterior cingulotomy, and response to the procedure has been associated with higher baseline metabolism in the subgenual prefrontal cortex and thalamus, although this has not yet been replicated. In addition, post-procedure metabolism in the subgenual cingulate cortex correlated with the improvement in depression severity scores [77]. Other neurosurgical targets with mixed efficacy have included orbitomedial lesion, subcaudate tractomy, and anterior capsulotomy [78] interrupting fontal-limbic white matter communication pathways.

*Non-Pharmacological, Non-Somatic Treatments*

Non-pharmacological and non-somatic therapies such as sleep deprivation, psychotherapy, cognitive behavioural therapy and intrapersonal therapy have received some attention in terms of imaging studies attempting to identify potential predictors of response. Recovery from depression following sleep deprivation has relatively consistently been associated with elevated baseline cingulate metabolism relative to non-responders [79, 80] and with normalization of glucose metabolic rates in anterior cingulate cortex (BA24), that persisted following addition of paroxetine treatment in 2 further studies [81]. In contrast, in healthy volunteers and non-responding depressed patients, cingulate metabolism did not change following sleep deprivation [80]. In

addition to metabolism studies, [$^{1}$H]MRS has been applied before and following sleep deprivation therapy. The reduction in anterior cingulate Glx peak (glutamate, glutamine and GABA) in responders correlated with improvement in depressive symptoms following combined treatment using sleep deprivation and light therapy. Finally, elevated dopamine release was detected in sleep deprivation responders relative to non-responders using SPECT and [$^{123}$I]IBZM [82].

The in vivo correlates of response to talk-based therapies such as cognitive behavioural therapy or intrapersonal therapy have received far less attention to date. One functional MR study detected an association between response to cognitive behavioural therapy and low baseline sustained reactivity to emotional stimuli in the subgenual cingulate and high baseline reactivity in the amygdala suggesting that response may be more likely in those with disrupted emotion regulation at baseline [83]. In MDD, PET studies examining glucose metabolism identified prefrontal, cingulate and temporal lobe metabolism normalized with response to intrapersonal therapy [43] and an inverse relationship between ventral frontal, cingulate and anterior insula metabolism and improvement in symptom scores in response to intrapersonal therapy [84]. Increased limbic blood flow in MDD in response to intrapersonal therapy has been corroborated by a separate neuroimaging technique and research group using SPECT and $^{99m}$Tc-hexamethylpropyleneamine oxime [85]. Distinct metabolic responses to cognitive behavioural therapy versus paroxetine treatment have been identified among responders: cognitive behavioural therapy resulted in increased metabolism in the hippocampus and cingulate cortex and decreases in the dorsal ventral and medial frontal cortex, whereas paroxetine response was associated instead with increased metabolism in prefrontal regions and decreased metabolism in the cingulate cortex and hippocampus [86], implicating distinct mechanisms of action leading to improvement in depressive symptoms in response to cognitive behavioural therapy versus paroxetine. Response to venlafaxine or cognitive behavioural therapy was associated with reduced orbitofrontal and dorsomedial prefrontal cortex metabolism. However, these treatments differentially affected metabolism in the posterior cingulate (BA29), reduced metabolism was associated with response to cognitive behavioural therapy and increased metabolism with response to venlafaxine. Unique in the responders to cognitive behavioural therapy was a reduction in metabolism in the thalamus, and an increase in the subgenual cingulate/ventromedial frontal cortex (BA32) [87, 88]. One study has examined differences in responders to cognitive behavioural therapy versus psychotherapy and using a path modelling meta-analysis identified limbic-cortical and cortical-cortical connectivity path differences as differentiating between the two [89].

The variety in methodological approaches employed in these studies adds to the challenges of replicating any potential predictors of response identified. Refinement and consensus on defining remission would be useful in comparison across studies. Variation in dosing regimens and medication classes involved additionally makes interpretation difficult. Large-scale neuroimaging studies that have the power to

compare between treatment types, as well as between responders and non-responders are likely to be fruitful in progressing the field toward the identification of clinically useful predictors of response. Challenges for these studies into the future are most likely to include accounting for variation in outcome due to genotype, and genotype-by-treatment interactions.

### The Future of Neuroimaging in Benefiting Individuals with Depression

It is clear from the range of studies published to date that advances in brain imaging acquisition and analysis techniques have experienced a fast growth rate over the past couple of decades. Subsequently, the picture of the neurochemical pathophysiology of depression we understand today has been transformed by an increasingly complex and broad-ranging spectrum of molecular disruption associated with clinical depression. The variance across individuals in many quantitative neuroimaging parameters is an impediment to the development of clinically useful classification tools. Technical advances that improve sensitivity may reduce this variance but a major contribution comes from factors specific to the individuals scanned. These include gender, age, genetic liability and make-up, environment, stressful and early life events, illness profile and course, mood state at the time of scanning, previous treatment exposure, psycho-education, social support networks, family circumstances as well as indirect genetics factors that influence individuals' personality factors and resilience including optimism, neuroticism, intrapersonal sensitivity, obsessive tendencies, and adaptability or flexibility. Indeed, even entirely external forces of nature influence imaging studies such as average daily sunshine hours (and therefore potentially the latitude a study is carried out at), which was recently reported to influence 5-HT transporter binding levels across the year. Taking all such factors into account will become increasingly difficult if not impossible and will eventually require 1 of 2 strategies to be adopted to answer a given question: large-scale, longitudinal population-based studies aimed at prodrome characterization, involving genotyping, and examining imaging traits versus state pathophysiology [90] or hypothesis-driven approaches with narrowly defined inclusion criteria. However, selecting a well-defined homogenous patient sample while scientifically informative, may thwart generalizability of findings to presenting clinical populations.

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# Animal Models of Depression – Where Are We Going?

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**Abstract**

The wide spectrum of disruptions that characterises depression and bipolar illness highlights the difficulties researchers are posed with as they try to mimic these disorders in the laboratory. Nonetheless, numerous attempts have been made to create rodent models of mood disorders, or at least models of the symptoms of depression. However, despite many advances, there are no satisfactory animal models available. The need for improved animal models for identifying new antidepressants and providing insights into the neuropathology underlying the disease is critical. Currently, there is a shift away from traditional animal models to more focused research dealing with an endophenotype-style approach, genetic models and studies, and incorporation of new findings from human neuroimaging. Such approaches are opening up more tractable avenues for understanding the neurobiological and genetic bases of these disorders. Further, advances in the clinical dissection of psychiatric illnesses using molecular genetics coupled with functional neuroimaging techniques promise to yield better translational animal models and hence more fruitful therapeutic targets.



'A major problem in the search for new antidepressant drugs is the lack of animal models which both resemble depressive illness and are selectively sensitive to clinically effective antidepressant treatments.' So opens the seminal *Nature* paper by Porsolt et al. [1], which introduced the forced swimming test (FST) model of antidepressant action. However, despite over 30 years of progress, this statement remains as true today as it did when Porsolt believed that the FST would bridge the gap from preclinical research to humans. Major depression is a leading medical and social problem affecting over 340 million people worldwide and is predicted to become the second most common cause of global disease burden by 2020 [2]. While antidepressant treatment can effectively improve the quality of life of patients, they all work with a delayed onset of action and 30–40% of patients do not respond to treatment [3]. A

variety of compounds with novel mechanisms of action and very promising preclinical data supporting an antidepressant-like signature have undergone clinical testing and failed to gain approval for use [4]. There are a number of reasons for the failure of novel antidepressants including the high placebo rate, and the arbitrarily set diagnostic criteria (for a review, see Frazer and Morilak [5]). Another reason is the lack of direct translation of results from preclinical animal models to patients, which has led in the last few years to a number of different approaches being employed. Criticism has been directed towards the 'traditional' preclinical models, which can often be better described as tests of antidepressant activity (table 1). However, many of these paradigms have greatly advanced our understanding of depression. Additionally, research in the clinical arena has slowly become more sophisticated and is providing potential avenues to incorporate into preclinical paradigms. These emerging findings will not only enable closer comparisons to be made but also to determine whether findings from preclinical models remain true in humans. With this in mind there are a number of new research directions being taken currently, which aim to provide more information regarding the pathophysiology of depression and ultimately lead to antidepressants with novel mechanisms of action. These approaches are discussed in the following review, which include endophenotype-based and social stress-based models, genetic and translational approaches.

## Traditional Animal Models of Depression

Depression is a multifaceted, heterogeneous disease and as such there are a variety of different symptoms, often occurring in opposite directions, for example appetite, which constitute the current diagnostic manuals (DSM-IV and ICD-10; table 1). A perfect situation for an animal model would be to have identical causative factors, symptomatology and treatment modalities [6]. However, a number of symptoms of depression are clearly not measurable in preclinical paradigms, such as recurrent thoughts of death or suicide, or excessive thoughts of guilt. Additionally, given the paucity of understanding concerning the causative factors, and the pathophysiology of the disease state in humans, this occludes basing animal models solely on aetiology [6].

Despite these difficulties, a number of diverse animal models of depression have been widely utilised and many show substantial construct validity (i.e. antidepressant administration reverses the behavioural parameters assessed). These animal models are often referred to as tests of antidepressant-like activity given that they have all been validated since the introduction of clinically approved medications [7]. This also renders the predictive validity of the models unclear until a novel-acting compound from preclinical testing is successfully applied in man. There have been numerous reviews written regarding the majority of these animal models, which are summarised below and in table 1.

**Table 1.** Animal models of depression: benefits and drawbacks (adopted from Cryan and Slattery [4])

| Model | Advantages | Disadvantages |
|---|---|---|
| Forced swimming test/tail suspension test | Ease of use, high throughput, responsive to ADs, interlaboratory reliability | Acute response, model of antidepressant action, reliant on motor function |
| Olfactory bulbectomy | Requires chronic AD, translational neurochemical and structural changes | Not widely utilised, behaviour mainly relies on motor function |
| Reward paradigms | Respond to some antidepressants, analogous to core symptom of depression | Not well validated, low throughput |
| Early-life stress | Early-life stress increases MDD likelihood in humans, deficits in reward | Low reliability, low throughput |
| Chronic mild stress | Stress increases chance of MDD in susceptible patients, requires chronic antidepressant | Low reliability, low throughput |
| Social stress paradigms | Face validity, respond to chronic AD treatment, mimic numerous endophenotypes | Low throughput, differ between species |
| Learned helplessness | Responds to some ADs, causes depression-like symptoms | Requires extreme stressors, not all animals become helpless |
| Selected breeding/inbred strains | Enables comparison of susceptible and resistant animals | Low throughput, expensive |
| Drug withdrawal paradigms | Face validity, responsive to ADs, model core symptom of depression | Low throughput, expensive |

AD = Antidepressant.

The most widely utilised preclinical tests employed are the FST and the tail suspension test (TST). These tests are based on the observation that when a mouse or rat is placed in an inescapable situation [i.e. cylinder filled with water, or suspended by their tail (only mice)], after initial escape-directed behaviour, the animals quickly adopt an immobile posture [8, 9]. This switch in behaviour is hypothesised to reflect either a failure to persist with escape-directed behaviour or a passive behaviour to cease active forms of coping with the stressful stimuli [4, 6, 10]. Despite differences in the tests between mice and rats, these tests have proven to be highly selective for

antidepressants, with all clinically effective drugs prolonging the time that animals spend in active behaviours. While antidepressants are effective in these tests, it is becoming more apparent that different biological substrates underlie the 2 tests [4, 8]. For example, $GABA_B$ receptor antagonists and knockout mice have been shown to have antidepressant-like properties in the FST but not TST [10]. This emphasises the importance of employing more than 1 test in preclinical depression research. One of the main criticisms of these tests is that they are sensitive to acute antidepressant treatments, whereas in the clinical setting long-term treatment is required to induce therapeutic effects. Moreover, there is very little neurobiological evidence focused on the mechanisms of how antidepressants elicit their effects in these tests. However, recent data indicate that the hippocampus and infralimbic cortex are crucial for FST-induced effects [11, 12].

The learned helplessness model of depression utilises a similar set of behaviours as that in the FST and TST. However, in this instance, animals are presented with inescapable shocks, which, in a subset of animals, result in a reduced number of escape attempts when confronted with an escapable situation. Unlike the FST and TST, pharmacological reversal of helpless behaviour requires at least 3–5 days of antidepressant administration. This makes the model more attractive in some eyes, as it more closely resembles the clinical situation. However, it can be difficult to establish in the laboratory and while it can be beneficial to study the differences in animals that show helpless behaviour versus resistant animals, this may not always be the aim of a study. Additionally, the depressive symptomatology does not persist beyond a few days following the shocks.

The removal of the olfactory bulbs has been shown to result in numerous behavioural, neuroendocrine, neurochemical and immune alterations which are observed in depressed patients [6, 13]. However, these changes are not primarily due to the loss of smell as inducing anosmia (i.e. via zinc sulphate) does not result in the constellation of changes induced by olfactory bulbectomy (OB). Thus, many of the changes observed including enlargement of the ventricular system, 5-HT hyper-innervation of the frontal cortex, and disinhibition of the amygdala are specific to OB. The main behavioural readout following removal of the olfactory bulbs is hyperactivity in a brightly lit open field. These changes are only reversible following chronic (>14 days) antidepressant treatment, which is a clear advantage over tests that are responsive to acute treatment. Despite the questionable face validity of the test, the resultant changes and pharmacological profile of OB make it a highly attractive model for studying the underlying aetiology of depression. Recently, we have shown, using intracranial self-stimulation (ICSS), that OB animals have marked reward deficits making it an interesting paradigm for studying anhedonia (see below). Further research focusing on how antidepressants reverse the behavioural, physiological and immune changes will allow for greater utility of the model.

The chronic mild stress paradigm is based on the initial discovery by Katz [14] that exposure to severe predictable stressors led to an anhedonic phenotype in rats.

Reducing the severity of the stressors (e.g. social instability, 24-hour light exposure, restraint, food and water deprivation) but prolonging their duration resulted in the chronic mild stress paradigm. This retains the end readout of anhedonia, which can be assessed using sucrose preference or sensitivity to drugs of abuse, but is more ethical to perform. The resulting anhedonia is long-lasting and can be reversed only by chronic treatment with antidepressants. However, there is controversy in the literature surrounding the reliability and robustness of the model, and it seems that employing numerous different stressors can reduce interlaboratory comparability [15].

The majority of the tests described above are stressor based and while there is evidence for a link between stress and depression, this does not always follow. Another problem with animal models is the requirement for repeatability between laboratories, which implies strictly controlling all possible elements of the test. However, even if all factors within different laboratories are controlled as tightly as possible, the results can often be divergent [16]. Additionally, as was shown with the recent modifications introduced to the traditional FST, a degree of flexibility is required to refine the model [6, 17]. Ultimately, validation of the utility of such animals awaits the clinical approval of a novel compound, which is active in the models.

## Endophenotype-Style Approaches

The majority of the more traditional models of depression, such as the FST and TST, do not possess strong face validity (i.e. similar symptoms to the human situation). However, there are a number of different symptom clusters that can be relatively easily modelled in rodents. This has led to an endophenotype-based approach to study psychiatric disorders in preclinical laboratories, which attempt to assess only 1 symptom, or marker, of the disease rather than the whole syndrome [6, 18]. This has the benefit of simplifying a complex disorder, such as depression, into individual behaviours, which are more easily measurable in both patients and laboratory animals. By assessing an individual symptom, it also reduces the likelihood of interlaboratory variations and the number of genes that underlie the measured readout. An additional benefit of this approach is that the model may lead to increased understanding of a symptom relevant for a number of psychiatric disorders, given their substantial comorbidity. However, this may reduce the translation of the findings to the human disorder, but a more endophenotype-based approach in clinical studies may also be beneficial for uncovering the aetiology of specific symptom clusters. Indeed, in the upcoming DSM-V, there is a proposal to include 'mixed anxiety depression' as a new addition to the mood disorders section (www.dsm5.org). There exist a number of criteria, which have been proposed to evaluate the relevance of different endophenotypes, including specificity to the disease of interest, heritability and biological

relevance to the disease state [18]. Therefore, depression can be broken down to a number of symptomatic clusters and assessed independently in basic research. The endophenotypes of depression that can be easily modelled in animals include psychomotor alterations, anhedonia (low mood), appetite and weight alterations and cognitive deficits.

### *Anhedonia*

Anhedonia, the loss of pleasure or interest in normally rewarding stimuli, represents an extremely feasible symptom to study, both in humans and in preclinical research, which coupled with it being a core symptom of depression makes it an attractive proposition. Anhedonia has been used as a behavioural endpoint for a number of the existing animal models of depression, such as chronic mild stress and maternal separation, with sucrose preference being measured [19].

It is possible to study anhedonia following particular manipulations, genetic or environmental, utilising paradigms that were initially used in the field of drug addiction, for example, progressive ratio responding or ICSS. Numerous researchers have used such readouts to assess the effect of stress exposure or withdrawal from drugs of abuse [20] on this endophenotype of depression. Moreover, two recent studies have combined the OB model with ICSS. While anhedonia was observed immediately after bulbectomy, this lasted only 7 days [19] and conflicting results were observed in relation to the ability of cocaine to reduce the ICSS response threshold [21]. More extensive use of the ICSS procedure in preclinical research would provide invaluable insights into the time course of alterations to the brain reward system and anhedonic-like behaviour.

Recent studies have shown that the reward system appears to be hypersensitive in severely depressed patients [21–24]. The hypersensitivity was associated with decreased activity of a number of regions of the reward circuitry as well as cortical regions following drug administration. These findings resemble the findings from recent animal studies revealing increased cocaine preference following chronic stress exposure [25]. Interestingly, in this study, increased brain-derived neurotrophic factor signalling in the ventral tegmental area-nucleus accumbens pathway was found to underlie, at least in part, the depressed phenotype. Thus, converging evidence from rodent and human studies supports an alteration in the brain reward system in the aetiology of rodent and human models. Further study of the brain reward system in both human and preclinical research could greatly increase the understanding of anhedonia in depression.

A number of studies have also shown that depressed patients display decreased brain activity when processing positive, but not negative stimuli compared with controls, particularly in the orbitofrontal cortex [26, 27]. This region has been shown in numerous human and primate studies to be important in reward expectation and

value [28–30]. This suggests that depressed patients exhibit decreased cognitive processing of positive stimuli despite having intact behavioural responses in this relatively easy task.

It is possible to conceive of similar tasks, which could be applied in preclinical research to assess such response biases and brain activity. A task favouring a reward for a particular stimulus could then be the final readout following behavioural, genetic or pharmacological manipulations, which would enable greater insight into the circuitry involved and a closer correlation with human studies. Recently, such an approach has been investigated with much promise in the learned helplessness model [31].

### *Cognitive Function*

Cognitive impairments, including decreased ability to concentrate, decreased learning and memory and deficits in executive function (the ability to deviate from a stereotyped response to stimuli), are observed in depressed patients; indeed decreased concentration is a DSM-IV criteria. Although impairments have been reported in attention, working memory, verbal fluency and planning in depressed patients, such findings are not always consistent (for a review, see Rogers et al. [32]). This is likely to represent the heterogeneity of the patients used in the tests as well as methodological differences. The observed deficits in cognitive function can be partially explained by neuroimaging studies demonstrating decreased activity in many regions of the frontal cortex, as executive function has been shown to be dependent on these regions [32, 33]. Additionally, the hippocampus has been shown to be involved in long-term memory retrieval and decreased hippocampal volume is amongst one of the most consistent findings in human studies. The hippocampal volume loss is also dependent on the duration of major depressive disorder (MDD); rather than the severity of the symptoms [34]. Repetitive stress in rodents has been shown to cause dendritic shortening in the CA3 field of the hippocampus, as well as in the medial prefrontal cortex but increased dendritic growth in the amygdala [35].

Increased understanding of this phenomenon could lead to increased understanding of the volume loss and decreased activity seen in the frontal cortex, or the increased activity in the amygdala of MDD patients.

However, to date, cognition has not been greatly studied in preclinical depression research. The use of tasks, such as the 5-choice serial reaction time task, in which rats must nose-poke 1 of 5 chambers, in which a light stimulus is randomly allocated to receive a food reward, provides information about attention and motivation. Translational touch screen-based cognitive tests are also being developed which parallel clinical cognitive testing [36]. Together, approaches will be used to assess cognitive effects of behavioural or genetic manipulations, which are purported to relate to the pathophysiology of depression.

*Sleep Disturbances*

Sleep disturbance is a key aspect of depression, with 90% of patients suffering from MDD complaining about their sleep, and can be the reason why they search out medical help [37]. Although sleep disturbances are common in numerous psychiatric illnesses, the high level of disturbance seen in MDD suggests that study of the sleep architecture could shed light on the underlying pathophysiology of depression. Additionally, almost every clinically approved antidepressant, as well as electroconvulsive therapy, increases REM latency and decreases total REM sleep as well as having effects on overall sleep architecture (for a review, see Wilson and Argyropoulos [38]). In support, agomelatine, the first approved antidepressant, which is not thought to elicit its effects primarily through monoaminergic effects, modifies sleep architecture via the melatonin system. Additionally, the monitoring of sleep patterns in rodents using EEG techniques demonstrates strong predictability, with differing classes of psychoactive compounds displaying commonalities. Therefore, novel compounds can be examined and compared with a large database of compounds for their effect on sleep architecture, which may indicate the likelihood of an antidepressant-like effect (see also inbred section). Similarly, knockout mice can also be employed to determine the role of a specific protein in sleep architecture [39]. Increased utilisation of sleep monitoring may help to generate more confidence in specific models and gain further insights into this endophenotype of depression. In addition, sleep disturbances and circadian dysfunction are hallmarks of bipolar disorder. Recently, McClung and colleagues [40] have shown that mice that lack the *clock* gene have many of the hallmarks of manic depression that can be reversed by the mood stabiliser lithium.

## Depression and Comorbid Indications

Depression is often reciprocally associated with cardiovascular disease; for example, depression can follow a heart attack as well as give rise to enhanced risk for cardiovascular disease [41, 42]. However, very few studies have assessed cardiovascular function in animal models of depression. It has been demonstrated that OB rats exposed to an open field display not only hyperlocomotor activity but also elevated heart rate and blood pressure. These behavioural and physiological symptoms are normalised following chronic antidepressant administration [43]. Further studies may help to elucidate potential mechanisms which link depression and cardiovascular diseases [44]. Additionally, substantial comorbidity exists between MDD and functional bowel disorders such as irritable bowel syndrome. Recent studies have focused on characterising early-life stress and genetic models of such conditions, which may offer novel insights into developing therapies for both disorders [45, 46].

## Social Stress-Based Models

There has been a resurgence of interest in developing more stress-relevant animal models than the majority currently employed, due to the lack of understanding of the aetiology and discovery of truly novel-acting pharmacotherapies for stress-related disorders such as major depression [47]. Therefore, given the evidence purporting social stress to be a risk factor for the development, not only of cardiovascular diseases, but also of depression and anxiety in vulnerable individuals [48], recent attempts have focused on the development of social stress paradigms [25, 49, 50]. Such paradigms are believed to be more relevant to the human situation than non-social stress paradigms (e.g. repeated restraint [4]). Social defeat [49], chronic subordinate colony housing in mice [53] or chronic subordination in tree shrews [51] have been shown to result in numerous behavioural and physiological consequences, which closely resemble those resulting from chronic stress in humans. These alterations are also believed to persist for a long time after the termination of the stressor. Chronic social defeat was shown to alter anhedonia-type readouts, cocaine and sucrose preference, but interestingly not to alter behaviour in traditional tests such as the FST and TST [25]. Such findings demonstrate the need in preclinical models to assess multiple behavioural readouts. A major advantage of chronic social stress paradigms, used in rodents or tree shrews [51], is that the time required for therapeutic efficacy more closely resembles that required for clinical benefit of current antidepressants [3]. Thus, social stress paradigms can provide insight into novel factors involved in the aetiology of stress-based disorders. Such models have enhanced our knowledge of underlying molecular maladaptations caused by chronic stress exposure, and continue to so.

## Genetic Predisposition

Genetic factors play a significant role in the development of MDD and are also known to interact with environmental factors. A meta-analysis performed by Sullivan et al. [52] reported that the heritability of major depression is approximately 30–40%, which the authors suggest is likely to be underestimated due to current diagnostic criteria and incomplete data sets. In some individuals, major depression appears to be preceded by stress, while in the majority of cases such stress does not lead to the development of depression [53]. Similar variation to stressors has also been shown in preclinical studies, with some animals displaying high or low behavioural responses. For example, approximately 70% of rats exposed to inescapable stressors subsequently display helpless behaviour [54]. These observations have formed the basis of a number of selective breeding programs, where high or low responders have been selected and bred together. Moreover, it has been shown that when these rodents are selectively bred, the progeny display similar behaviours in the test with increasing frequency, which leads to the generation of divergent lines possessing 'depressogenic' or

'resistance' traits. A number of such lines have now been assessed and the results suggest that such an approach can lead to important information regarding the underlying pathophysiology of depression.

### *Inbred Strains*

It has been well described that strains of rats and mice behave differently in numerous behavioural paradigms and responses to drugs [55–59]. This has led to several strains being suggested to display depression-like phenotypes, which in the case of rats include the Wistar-Kyoto (WKY) and Fawn-Hooded strains. WKY rats demonstrate numerous behaviours which are akin to depressed patients, including hyperresponsivity to stressors, altered hypothalamic-pituitary-adrenal axis activity [60], increased immobility time in the swim test [61, 62] and increased anxiety-like behaviours [63, 64] compared with other strains. Additionally, WKY rats appear to have altered responsivity to antidepressant administration compared with other strains, although both reduced [65, 66] and increased [67] response to tricyclic antidepressants have been reported. However, and similar to the heterogeneity of the human situation, larger behavioural and genetic variations have been reported within this strain, which is unusual for an inbred strain [62] and may reduce the opportunity of determining genetic influences on the behavioural phenotype.

The alcohol-preferring Fawn-Hooded rat strain is also purported to reflect an inbred strain, and also demonstrates depression-like behaviour. Fawn-Hooded rats display a high immobility time in the FST [65, 68] compared with other strains. This is of interest given the high comorbidity between depression and drug addiction, including alcoholism and the theory that similar neurochemical abnormalities may underlie both disorders [69]. Recently, these behavioural traits of Fawn-Hooded rats were shown to dissociate, as chronic treatment (2 mg/kg) with the mGluR5 receptor antagonist, MTEP, reduced alcohol intake but did not affect anxiety or depression-related behaviours [70]. This separation of traits may relate to the dose and tests that were used or alternatively it means that mGluR5 receptor antagonism may not represent a novel antidepressant therapy. However, recent studies have demonstrated an antidepressant-like effect of mGluR5 receptor antagonism in outbred strains.

### *Genetic Manipulation*

A main reason for the large increase in the utilisation of mice in preclinical testing in the late 1980s/early 1990s was the ability to generate mice in which specific proteins could be ablated. This led to an explosion of mice research, which assessed the impact of knockout of a specific receptor, enzyme or transporter on behaviour.

Such mice provide insight into receptors where there is a lack of pharmacological agents and have aided discovery of numerous potential targets underlying depression, which may give rise to novel antidepressants. However, care must be taken when using knockout mice, as compensatory changes can occur due to the lifelong ablation of a protein and it may in fact be such alterations that result in the behavioural phenotype. More recently, inducible and site-specific knockouts have been generated, which enable the role of proteins to be assessed in adult mice negating the compensatory effects. Similar strategies can also be used to knock-in specific genes, which lead to an overexpression of the protein. The implication of more and more mouse behavioural models has also expanded the strength of genetically-modified mice and as such they represent a powerful tool with which to study the role of specific proteins in depression. The recent successful administration of short-interfering RNA in vivo [71, 72] has opened the possibility of selectively downregulating a target gene in a temporal and spatial fashion. Although a similar technique to that of antisense oligonucleotide administration, to date short-interfering RNA does not appear to be associated with the side effects of the former, which include immune system activation. Additionally, the success rates for the design of effective short-interfering RNA constructs appear to be much greater than those associated with antisense oligonucleotide design (for a review, see Thakker et al. [73]). Therefore, if a specific protein is postulated to be involved in the pathophysiology of depression, from either animal studies or from clinical studies, short-interfering RNA administration could be used to assess the behavioural effects of decreasing the protein. Similar studies have been performed using viral vectors, and such genetic manipulations can provide important information regarding the role of a specific target in both the aetiology and treatment of depression.

### Incorporation of Human Findings

A substantial barrier in preclinical psychiatric research has been the lack of knowledge regarding the cause and pathophysiology of the illness in humans. Indeed, in specific instances where there has been a very definable cause, such as the triple GGG motif present in Huntington's disease, there has been success in creating an animal model, which is a transgenic mouse expressing the human *huntingtin* promoter. Unfortunately, regarding depression there is no such information; indeed, presently there would be no consensus as to which brain regions, genes and causative factors are relevant.

Recent advances in human neuroimaging studies have identified a number of abnormalities in metabolism and volume in a variety of brain regions. Additionally, a number of studies are now finding a number of polymorphisms in certain genes, which in some cases are proposed to increase the likelihood of patients to become depressed. Complementary preclinical imaging approaches, for example c-Fos or

small animal fMRI, can attempt to relate metabolic and structural alterations in animals to those which are common in human patients.

The recent landmark finding that inactivation of BA25 using deep brain stimulation could alleviate depressive symptoms in treatment-resistant patients [74] gave rise to the possibility of examining the effects of a similar treatment in an animal model of antidepressant activity. Analogous to the human situation, inactivation of the infralimbic cortex, the rodent correlate of BA25, resulted in an antidepressant-like phenotype in normal rats [12]. More pertinently, this finding could be replicated in rats with an innate high level of depression-related behaviour, analogous to the findings in treatment-resistant patients. These results show that it is possible to replicate findings from a clinical trial in rodents. Airan et al. [11] employed a novel hippocampal imaging approach to determine that chronic stress-induced alterations in dentate gyrus function directly correlated with immobility in the FST. Moreover, such studies provide a greater understanding of the neurocircuitry underlying behavioural responses in tests such as the FST, which will benefit future studies employing such tests.

## Conclusions and Outlook

Presently, with the advances in techniques employed to study affective disorders in humans, there is an opportunity to incorporate such findings into preclinical research. In this context, while translational medicine in basic research and drug discovery efforts necessitates valid animal models of human disease, this approach is a two-way bridge. There is increasing pressure on preclinical research to provide detailed information on the relation between pharmacodynamic and pharmacokinetic measures and occupancy at relevant drug targets and suggestions for relevant biomarkers and translational measures to facilitate the entry into clinical development; however, the converse is also essential [3, 75]. The development of clinical research tools that are informed by, and can parallel rather than specifically recapitulate, rodent models of depression may well be the most fruitful path to understanding these diseases. Additionally, the increased awareness of the need for cooperation between psychiatrists and behavioural neuroscientists has led to a more endophenotype-based approach to study single symptomatic clusters. Such approaches have been employed by investigators assessing cognitive dysfunction in neuropsychiatric disorders such as schizophrenia and Huntington's disease with considerable success [76]. That said we should not neglect older traditional models as we still have a lot to learn from them in terms of the mechanism of current antidepressant action. Thus, investigations into understanding how antidepressants and chronic stress are able to induce divergent behavioural effects in such paradigms may offer insight into the circuits underlying depression and facilitate the development of novel brain circuit-targeted antidepressant approaches.

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# Genetic Models of Depression and Antidepressant Response

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**Abstract**

Scientific approaches for studying the genetics underlying depression and antidepressant responses using available animal models are reviewed. Numerous examples of genetically defined inbred rodent strains with divergent responses on tests of depressive behavior provide opportunities to identify genes that may influence the risk of major depressive disorder (MDD). Rodent strains that differ dramatically in behavioral responses to pharmacologically distinct classes of antidepressants can be used to identify genes that might predict either sensitivity or resistance to the therapeutic effects of drugs in MDD. Rodent strains with deficient monoamine synthesis have been used to study the pathology of MDD and the mechanism of drugs used to treat MDD.



Major depressive disorder (MDD) is a chronic debilitating illness that exerts a huge cost, economically and emotionally, on society [1]. The clinical symptoms required for diagnosis of an episode of MDD are: depressed mood, markedly diminished interest or pleasure in activities, significant weight loss or weight gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive or inappropriate guilt, diminished ability to think or concentrate, and recurrent thoughts of death or suicide (DSM-IV). This cluster of symptoms must be persistent, at least 2 weeks in duration. Despite the availability of a number of clinical treatments, approximately 30–40% of patients diagnosed with depression are treated ineffectively because of limiting side effects or a treatment-resistant form of the disorder [2].

Despite much research, a specific etiology for MDD is unknown. A significant genetic component underlying MDD is suggested by studies showing a higher concordance of MDD in identical twins compared to fraternal twins [3]. However, MDD has less heritability than many other psychiatric disorders such as bipolar disorder and schizophrenia, supporting the likelihood that environmental factors are also

responsible for producing MDD [4]. The presence of environmental stress is highly correlated with the onset of MDD and patients with greater reactivity to stress are more likely to develop mood disorders [5]. The etiology of MDD likely involves a combination of genetic and environmental factors that may interact distinctly for different individuals. In addition, patterns of response or resistance to the same antidepressant drug have been observed to run in families [6] and a number of genes have been associated with treatment response [7]. If the antidepressant response itself is heritable, a substantial component of treatment failures may be due to some combination of genetically predisposing characteristics.

Animal models of depression refer to experimental procedures or animal preparations which model aspects of MDD in humans. These animal models are broadly subdivided into screening models, used primarily for identifying potential therapeutic effects of established and novel compounds, and simulation models, where the emphasis is more on understanding the theoretical basis for MDD. General reviews are available that describe the various behavioral tests used to measure the effects of antidepressant treatments in animals [8–10] and stress predisposition models that are meant to simulate conditions that produce depressive behavior [11].

The goal of this chapter is to review some of the scientific approaches available for studying the genetics underlying depression and antidepressant responses using available animal models. The numerous examples of genetically defined inbred rodent strains with divergent responses on tests of depressive behavior provide an opportunity to identify genes that may influence the risk of MDD. In addition, rodent strains which show differing responses to antidepressants can be used to identify genes that might predict either sensitivity or resistance to the therapeutic effects of drugs in MDD.

## Rat Strains Showing Exaggerated Stress-Induced Behavioral Depression

### *Flinders Sensitive Line*

The Flinders line rats are perhaps the most thoroughly characterized genetic animal model of depression [12]. They were developed at Flinders University in Australia by selective breeding of outbred Sprague Dawley (SD) rats for differences in the effects of the anticholinesterase diisopropyl fluorophosphate on temperature, drinking and body weight. The Flinders sensitive line (FSL) rats are more sensitive to diisopropyl fluorophosphate and cholinergic agonists than Flinders resistant line (FRL) rats, which often serve as controls for the FSL rats. Depressed humans show elevated sensitivity to cholinergic agonists [13], which led to the original proposal of the FSL rats as an animal model of depression [14].

A large body of behavioral, neurochemical and pharmacological literature supports the claim that FSL rats present a phenotype closely resembling many symptoms

of MDD [15]. On the behavioral level, FSL rats show decreased bar pressing for water or food reward than FRL rats, mimicking the decreased pleasure-seeking behavior, or anhedonia, seen in depressed humans. FSL rats also demonstrate a 'depressive-like' pattern of avoidance behaviors, by showing a decreased tendency to actively avoid an aversive stimulus such as foot shock and an increased tendency toward passive avoidance. Furthermore, FSL rats display exaggerated immobility in the forced swimming test (FST), i.e. twice the amount of immobility compared to FRL rats. In addition, FSL rats subjected to chronic mild stress demonstrate a greater decrease in saccharin preference, suggesting enhanced vulnerability to the anhedonic effects of stress. Furthermore, the elevated FST immobility displayed by FSL rats is reduced by chronic treatment with a number of antidepressant drugs, including the tricyclics imipramine and desipramine and the selective serotonin reuptake inhibitor (SSRI) sertraline.

FSL rats also mimic depressed humans in showing greater REM sleep and a shorter interval between REM episodes than FRL rats. These differences occur in the basal state and, unlike the above-mentioned behavioral differences, do not require exposure to stressful conditions.

Because there is a substantial comorbidity between depression and anxiety, it was of interest to determine if FSL rats could model enhanced anxiety in addition to a depressive-like phenotype. However, FSL rats did not differ from FRL rats in the elevated plus maze, a well-accepted test for anxiety. Therefore, FSL rats appear to show behavioral specificity as an animal model of depression, without a prominent anxiety component.

A number of studies have attempted to understand the neurochemical differences between FSL and FRL rats that underlie their interesting behaviors. When FSL and FRL rats were crossbred to produce F1, F2 and backcross progeny, the immobility time in the FST did not correlate with the hypothermic response to an anticholinesterase, but rather correlated with the hypothermic response to 8-OH-DPAT, a 5-$HT_{1A}$ receptor agonist [16]. There have since been numerous reports of serotonergic dysfunction in FSL rats, including increased tissue levels of 5-HT and 5-HIAA, which can be normalized by chronic antidepressant treatment [15]. No genetic linkage studies have been performed with the Flinders line rats despite the fact that they provide an excellent opportunity to map genes regulating susceptibility to depression.

### *Wistar-Kyoto Rats*

The Wistar-Kyoto (WKY) rat has been proposed as an animal model of depressive behavior because it consistently demonstrates exaggerated behavioral and physiological responses to stress across a variety of situations in comparison to other strains. The WKY line was initially bred from outbred Wistar rats as the control strain for

the spontaneously hypertensive rat [17]. Compared to other strains, such as inbred Fisher 344, Wistar or outbred SD rats, WKY rats display elevated FST immobility [18, 19], high susceptibility to learned helplessness [19, 20], high emotionality and freezing behavior in response to stress and decreased exploratory activity in the open field [21]. Furthermore, WKY rats secrete greater levels of stress hormones in response to forced swimming [22] and restraint or cold stress [23] and are very susceptible to gastric ulceration [19].

In addition to an increased depressive-like phenotype, WKY rats also exhibit increased anxiety-like behavior compared to other strains. Anxiety-like responses have been measured in the open-field test [24], elevated plus maze [25], defensive burying test [19, 25], and novelty-suppressed feeding [26]. WKY rats display other behaviors indicative of increased anxiety including higher levels of basal and stress-induced alcohol consumption compared to Wistar and SD rats [27–29] and faster acquisition, combined with more intertrial interval responses, in an active escape/avoidance procedure [30] compared to SD rats. These effects suggest that a contributing factor to the WKY behavioral phenotype is increased salience attributed to aversive stimuli.

Using quantitative trait loci (QTL) analysis in the segregating F2 generation of a WKY × Fisher 344 cross [31], Redei and colleagues [31] identified multiple QTL for depressive behavior in the FST and the plasma corticosterone response to restraint stress. None of the identified QTL for behavioral despair and stress corticosterone were similar, supporting an earlier finding that separate genetic pathways are involved in behavioral stress and endocrine responsiveness in this intercross. This discovery of rat chromosomal loci regulating depressive behavior in the FST illustrates the utility of the QTL approach and has directly nominated syntenic regions of the human genome for containing depression susceptibility genes.

### *Selective Breeding of Rats*

Weiss et al. [32] selectively bred SD rats to produce rats with either high or low motor activity in a modified FST. In a 15-min swimming test, swim low-active (SwLo) rats show little struggling and much floating, while swim high-active (SwHi) rats display the reverse. Further experiments showed that these strains do not differ in daily spontaneous locomotor activity, but do exhibit activity differences in response to acute challenges such as those encountered in the open field, novel home cage and Porsolt-type swimming test. Under these conditions, SwHi rats show more active behavior, whereas SwLo rats exhibit a deficit of this response. These lines have also been tested with various classes of antidepressants in the FST. The results showed that chronic but not acute treatment with norepinephrine (NE) reuptake inhibitors and monoamine oxidase inhibitors could increase the activity of SwLo rats, but an SSRI was ineffective. No antidepressants were active in the SwHi rats [33, 34].

**Response to Antidepressant Drugs in Wistar-Kyoto Rats**

Most studies of the behavioral effects of antidepressant drugs in rats have used common laboratory strains, such as SD, Wistar or Fisher rats. Because the purpose of most studies with antidepressants has been to screen new compounds for drug discovery, most rodent strains used must be responsive to established classes of antidepressants as a reference standard. Accordingly, few differences in behavioral responses to antidepressant drugs have been reported between these rat strains or suppliers.

Concern has been raised that current screening techniques for antidepressant drugs may not be able to detect compounds with novel mechanisms. There can be no factual response to this issue until a novel antidepressant is developed that is undetectable by current screens. However, the validity of this concern may be more justifiable when considering that screening techniques are conducted mostly with a restricted set of rodents that are genetically similar and unstressed, or 'normal'. Behavioral screening procedures may have greater sensitivity for detecting novel drugs if they are applied to rodent strains that are selected for special characteristics, such as an exaggerated depressive phenotype that resembles the clinical characteristics of MDD. WKY rats exhibit hyperresponsive neuroendocrine and behavioral responses to stress that exceed other rat strains and are especially prone to develop stress-induced depressive disorder. The value of using special rodent strains may come from their increased face validity with respect to human mood disorders. This section will consider the response to antidepressant drugs of WKY rats. These rats have been examined for their response to conventional antidepressant drugs and may be suitable for measuring the effects of novel compounds.

WKY and SD rats not only differ in baseline FST behavior but also in their response to pharmacologically different types of antidepressant drugs in the FST. The noradrenergic antidepressant desipramine produced a reduction of immobility in WKY rats, just like it does in SD rats, but these effects were shown relative to the higher baseline value of immobility displayed by the WKY rats [35]. The behavioral response of WKY rats after treatment with desipramine was similar to SD rats that did not receive drug treatment, although it would be unwise to characterize this behavioral change as a 'normalization'. Changes in sensitivity to desipramine were difficult to quantify with differing baseline values, but a lower dose of desipramine was behaviorally effective in WKY rats compared to SD rats. These results are similar to the antidepressant-like effects of chronic desipramine treatment in the FST reported in WKY rats [36–38]. These results may be associated with the role of the locus coeruleus, the main source of forebrain NE, in behavioral activation and the response to stress [39]. WKY rats express genes involved in NE synthesis and metabolism (e.g. tyrosine hydroxylase and monoamine oxidase A and B) at higher levels than SD rats in the locus coeruleus [40].

A particularly interesting component of the WKY pharmacological phenotype is insensitivity to the antidepressant effects of SSRIs [18, 37, 38]. WKY rats fail to

respond behaviorally to SSRI treatment despite the fact that these compounds produce their normal neurochemical effects on extracellular 5-HT levels in the strain [41, 42]. WKY rats are also insensitive to the antidepressant-like effects of the 5-$HT_{1A}$ receptor agonist 8-OH-DPAT [18]. Inherent differences in 5-HT metabolism or 5-HT receptor distribution may underlie the differences between the strains [43].

A key recent finding has been the discovery of increased expression of the κ-opioid receptor (KOR) in the locus coeruleus of WKY rats compared to SD rats [40]. There is a substantial literature linking the dynorphin-KOR system with stress and depression. Exposure to stressors that produce end points of depression in behavioral models, such as learned helplessness and the FST, increases the release of endogenous dynorphin and induces a negative affective state, including increased anxiety and dysphoria-related behaviors [44, 45]. KOR antagonists and genetic deletion of either KORs or the prodynorphin peptide block the depressive and anxious phenotype of elevated dynorphin levels and provide strong support for the role of KOR signaling in mood states [46–48].

KOR antagonists represent a novel therapeutic target which may be beneficial in the treatment of depressive and anxiety disorders. However, the effects of systemic administration of KOR antagonists have been difficult to measure in behavioral models. Using WKY rats, systemic administration of KOR antagonists was shown to produce antidepressant-like effects in the FST, whereas they were inactive in SD rats [49]. Utilizing topographical differences in cellular activation between the strains, the KOR antagonist nor-BNI was shown to increase c-fos expression in the shell of the nucleus accumbens and the piriform cortex in those rats that demonstrated antidepressant behavioral effects in the FST. In confirming the significance of this pattern, local administration of nor-BNI into the piriform cortex was shown to produce antidepressant behavioral effects. Acute administration of the KOR antagonist nor-BNI also reduced latencies in the novelty-induced hypophagia test [50]. Classically, antidepressants require chronic administration for weeks to produce responses in the novelty-induced hypophagia test using SD rats [51, 52], but the rapid response of WKY rats could indicate that KOR antagonists have the capacity to produce antidepressant effects with a short latency. Thus, WKY rats can serve as a model for measuring the behavioral effects of KOR antagonists on behaviors associated with depression and anxiety. In contrast, SD rats may be significantly less sensitive to these effects because of differences in baseline depressive and anxiety behaviors.

## Mouse Strains Showing Increased Depressive Behaviors

### *Inbred Strains of Mice*

Commercially available inbred mouse strains have been surveyed for depressive behaviors by measuring variations of immobility in the FST or tail suspension

test (TST). Testing 4 inbred mouse strains, Yoshikawa et al. [53] reported that C57BL/6J mice demonstrate higher immobility values in the TST and FST than C3H/He mice. Intercrossing these lines to produce F1 and F2 animals and testing them for TST and FST immobility produced progeny with immobility values intermediate of the parents and a distribution suggesting high heritability. Since FST and TST immobility values for individual animals were not correlated, these tests may involve separate genetic determinants of behavioral depression [53]. The authors successfully mapped several QTL regulating immobility in the segregating F2 population, including loci on chromosomes 8 and 11 which overlap between the 2 behavioral measures [53]. Two of the QTL involved in TST immobility contain genes encoding $GABA_A$ receptor subunits, and the authors noted decreased $\alpha_1$-subunit mRNA expression in the frontal cortex of C57BL/6 mice compared to C3H/He mice. Additional TST and FST strain surveys have supported and elaborated on the immobility differences between inbred strains of mice [54–56]. Strain differences in baseline immobility are not correlated with a greater or reduced response to antidepressant drugs.

Shanks and Anisman [57] examined learned helplessness behavior induced by inescapable shock in 6 strains of mice. In this paradigm, some strains showed pronounced escape deficits (BALB/c and C57BL/6) indicating learned helplessness behavior, whereas the deficits were smaller (A/J) or entirely absent (DBA/2) in other strains. In a follow-up study [58], the authors measured alterations in the levels of monoamine neurotransmitters following inescapable foot shock in different brain regions in the same 6 strains. The effects of stress on NE, dopamine and 5-HT content varied across strains, but did not correlate with strain differences in learned helplessness.

### *Selective Breeding of Mice*

Vaugeois et al. [59] selectively bred CD-1 mice for high and low immobility on the TST and performed a behavioral, neurochemical and electrophysiological characterization of these lines [60]. After 8 generations of breeding, helpless (HL) mice spent about 200 s immobile in a 6-min TST, while nonhelpless mice (NHL) spent fewer than 10 s immobile [60]. This difference extended to the mouse FST, where HL mice showed 2–3 times greater immobility than NHL mice. Several other features of the HL line supported a more general role for these mice as a genetic mouse model of depression. HL mice responded to both noradrenergic and serotonergic antidepressants in the TST and FST. HL mice showed elevated basal levels of corticosterone and consumption of a palatable 2% sucrose solution was reduced. Abnormalities of the 5-HT system suggestive of human depression were noted in HL but not in NHL mice [60]. These mouse lines are excellent candidates for genetic analysis that could lead to the identification of genes regulating behavioral despair.

Opportunities for exploring genetic contributions to the regulation of complex behavior and the pathophysiology of psychiatric disease have increased exponentially with the development of gene targeting technology. The function of particular genes of special interest to psychiatric diseases can now be examined in lines of mice generated with specific physiological changes. Mice have been generated to study the function of hundreds of candidate genes in neurobiology and these mice can be screened for behavioral phenotypes using relevant test batteries [61]. The identification of mice that produce changes in depressive behaviors from the library of candidate genetic lines could provide an original opportunity to identify genetic mechanisms that may be associated with depression.

From a Medline survey of genetic mutations that have been tested in the FST or TST, many mouse lines were identified that demonstrate behavioral effects similar to the effects of antidepressant drugs. Since these mice show stable phenotypes that resemble antidepressant treatments, they may provide opportunities to explore potential novel treatments for depression [62, 63].

In contrast, considerably less frequent are the relatively few mouse strains identified from the Medline survey with a depressive behavioral phenotype. Some of these mouse strains are listed in table 1. They are divided into strains that demonstrate increased depressive behavior without signs of anxiety and lines that show increases in both depressive and anxiety behaviors. The most common test used to study depressive behavior was the FST, but the TST sucrose drinking and progressive ratio schedules were also used to confirm initial findings with the FST. There are undoubtedly additional mutations that will be added to this list. The finding that genetic mutations can produce increased depressive behavior with and without anxiety is similar to different varieties of psychiatric illness that exist. Although clinical depression is often comorbid with increased anxiety and this comorbidity can make clinical treatment more difficult, MDD also appears without prominent signs of anxiety. Because these different phenotypes appear to have a separate neurobiology, the existence of these varieties of behavior patterns may caution psychiatrists against blending these categories together in a single entity. It is presently unknown whether the various patterns of increased depressive behavior in mice may convey differential sensitivity for measuring the effects of different types of antidepressant drugs.

## Measuring Antidepressant Responses in Different Mouse Strains

Mouse strains vary dramatically in their response to antidepressant drugs on behavioral tests. Because background mouse strain is a major determinant of the sensitivity for detecting antidepressants with varying pharmacological selectivity and mechanisms [64–66], the consideration of mouse strain is of paramount importance when

**Table 1.** Mouse strains showing increased depressive behaviors

**a** Increased depression without change in anxiety

| Mutation | Depression tests | Anxiety tests | Reference |
|---|---|---|---|
| Aromatase<br>(females only) | FST<br>sexual behavior | elevated plus maze<br>open field | 85<br>86 |
| BDNF (+/–)<br>(females only) | FST<br>sucrose preference | elevated plus maze<br>open field | 87 |
| Desert hedgehog | FST | light-dark | 88 |
| 5-$HT_{3A}$ | FST | elevated plus maze<br>open field | 89 |
| Melatonin R-1 | FST | open field | 90 |
| Vmat2 (+/–) | FST<br>TST<br>sucrose drinking<br>learned helplessness | open field | 91 |

**b** Increased depression and increased anxiety

| Mutation | Depression tests | Anxiety tests | Reference |
|---|---|---|---|
| $\alpha_{2A}$-Adrenergic receptor | FST | open field<br>light-dark | 92 |
| CRF-R2 | FST | elevated plus maze<br>open field<br>light-dark | 93 |
| GABA γ2 subunit | FST | elevated plus maze<br>light-dark<br>novelty-suppressed feeding | 94 |
| Homer1 | FST<br>progressive ratio | novel objects | 95 |
| 5-HT transporter | FST<br>TST | elevated plus maze<br>open field | 96<br>97 |
| δOR | FST | elevated plus maze<br>light-dark | 98 |

designing tests to evaluate compounds with novel mechanisms. Several strain surveys have been conducted of the behavioral response of antidepressants in the FST [55, 67] and the TST [56, 68]. The inbred BALB/c strain and the outbred Swiss strain appear the most responsive to different classes of antidepressant drugs. However, the

inability to respond to a class of antidepressants may also be important because it can also be used to develop a model of treatment resistance.

The background strain also determines the phenotypic response on tests of depressive behavior conducted with lines of genetically deficient mice [62]. For example, SERT knockout mice generated on a 129 background demonstrate reduced immobility in the TST but show increased immobility in the same test if generated on a C57BL/6 background. This differential sensitivity recapitulates variations in response to SSRIs between these inbred mouse strains. It is not surprising to find that mice from a 129 background show an antidepressant-like phenotype to SERT deletion because they are highly responsive to the antidepressant effects of SSRIs, whereas C57BL/6 mice that did not develop the antidepressant-like phenotype are relatively poor responders to the antidepressant effects of SSRIs [55, 56].

## Murine Models of Deficient Monoamine Synthesis

Most antidepressant drugs produce their pharmacological effects by increasing extracellular levels of monoamine transmitters by inhibiting their reuptake. Mouse models of monoamine deficient transmission offer an opportunity to evaluate the role of brain monoamines in maintaining behavior on tests for depressive behavior. In addition, mice with deficient synthesis of NE or 5-HT can be used to evaluate the role of these transmitters in producing responses to antidepressant treatments.

### *Deficient Synthesis of Norepinephrine*

Dopamine-β-hydroxylase (DBH) is the rate-limiting enzyme for the biosynthesis of brain NE and epinephrine. Disruption of the *Dbh* gene causes a specific and complete inhibition of NE synthesis thus circumventing problems associated with the use of nonselective and toxic depleting agents [69]. Studies have examined the effects of NE deficiency using mice with genetic deletion of *dbh* in the FST and TST. Baseline behavior in the FST and TST was unaltered by NE deficiency in the TST [70, 71]. This would suggest that endogenous NE does not appear to be necessary for the tonic regulation of baseline performance in these tests.

In contrast, NE is necessary for reductions of immobility in these tests produced by antidepressants. The selective NE reuptake inhibitors desipramine and reboxetine were completely ineffective in either the FST or TST in mice deficient in NE [70, 71]. Furthermore, repletion of NE using the precursor L-DOPS restored the behavioral effects of desipramine in $Dbh^{-/-}$ mice, indicating the lack of NE was responsible for the deficit of behavioral response. Additional experiments have shown that deficits in response under conditions of NE deficiency extend to other antidepressants, particularly reboxetine, bupropion and the monoamine oxidase inhibitor pargyline.

Although it was expected that antidepressants that increase NE transmission would be ineffective, it was unexpected that behavioral responses to the SSRIs fluoxetine and paroxetine in the TST were also blunted in *Dbh*$^{-/-}$ mice [71]. The diminished response to SSRIs in *Dbh*$^{-/-}$ mice was not due to an alteration in SERT binding or kinetics or any other detectable changes in 5-HT or metabolite concentrations measured ex vivo. Repletion of NE levels with the precursor L-DOPS restored the behavioral effects of paroxetine in the TST [71] indicating that NE participated in the behavioral effects of this SSRI. Among the SSRIs tested, however, citalopram was the only drug whose effects were maintained with complete NE deficiency.

The results of a series of microdialysis studies were used to develop a model to account for the deficient SSRI behavioral response produced by NE deficiency. Systemic fluoxetine failed to increase extracellular 5-HT levels in *Dbh*$^{-/-}$ mice, whereas the effects of citalopram were only marginally reduced [71]. Thus, a critical role for NE, particularly innervation of the dorsal raphe nucleus, in the regulation of extracellular levels of 5-HT by SSRIs was proposed on the basis that fluoxetine is only marginally more selective for 5-HT than NE transporters. It was hypothesized that fluoxetine might employ NE transmission as a mechanism to increase 5-HT levels in vivo. Mice with selective destruction of NE neurons in the dorsal raphe nucleus were unable to respond behaviorally or to increase extracellular hippocampal 5-HT levels in response to fluoxetine [72], just like *Dbh*$^{-/-}$ mice. In contrast, the effects of citalopram were unaffected by NE neuronal destruction and this was thought to occur because citalopram is so much more selective for SERT than is fluoxetine. Subsequent studies showed that combining selective NE and 5-HT reuptake inhibitors together produced synergistic behavioral effects when administered at doses that are individually behaviorally inactive [G.V. Carr and I. Lucki, unpubl. results]. The cooperative effects between NE and 5-HT systems may be one reason that dual-action antidepressants, such as venlafaxine or mirtazapine, are viewed as among the most effective drugs used to treat depression.

### *Deficient Synthesis of Serotonin*

#### *Strain Variations of tph2 Activity*

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme for the biosynthesis of brain 5-HT. A number of studies have compared mice with naturally occurring variations in 5-HT synthesis capacity, or genetic deletions targeted at the *tph2* gene on depression-related behaviors or the behavioral effects of SSRIs.

Murine studies indicate that strain differences in a C1473G polymorphism in the *tph2* gene alter the rate of 5-HT synthesis. The 1473C allele is highly conserved across the species. However, the 1473G form is found in several inbred mouse strains and confers a 50% reduction in Tph2 enzymatic activity [73] and in vivo brain 5-HT synthesis and tissue content [74, 75].

Some murine studies suggest that the C1473G polymorphism in *tph2* modifies the behavioral response to SSRIs in the FST [74, 76]. C57BL/6 and 129Sv mice, 2 strains carrying the 1473G allele, were reported to respond to acute SSRI treatment in the FST. In contrast, 2 strains with the 1473G allele, BALB/c and DBA/2 mice, were reported to be refractory to the effects of SSRIs in the FST. These behavioral results may vary according to endogenous differences in 5-HT synthesis.

However, other studies have reported results that are not consistent with this view. In a prior survey of 13 strains, DBA/2J and BALB/cJ mice were reported to respond significantly to acute treatment with fluoxetine in the FST [55]. In contrast, the A/J mouse strain which carries the 1473G allele was reported to be resistant to the behavioral effects of fluoxetine. There were a number of important differences between these sets of studies. First, mice used in the studies of Cervo et al. [74] and Guzzetti et al. [76] were obtained from Charles River Laboratories (Calco, Italy), whereas mice used in the studies of Lucki et al. [55] were obtained from Jackson Laboratories (Bar Harbor, Me., USA). Therefore, substrain differences may account for the different behavioral results obtained in these studies. However, since these substrains do not differ with respect to the C1473G allele, the results do not support this polymorphism in the *Tph2* gene as a good predictor of responsiveness to acute SSRI treatment in the FST.

The results of a survey of 8 mouse strains using the more selective SSRI citalopram in the TST further supports this conclusion [56]. DBA/2J and BALB/cJ mice were 2 of the more responsive strains to citalopram in the TST and both of these strains carry the 1473C allele. In contrast, C57BL/6J and A/J mice were 2 of the least responsive strains to citalopram in the TST and both strains carry the 1473G allele for *tph2*. Thus, results with the TST, a second behavioral test for antidepressant activity in mice, also failed to support the C1473G allele as a predictor of the behavioral effects of SSRIs.

*Targeted Mutations of Tph2*

Mutant mice that are deficient in 5-HT have been generated with targeted genetic deletions of *Tph2* or both *Tph1* and *Tph2*. *Tph2*$^{-/-}$ mice demonstrated a 92% depletion of tissue 5-HT, whereas mice with dual *Tph1* and *Tph2* deletions showed a near-total 98% deletion of forebrain 5-HT content. It is important to note that these mice were generated on a mixed 129C57BL/6 genetic background [77] because mice generated with Tph2 deletions on a single genetic background demonstrated severe developmental deficits [78]. When given tests for depression-related behaviors [77], mice with deletion of *Tph2* were significantly more immobile in the TST. Performance on the FST was not evaluated because of abnormal swimming behavior. The increased immobility in the TST was also shown recently by a *Tph2* knock-in mouse line with reduced TPH activity [79].

These findings are significant because it is the first time that rodents have demonstrated increased depressive behavior after the depletion of 5-HT. Pharmacological depletion of 5-HT by the administration of the TPH inhibitor parachlorophenylalanine (PCPA) or lesions of 5-HT neurons does not produce a depressive phenotype

in rodents in the FST and/or TST [80–83]. Generally, PCPA causes a substantial but incomplete depletion of forebrain 5-HT tissue content in rats (80–95%) and mice (60–70%) [80, 81]. Nevertheless, the inhibition of 5-HT synthesis was still sufficient to block the antidepressant-like effects of SSRIs [80–82].

The appearance of a depressive behavioral phenotype in a genetic model may be due either to larger depletions of tissue 5-HT than can be produced by using a drug like PCPA or to the long-term depletion of 5-HT during a critical developmental period. Considering the accumulating evidence for associating 5-HT insufficiency with vulnerability to depression in humans [84], the *Tph* mutants provide the first evidence in animals for a pathological role of 5-HT depletion in depressive behavior. Furthermore, since *Tph* mutants would be unlikely to respond to the behavioral effects of SSRIs, they would represent a logical and effective model of treatment resistance.

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# The Role of 5-$HT_{2C}$ Receptors in the Antidepressant Response: A Critical Review

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**Abstract**

There is evidence for altered 5-$HT_{2C}$ receptor function in anxiodepressive disorders and many antidepressant drugs have a primary action at these receptors. Although the notion of 5-$HT_{2C}$ receptor desensitization following prolonged treatment with drugs that increase the synaptic availability of 5-HT is well anchored in the literature, this concept is based mainly on in vitro assays and/or behavioral assays that have little relevance to depression. Our objective herein is to provide a comprehensive overview of the studies that have assessed the effects of antidepressant treatments on 5-$HT_{2C}$ receptors. Relevant neurochemical (receptor binding and mRNA levels), physiological (5-$HT_{2C}$-receptor-induced hyperthermia and hormone release) and behavioral (5-$HT_{2C}$-receptor-induced changes in feeding, anxiety, defense and motor activity) data are summarized and discussed. Our extensive analysis of the literature clearly evidences a crucial lack of studies investigating changes in 5-$HT_{2C}$ receptor function in selected brain areas following antidepressant treatments, mainly because adequate tools are lacking. Although the notion of an overall desensitization of 5-$HT_{2C}$ receptors by antidepressant drugs has been challenged, the hypothesis appears nevertheless well supported by clear-cut data in selected brain areas. Setting the record straight about drug-induced changes in 5-$HT_{2C}$ receptor function should help in determining which pharmacotherapeutic strategy is the best in anxiety and depression.



## The Relevance of 5-$HT_{2C}$ Receptors in Depression

Over the last few years, growing evidence has suggested an important involvement of 5-$HT_{2C}$ receptors in both the etiology and the therapeutics of depression. Already in the mid 1980s, reductions of 5-$HT_2$ receptor binding had been observed with either acute treatment with the antidepressant drug mianserin or repeated treatment with the agonist trifluoromethylphenylpiperazine (TFMPP) [1], now known as preferential 5-$HT_{2C}$ receptor ligands. The 5-$HT_{2C}$ receptor was first known as the 5-$HT_{1C}$ receptor,

which was pharmacologically identified around the same period and described at high density in the choroid plexus where it increases phosphatidylinositol (PI) turnover by activating phospholipase C (PLC) [2]. It was later renamed 5-$HT_{2C}$ based on homology with the 5-$HT_2$ receptor class [3]. Because this receptor is present at the highest concentration in the choroid plexus, a structure not traditionally involved in depression, doubts were first raised against the idea of its relevance in the therapeutics of depression. However, in addition to this location, 5-$HT_{2C}$ receptors were also found to be concentrated in the septum, the hippocampus, the amygdala, the striatum, the frontal cortex, the periaqueductal gray (PAG) and the brainstem of primates and rodents [4–7], i.e. structures that have all been identified as being strategically important in studying brain mechanisms underlying emotions and related behaviors.

To date, there are 3 main pieces of evidence supporting the implication of 5-$HT_{2C}$ receptors in the mechanisms of action of antidepressant drugs. First, as anticipated by Blackshear et al. [1] in their study with mianserin, there is the fact that many classes of antidepressant drugs act as direct antagonists with moderate to high affinity at these receptors [8, 9]. This is notably the case of classical tricyclic antidepressant drugs such as amitriptyline, imipramine, chlomipramine, amoxapine, doxepin and nortriptyline. In addition, antidepressant drugs that selectively block noradrenaline reuptake such as desipramine and maprotiline and atypical antidepressant drugs including mirtazapine, trazodone and nefazodone also act as direct 5-$HT_{2C}$ receptor antagonists. Some selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and citalopram, also exert some antagonistic action at 5-$HT_{2C}$ receptors [10, 11]. Even more interestingly, the recently introduced antidepressant drug agomelatine, initially developed as an agonist at $MT_1$ and $MT_2$ melatoninergic receptors, was subsequently shown to block 5-$HT_{2C}$ receptors [12], and extensive studies demonstrated that its antidepressant action in fact results from the combined blockade of 5-$HT_{2C}$ receptors and activation of $MT_1$ and $MT_2$ melatoninergic receptors [13]. Although there are no data yet concerning the effect of a selective 5-$HT_{2C}$ receptor antagonist in depressed patients, a clear-cut antidepressant efficacy has been reported in a small-scale study for ritanserin, which has potent 5-$HT_{2C}$ receptor antagonist properties [14].

On the other hand, increased 5-$HT_{2C}$ receptor function has been reported in depressed patients and after potentially depressogenic stress in validated animal models. In particular, greater 5-$HT_{2C}$-receptor-mediated increases of prolactin and cortisol secretions were found in depressed patients compared to a control group [15]. Furthermore, cys23ser polymorphism-associated changes in 5-$HT_{2C}$ receptor functions [16] were found to influence the vulnerability to affective disorders [17, 18]. In rats, *m*-chlorophenylpiperazine (mCPP)-induced penile erection, which is mediated by 5-$HT_{2C}$ receptor activation, was found to be increased after chronic unpredictable stress, causing depression-like symptoms [19]. The hypothesis of a causal relationship between hypersensitive 5-$HT_{2C}$ receptors and at least one of these symptoms, anhedonia, is supported by the finding that chronic administration of a preferential 5-$HT_{2C}$ receptor agonist (RO 60-0175) prevents its occurrence during stress [19], maybe

because it desensitizes or downregulates 5-$HT_{2C}$ receptors. In line with the idea of a close relationship between 5-$HT_{2C}$ receptor function and stress responses, higher levels of 5-$HT_{2C}$-receptor-like immunoreactivity were found in the hippocampus of rats subjected to the social isolation stress [20]. In addition, increased sensitivity of the 5-$HT_{2C}$ receptors mediating inhibition of accumbal dopamine release was observed in the Flinders sensitive line rat model of depression [21]. Recently, the behavioral deficits associated with the learned helplessness model of depression were shown to be prevented by administration, before testing, of a 5-$HT_{2C}$ receptor antagonist [22]. All these convergent data indicate that 5-$HT_{2C}$ receptors play a particularly important role in models of depression involving chronic or repeated stress.

Furthermore, under acute conditions, notably in studies using the forced swimming test (FST) and the tail suspension test (TST) commonly applied to screen drugs for antidepressant activity, 5-$HT_{2C}$ receptors seem to play a role in modulating the corresponding stress-induced behaviors. Thus pharmacological blockade of 5-$HT_{2C}$ receptors, as much as genetic deletion of 5-$HT_{2C}$ receptors, enhanced the reduction of immobility induced by an SSRI in the TST [23, 24]. Consistently, acute administration of 5-$HT_{2C}$ receptor antagonists decreased immobility in the FST [9, 25]. However, these results apparently contrast with a series of studies with relatively selective 5-$HT_{2C}$ receptor agonists that similarly decreased immobility in the FST when injected 3 times over 24 h [26, 27]. Indeed, under such conditions, the agonists might have induced a rapid 5-$HT_{2C}$ receptor desensitization, which would make these data consistent with those obtained after acute treatment with 5-$HT_{2C}$ receptor antagonists. However, according to Rosenzweig-Lipson et al. [27], this possibility is rather unlikely because coadministration of a 5-$HT_{2C}$ receptor antagonist prevented the antidepressant-like effect of 5-$HT_{2C}$ receptor agonists in the FST. On the other hand, a relatively rapid antidepressant-like effect of 5-$HT_{2C}$ receptor agonists has also been found in the bulbectomy model of depression [27, 28]. These data led to the hypothesis that 5-$HT_{2C}$ receptor agonists are endowed with antidepressant potentialities, and that crucial components of the 5-$HT_{2C}$ receptor agonist action relevant to their therapeutic efficacy do not desensitize.

In view of these complex and rather discrepant conclusions regarding the positive or negative implication of 5-$HT_{2C}$ receptors in depression and/or antidepressant action, further analyses of relevant data are needed in order to decipher their real role, if any, in psychoaffective disorders.

## Antidepressant Drugs Acting at 5-$HT_{2C}$ Receptors Moderate Stress and Anxiety

### *Anxiety and Defense Behaviors*

Anxiety is certainly one of the most important comorbidity in depression, with 58% of patients with major depression fulfilling criteria for an anxiety disorder [29]. Vice versa, most anxious patients also experience at some point a major depression. As

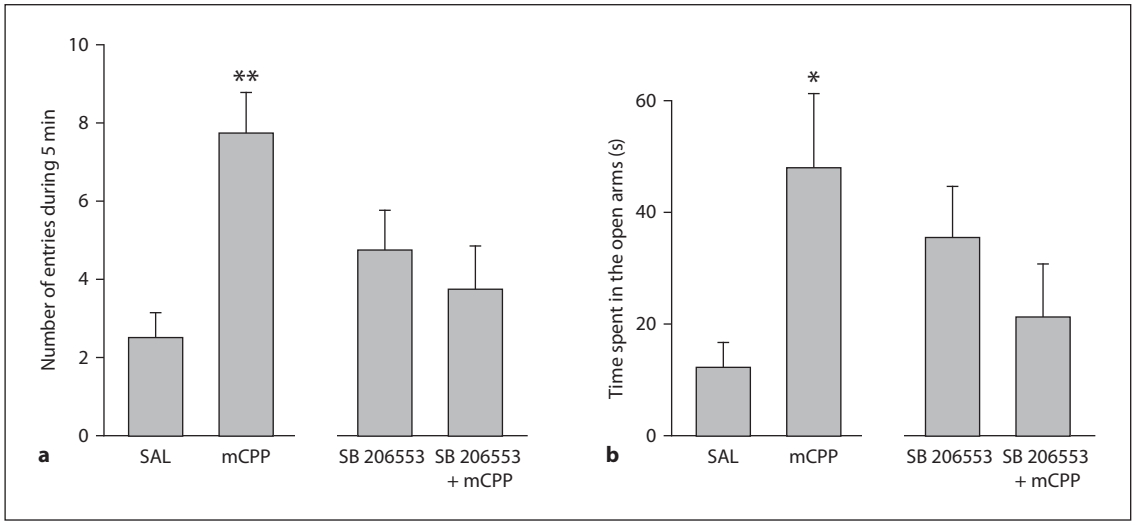


**Fig. 1.** Paradoxical anxiolytic-like effect of mCPP in mice subjected to the elevated plus maze. Administration of mCPP (1 mg/kg, i.p.) to C57BL6/J mice increased both the number of entries (**a**) and the time spent in the open arms (**b**). This effect was not observed in mice coadministered with the selective 5-$HT_{2C}$ receptor antagonist SB 206553 (1 mg/kg, i.p.). Data are mean ± SEM (n = 6). *p < 0.05, **p < 0.01; comparing mCPP to saline (SAL) using Student's t test.

much as 68% of depressed patients with comorbid anxiety had been anxious for over 10 years before eventually developing depression [30]. Furthermore, depression and anxiety share many overlapping symptoms and antidepressant drugs are also commonly used to treat anxiety [31]. In this context, 5-$HT_{2C}$ receptors have to be under focus because, beside obesity, anxiety is one of the most prominent target of drugs acting at these receptors.

In the mid 1980s, it was found that the preferential 5-$HT_{2C}$ receptor agonist, mCPP, induces intense symptoms of anxiety and panic attack in humans [32]. Preclinical studies using models such as the social interaction test confirmed the role of 5-$HT_{2C}$ receptors in anxiety [33]. In particular, SSRIs which exert anxiolysis after long-term treatment, but generate anxiety during the initial phase of treatment [34], were found to exert their dual action through 5-$HT_{2C}$-receptor-mediated mechanisms in both rats and mice [35–37]. Also, in the social interaction test, acute administration of a neutral 5-$HT_{2C}$ receptor antagonist (SB 242084) was reported to exert anxiolysis, but only in stressful or anxiogenic contexts [36, 37].

However, studies with 5-$HT_{2C}$ receptor agonists in other standard models of anxiety, such as the elevated plus maze, generated conflicting data. Although systemic mCPP administration was generally found to exert an anxiogenic effect, consistent with the observations in humans and in the rodent social interaction test [20, 38–46], either no change or even an anxiolytic effect of acute 5-$HT_{2C}$ receptor agonist administration was also reported by several authors [28, 45–47] (fig. 1). These discrepancies

apparently depended on the species or the strain used, the lighting conditions, or the specificity of the agonists. However, most importantly, dual anxiogenic-anxiolytic effects of 5-$HT_{2C}$ receptor agonists could also be induced by direct intracerebral administration. Thus, microinjection of 5-$HT_{2C}$ receptor agonists into the amygdala was shown to induce anxiogenic-like effects [48, 49], while more recent studies performed in the same elevated plus maze conditions revealed that microinjection of these agonists into the PAG induced instead an anxiolytic-like effect [50, 51]. Because of such major regional differences, it is unclear which of these 5-$HT_{2C}$ receptor systems modulating anxiety, the excitatory forebrain or the inhibitory midbrain systems, should prevail over the other following acute systemic injection of a 5-$HT_{2C}$ receptor agonist in rodents. Furthermore, it has to be emphasized that direct activation of 5-$HT_{2C}$ receptors in the dorsal PAG inhibits active defense behaviors such as escape [52]. Because the elevated plus maze mixes both active and passive defense mechanisms [53], these data could explain, at least some, of the above discrepancies.

The contradictions about the effects of 5-$HT_{2C}$ receptor ligands in screening models of antidepressant activity could also be related to the dual effects of 5-$HT_{2C}$ receptor activation on defense reaction as both the FST and the TST are models based on escape-related behaviors [54]. The tail suspension and water immersion stresses trigger active defense mechanisms, i.e. attempts to escape the stressful situation, that compete with passive defense mechanisms, expressed as tonic immobility, the so-called 'resignation' behavior. The fact that defense mechanisms markedly differ among strains and species might explain why 5-$HT_{2C}$ receptor activation exerts an antidepressant-like effect in rats [26, 27] but not in mice [55, 56] (fig. 2). The neuroanatomical pathways involved in the changes in behavior after acute administration of antidepressant drugs in models such as the TST and the FST thus need to be unveiled in order to possibly resolve the controversies about the antidepressant-like activity of 5-$HT_{2C}$ receptor agonists [26, 27] versus that of antagonists [9, 24, 25].

*Stress and the 5-HT System*

The stress response could be another factor influencing the effect of 5-$HT_{2C}$ receptor ligands in depression. There is an increased tonic activity of the hypothalamic-pituitary-adrenal (HPA) axis associated with major depression, most likely resulting from a deficit in the negative feedback regulation of the HPA axis. Interestingly, acute injection of the preferential 5-$HT_{2C}$ receptor agonist mCPP in humans induces not only anxiety, but also a major increase in ACTH and cortisol plasma levels [57]. In line with these observations, 5-$HT_{2C}$ receptors in the paraventricular nucleus (PVN) of the hypothalamus are well known to play a central role in the regulation of glucocorticoid secretion [58] (fig. 3a).

Neuroanatomical studies suggest a direct action of 5-HT through synaptic contacts between serotoninergic terminals and cells containing corticotropin-releasing

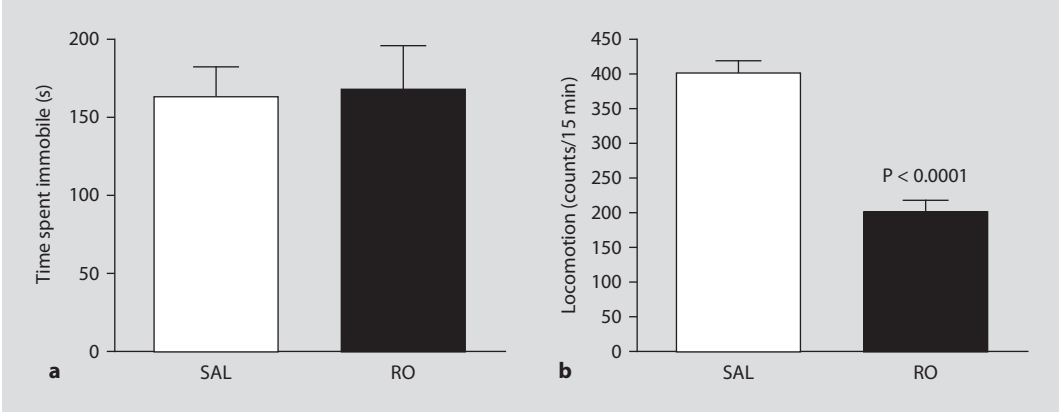


**Fig. 2.** Effect of the preferential 5-$HT_{2C}$ receptor agonist RO 600175 on locomotor activity and helpless behavior in mice. **a** Administration of RO 600175 (RO; 3 mg/kg, s.c.) to C57BL6/J mice did not alter the time spent immobile in the FST. The experiment was performed following the protocol of Cryan and Lucki [26]; mice were first placed into the swimming apparatus for 15 min and then 24 h later replaced in the same apparatus for the 6-min test session. Saline (SAL) and RO were injected three times (at 1, 5 and 23.5 h) before the test session. Data were acquired using a computer-based video tracking system (View Point, France). Data are mean ± SEM (n = 6). **b** Administration of RO 3 mg/kg, i.p. to C57BL6/J mice markedly decreased horizontal motor activity. Locomotor activity was measured using a computer-based photobeam apparatus (Actisystem II, Panlab, Spain). Data are mean ± SEM (n = 7–13). Statistical comparisons were made with Student's t test.

hormone (CRH) in the PVN [59]. 5-$HT_{2A/2C}$ receptors in the PVN are among the most important 5-HT receptors that mediate the stimulatory effect of 5-HT on glucocorticoid secretion during emotional stress. Indeed, a selective 5-$HT_{1A}$ receptor antagonist is, at best, only partially effective in reducing the stress-induced ACTH release, whereas 5-$HT_{2A/2C}$ receptor antagonists nearly completely abolish this response [60, 61]. The 5-$HT_{2C}$ receptor, rather than the 5-$HT_{2A}$ receptor, is the most abundant among all 5-HT receptors expressed in the PVN CRH neurons, as shown by laser dissection coupled with transcriptomic analyses in mice [58]. The enhancement of CRH secretion by the 5-HT releaser fenfluramine can be completely blocked by a 5-$HT_{2C}$ receptor antagonist, and genetic deletion of 5-$HT_{2C}$ receptors abolishes CRH surges induced by mCPP or the mixed 5-$HT_{2A/2C}$ agonist DOI [58]. Furthermore, neuroanatomical studies using Fos-IR, an indicator of cellular activity, and semi-quantitative in situ hybridization of CRH mRNA coexpression as well as in vitro electrophysiological recordings confirmed the existence of functional 5-$HT_{2C}$ receptors on CRH-containing neurons in the PVN [58]. 5-$HT_{2C}$ receptors also appear to control the activity of CRH neurons at the level of the amygdala and the bed nucleus of the stria terminalis (BNST; fig. 3a), a fact that might explain, at least in part, the anxiety-like behavior induced by 5-$HT_{2C}$ agonists, as CRH is a potent anxiogenic neuropeptide [62].

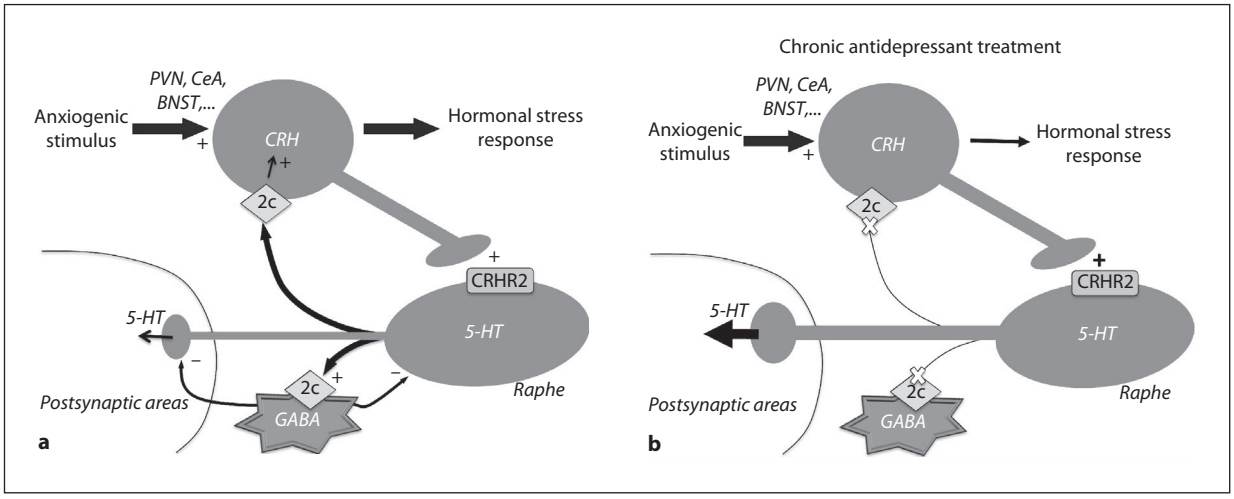


**Fig. 3.** Schematic representation of the putative involvement of 5-$HT_{2C}$ receptors in stress responses. **a** Activation of 5-$HT_{2C}$ receptors triggers the hormonal stress response through PVN CRH neurons, which inhibit the firing rate of 5-HT neurons and the release of 5-HT at postsynaptic sites during stress via GABAergic interneurons. **b** Long-term treatment with antidepressant drugs that increase 5-HT synaptic availability or having 5-$HT_{2C}$ receptor antagonist properties could decrease these effects of stress.

Some of the effects of CRH during stress are mediated by CRHR2 receptors on raphe 5-HT neurons [63]. Activation of these receptors occurs from projection of the central nucleus of the amygdala (CeA), which, in turn, triggers the release of 5-HT in several brain areas including the CeA itself [64–66] (fig. 3a). In a recent study in mice, we found that 5-$HT_{2C}$ receptor activation prevented the enhancement of brain 5-HT turnover and extracellular concentration triggered by restraint stress [37]. Because this inhibitory effect was not observed under basal conditions, in the absence of stress, it is conceivable that an indirect 5-$HT_{2C}$-receptor-mediated circuit exerting a negative influence on 5-HT release plays a pivotal role in moderating the high serotoninergic tone that occurs during stress. Indeed, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors are known to exert an indirect modulatory effect on the firing rate of 5-HT neurons in the raphe [67–69]. Activation of these receptors by 5-$HT_2$ agonists decreases the firing activity of 5-HT neurons via GABA-dependent mechanisms [70–73] (fig. 3a). Again, 5-$HT_{2A}$ and/or 5-$HT_{2C}$ receptor activation has no effect on basal 5-HT release [74–76], but an enhancing effect of 5-$HT_{2C}$ – but not 5-$HT_{2A}$ – receptor blockade on extracellular 5-HT levels has consistently been reported under conditions of elevated serotoninergic tone, such as after acute administration of high doses of SSRIs [24, 77, 78]. Clear-cut evidence showed that the latter effect in fact results from the suppression of 5-$HT_{2C}$-receptor-mediated inhibition of 5-HT neurons by local GABAergic interneurons and $GABA_B$ receptors [23] (fig. 3a).

Overall, it seems that 5-$HT_{2C}$ receptor activation has prominent effects on anxiety and 5-HT neuron activity during stress. These facts should be taken into consideration when studying the effects of antidepressant treatments and depressogenic stresses on 5-$HT_{2C}$ receptors.

## Implication of 5-$HT_{2C}$ Receptor Desensitization in the Effects of Antidepressant Drugs

### *In vitro Studies*

Many in vitro studies on cellular systems clearly indicated that 5-$HT_{2C}$ receptor function rapidly desensitizes in response to elevated concentrations of 5-HT. In CHO cells transfected with human 5-$HT_{2C}$ receptors, pre-exposure to a high concentration of 5-HT decreases the 5-$HT_{2C}$-receptor-mediated mobilization of calcium, and this occurs in a noticeable manner within 30 min of pretreatment [79]. The speed of desensitization does change depending on the second messenger system: the decrement in 5-$HT_{2C}$-receptor-mediated accumulation of arachidonic acid via phospholipase $A_2$ occurs at a much faster rate than that of PI via PLC. Furthermore, 5-$HT_{2C}$-receptor-induced inositol phosphate accumulation recovers within minutes, although the process mediated by phospholipase $A_2$ recovers faster [80]. Using 3T3 fibroblast cells, it was shown that 5-$HT_{2C}$ receptors are phosphorylated under basal conditions and, furthermore, that the phosphorylation process is increased by pretreatment with 5-HT. This enhancement of phosphorylation occurs before or is coincident with the decrease in 5-$HT_{2C}$-receptor-induced PLC activation [81]. Combinations of partner proteins interacting with 5-$HT_{2C}$ receptors via the PDZ recognition motif are accessory in the receptor desensitization/resensitization processes. Deletion of this PDZ recognition motif prevents receptor phosphorylation and delays resensitization of 5-$HT_{2C}$ receptors in 3T3 cells [82]. Furthermore, the PDZ-binding proteins, PSD-95 and MPP3, were shown to exert opposite effects on the desensitization of 5-$HT_{2C}$-receptor-mediated $Ca^{2+}$ response in cos-7 cells and in mouse cortical neurons [83]. Finally, another important process for receptor desensitization is internalization: 5-$HT_{2C}$ receptors expressed in HEK-293 cells were shown to be internalized into the endocytic recycling compartment following exposure to agonists, but not after exposure to antagonists or inverse agonists [84].

Antagonists and inverse agonists affect 5-$HT_{2C}$ receptor function in cellular systems through various mechanisms. First of all, many antidepressant drugs acutely block 5-$HT_{2C}$ receptor signaling consistently with their antagonistic action at these sites [85]. However, they also have long-term effects: the incubation of Sf9 cells expressing high levels of 5-$HT_{2C}$ receptors with various types of 5-$HT_{2C}$ receptor antagonists, including the antidepressant drug mianserin, induced a major downregulation of 5-$HT_{2C}$ receptors after drug washout [86], in accordance with a previous study using

cells cultured from rat choroid plexus [87]. Importantly, this effect was not produced by 5-$HT_{2C}$ receptor agonists [86]. However, other in vitro studies did show 5-$HT_{2C}$ receptor downregulation in cells exposed to 5-HT, DOI or mCPP [88].

5-$HT_{2C}$ receptors display constitutive activity on PLC signaling in many cellular systems that coexpress 5-$HT_{2C}$ receptors and Gαq proteins [86, 89]. Selective 5-$HT_{2C}$ receptor ligands such as SB 206553 and antidepressant drugs such as mirtazapine and mianserin behave as inverse agonists, while others such as SB 242084, trazodone and amitriptyline do not alter basal inositol phosphate, as expected of neutral antagonists [89]. Long-term exposure to inverse agonists, but not neutral antagonists, increases the cell surface expression of 5-$HT_{2C}$ receptors and the 5-$HT_{2C}$-receptor-mediated PLC signaling in HEK-293 cells and 5-HT-evoked $Ca^{2+}$ response in cortical neurons [89]. This paradoxical upregulation of 5-$HT_{2C}$ receptor function by inverse agonists is complex and depends on receptor expression levels, the temporal dynamics of the treatment and the second messenger systems involved [90, 91]. It might explain the occurrence of 5-$HT_{2C}$ receptor upregulation occasionally observed in vivo, in some brain areas, after treatment with antipsychotic drugs endowed with 5-$HT_{2C}$ inverse agonist properties [92, 93].

*In vivo Studies*

As seen above, many conditions can produce 5-$HT_{2C}$ receptor desensitization, and it is not clear which of these conditions are met in vivo. For example, are the synaptic 5-HT concentrations reached after SSRI treatment sufficient to produce the type of fast desensitization observed in cellular systems? Differential desensitization in various brain areas is expected to occur depending on 5-$HT_{2C}$ receptor density and constitutive activity. Most importantly, the time frame required to observe changes in 5-$HT_{2C}$ receptor desensitization/downregulation is most likely different under in vivo versus in vitro conditions; it was reported to occur within minutes to hours in vitro, while several days to weeks appear to be required in vivo. Furthermore, 5-$HT_{2C}$ receptors rapidly resensitize after agonist washout in vitro, while in vivo desensitization/tolerance and downregulation are most often observed for hours/days after the drugs have been eliminated through metabolism.

*Receptor Studies*

A large body of evidence was accumulated in the early 1980s showing a decrease in 5-$HT_2$ receptor binding after chronic treatment with various classes of antidepressant drugs [94]. However, this is mostly accounted for by decreased binding at 5-$HT_{2A}$ receptors which are expressed at higher densities than 5-$HT_{2C}$ receptors in all the brain areas, except the choroid plexus. Overall, only few studies investigated the effects of long-term antidepressant treatments specifically on 5-$HT_{2C}$ receptor binding. The prototypical radioligand used for 5-$HT_{2C}$ receptor binding is [$^3$H]

**Table 1.** Effects of subchronic or chronic treatments with 5-$HT_{2C}$ receptor ligands or antidepressants on brain 5-$HT_{2C}$ receptor binding sites and 5-$HT_{2C}$ receptor mRNA in rodents

**a** Effects on [$^3$H]mesulergine binding

| Class | Treatment | Doses mg/kg/day | Time days | Brain areas | Technique | Effect on $B_{max}$ | Reference |
|---|---|---|---|---|---|---|---|
| Agonist | mCPP | 2.5 | 4 | brainstem | autoradio-graphy | ↓ | 134 |
| | mCPP | 2.5 | 4 | cortex | autoradio-graphy | ↓ | 134 |
| | mCPP | 2.5 | 4 | hippo-campus | autoradio-graphy | = | 134 |
| | mCPP | 2.5 | 4 | hypo-thalamus | autoradio-graphy | = | 134 |
| | mCPP | 2.5 | 4 | striatum | autoradio-graphy | ↓ | 134 |
| | mCPP | 10 | 14 | cortex | autoradio-graphy | ↓ | 95 |
| SSRI | citalopram | 2.5 | 14 | choroid plexus | binding | = | 96 |
| | citalopram | 10–20 | 14 | choroid plexus | binding | ↑ | 96 |
| | fluoxetine | 2.5 | 14 | choroid plexus | binding | = | 96 |
| | fluoxetine | 10 | 14 | choroid plexus | binding | ↑ | 96 |
| | fluoxetine | 20 | 14 | choroid plexus | binding | = | 96 |
| Antagonist | mianserin | 10 | 14 | amygdala | binding | ↑ | 113 |
| | mianserin | 10 | 14 | CA1, CA2 | binding | ↓ | 113 |
| | mianserin | 10 | 14 | septum | binding | = | 113 |

mesulergine, an antagonist (or inverse agonist, depending on cellular systems). To obtain specificity for 5-$HT_{2C}$ sites, the [$^3$H]mesulergine binding assay needs to be performed in the presence of compounds such as spiperone to prevent binding at 5-$HT_{2A}$ sites. However, these compounds are in fact not specific for 5-$HT_{2A}$ receptors and partially remove some binding at 5-$HT_{2C}$ receptors, resulting in rather weak specific [$^3$H]mesulergine binding at 5-$HT_{2C}$ receptors (except in the choroid plexus).

**Table 1. b** Effects on 5-$HT_{2C}$ receptor mRNA

| Class | Treatment | Doses mg/kg/day | Time days | Brain areas | Technique | Effect on mRNA | Reference |
|---|---|---|---|---|---|---|---|
| Agonist | mCPP | 12 | 14 | hypo-thalamus | RT-PCR | = | 135 |
| | RO 600175 | 36 | 14 | hypo-thalamus | RT-PCR | = | 135 |
| TCA | amitriptyline | 10 | 70 | hippo-campus | hybridization | = | 136 |
| | imipramine/ Amoxapine | 10/5 | 32 | whole brain | N-Blot | = | 137 |
| SSRI | citalopram/ fluvoxamine | 10/50 | 32 | whole brain | N-Blot | = | 137 |
| | fluoxetine | | | prefrontal cortex | RT-PCR | = | 126 |
| Antagonist | mianserin | 10 | 32 | whole brain | N-Blot | = | 137 |
| | mianserin | 10 | 14 | amygdala | hybridization | = | 113 |
| | mianserin | 10 | 14 | septum/ hippo-campus | hybridization | = | 113 |

TCA = Tricyclic antidepressants.

Furthermore, a rather large proportion of [$^3$H]mesulergine binding, in the presence of spiperone, is still observed in various brain areas of mutant mice deficient in 5-$HT_{2C}$ receptors [7]. While keeping these shortcomings in mind, it is interesting to note that some studies did find a decrease in 5-$HT_{2C}$ receptor binding following long-term treatment with 5-$HT_{2C}$ receptor agonists (table 1a). Immunohistochemical investigations also showed a decrease in 5-$HT_{2C}$ receptor protein in the hippocampus following repeated mCPP treatment [95]. In contrast, in another study [96], chronic treatment with SSRIs (that have some affinity for 5-$HT_{2C}$ receptors) was reported to increase [$^3$H]mesulergine binding in the choroid plexus. Clearly, better tools are needed to determine the actual effects of antidepressant treatments on brain 5-$HT_{2C}$ receptor protein levels.

None of the studies that have examined the effects of long-term treatments with antidepressant drugs on 5-$HT_{2C}$ receptor mRNA levels found any changes in any of the brain areas examined (table 1b). However, it is important to note that long-term

**Table 2.** Effects of subchronic or chronic treatments with 5-$HT_{2C}$ receptor ligands or antidepressants on 5-$HT_{2C}$ receptor-induced hypolocomotion

| Class | Treatment | Doses mg/kg/day | Time days | Drug challenge | Effect on hypolocomotion | Reference |
|---|---|---|---|---|---|---|
| Agonist | RO 600175 | 36 | 14 | none | = | 135 |
| | mCPP | 10 | 28 | none | = | 138 |
| | mCPP | 2.5–5 | 14–15 | mCPP | ↓ | 95, 139, 140 |
| SSRI | paroxetine | 10–20 | 14–21 | mCPP | ↓ | 141–143 |
| | paroxetine | 5 | 21 | Ro 600175 | ↓ | 37 |
| | fluoxetine | 10 | 6 | mCPP | = | 35 |
| | fluoxetine | 10 | 14–21 | mCPP | ↓ | 141;144 |
| | fluvoxamine | 10 | 21 | mCPP | = | 143 |
| | fluvoxamine | 30–90 | 21 | mCPP | ↓ | 143 |
| | sertraline | 5–10 | 14 | mCPP | ↓ | 145;146 |
| | citalopram | 10 | 14 | mCPP | ↓ | 145 |
| TCA | desipramine | 10 | 21 | mCPP | = | 141 |
| | imipramine | 20 | 14 | mCPP | = | 142 |
| | clomipramine | 30 | 21 | mCPP | = | 141 |
| | clomipramine | 70 | 21 | mCPP | ↓ | 141 |
| MAOIs | phenelzine | 10 | 7 | mCPP | ↓ | 147 |
| | nialamide | 20 | 7 | mCPP | ↓ | 147 |

MAOIs = Monoamine oxidase inhibitors.

treatment with clozapine and olanzapine, 2 antipsychotic drugs with inverse agonist activity at 5-$HT_{2C}$ receptors, can modify the levels of 5-$HT_{2C}$ receptor mRNA and protein in several brain areas [92, 97–99].

*Functional Studies*

The widely spread notion that 5-$HT_{2C}$ receptors get desensitized following long-term treatment with antidepressant drugs arises mainly from studies on locomotor and thermoregulation functions. Acute administrations of preferential 5-$HT_{2C}$ receptor agonists such as mCPP produce hypolocomotor and hyperthermic effects, which are blocked by selective 5-$HT_{2C}$ receptor antagonists [100–104]. As shown in tables 2 and 3, long-term treatment with drugs that act as 5-$HT_{2C}$ receptor agonists or that

**Table 3.** Effects of subchronic or chronic treatments with antidepressants on 5-$HT_{2C}$-receptor-mediated hyperthermia

| Class | Treatment | Doses mg/kg/day | Time days | Drug challenge | Species | Effect on hyperthermia | Reference |
|---|---|---|---|---|---|---|---|
| Agonist | mCPP | 0.08 | 2–3 | none | human | ↓ | 148 |
| SSRI | paroxetine | 20 | 14 | mCPP | rats | ↓ | 142 |
| | paroxetine | 20–30 | 21 | TFMPP | human | ↓ | 149 |
| | fluoxetine | 5–10 | 14 | TFMPP | mice | ↓ | 144 |
| TCA | imipramine | 20 | 14 | TFMPP | rats | ↓ | 142 |
| | imipramine | 5 | 22 | mCPP | rats | ↓ | 150 |
| | clomipramine | 5 | 22 | mCPP | rats | ↓ | 150 |
| MAOIs | nialamide | 80 | 7 | 5MeODMT | rats | ↓ | 151 |
| | clorgyline | 1 | 22 | mCPP | rats | ↓ | 150 |
| Antagonist | mianserin | 1–5 | 7–14 | mCPP | rats | = | 105 |
| | ritanserin | 1 | 6–13 | mCPP | rats | = | 105 |
| | ritanserin | 5 | 6–13 | mCPP | rats | ↓ | 105 |

enhance the synaptic availability of 5-HT (SSRIs, monoamine oxidase inhibitors), but not noradrenaline reuptake inhibitors such as desipramine, desensitize 5-$HT_{2C}$-receptor-mediated hypolocomotion and hyperthermia. However, no change in the 5-$HT_{2C}$-mediated hyperthermic response was observed following a long-term treatment with mianserin [105]. It is noteworthy that mCPP also acts at 5-$HT_1$ receptors [101, 102, 104] and this may obviously bias the interpretation of data obtained with this drug (table 2). Nevertheless, in a recent study [37], long-term treatment with the SSRI paroxetine was found to significantly reduce the hypolocomotor effect of the more selective 5-$HT_{2C}$ receptor agonist, RO 60-0175.

On the other hand, studies of the effects of chronic antidepressant treatments on hypothalamic functions mediated by 5-$HT_{2C}$ receptor activation led to more complex and rather discrepant data. These data concern notably the 5-$HT_{2C}$-mediated hypophagic and anorectic effects which most likely involve melanocortin receptors in the arcuate nucleus [106] and the secretion of corticosterone and prolactin via the PVN [58, 107]. Indeed, more than half of the studies indicated a lack of tolerance to the inhibitory effect of 5-$HT_{2C}$ receptor activation on feeding (table 4a). Although evidence has been reported that tolerance to the effect of 5-$HT_{2C}$-receptor-induced corticosterone and prolactin release develops upon repeated administration of mCPP

**Table 4.** Effects of subchronic or chronic treatments with antidepressants or 5-$HT_{2C}$ receptor agonists on 5-$HT_{2C}$-receptor-mediated effects at the level of the hypothalamus

**a** Effect of agonists and antidepressants on 5-$HT_{2C}$-receptor-induced reduction of feeding and body weight

| Class | Treatment | Doses mg/kg/day | Time days | Drug challenge | Rodent strain | Food access | Body weight loss | Reference |
|---|---|---|---|---|---|---|---|---|
| Agonist | RO 600175 | 36 | 14 | none | Lister-Hooded | 24 h | = | 135 |
| | mCPP | 12 | 14 | none | Lister-Hooded | 24 h | = | 135 |
| | mCPP | 10 | 8 | none | Lister-Hooded | 24 h | = | 152 |

| Class | Treatment | Doses mg/kg/day | Time days | Drug challenge | Rodent strain | Food access | Effect on hypophagia | Reference |
|---|---|---|---|---|---|---|---|---|
| Agonist | RO 600175 | 36 | 14 | none | Lister-Hooded | 24 h | ↓ | 135 |
| | mCPP | 12 | 14 | none | Lister-Hooded | 24 h | ↓ | 135 |
| | mCPP | 2.5 | 4 | none | Wistar | 4 h | ↓ | 134 |
| | mCPP | 10 | 6 | none | CD1 | 24 h | = | 116 |
| | mCPP | 10 | 14 | mCPP | Lister-Hooded | 4 h | = | 95 |
| | mCPP | 10 | 28 | none | Lister-Hooded | 24 h | = | 152 |
| | (–)-trans-PAT | 4.2 | 4 | none | C57BL/6 | 30 min | ↓ | 153 |
| | WAY-161503 | 1.9 | 10 | none | Sprague Dawley | 4 h | ↓ | 154 |
| | | 0.73 | 15 | none | obese Zucker | 4 h | = | 154 |
| SSRI | sertraline | 5 | 14 | mCPP | Sprague Dawley | 1–2 h | = | 146 |

or antidepressant drugs (fig. 3b), not all studies agree with these findings (table 4b). Finally, spinal 5-$HT_{2C}$ receptors, that mediate penile erection [108], do not apparently desensitize upon chronic antidepressant treatment [109].

*Models of Depression and Anxiety*

Table 5 summarizes the effects of repeated treatments with 5-$HT_{2C}$ receptor ligands in models of depression and anxiety. Besides the 5-$HT_{2C}$-receptor-induced corticosterone release, only few of the models reviewed in tables 2–4 have some relevance

**Table 4. b** Effects of agonists and antidepressants on 5-$HT_{2C}$-receptor-induced hormonal activation

| Class | Treatment | Doses mg/kg/day | Time days | Drug challenge | Species / strain | Hormone studied | Hormonal activation | Reference |
|---|---|---|---|---|---|---|---|---|
| Agonist | mCPP | 0.08 | 3 | mCPP | human | ACTH | ↓ | 148 |
| | mCPP | 0.08 | 3 | mCPP | human | cortisol | ↓ | 148 |
| | mCPP | 10 | 14 | mCPP | Lister-Hooded | cortico sterone | ↓ | 95 |
| | mCPP | 10 | 15 | mCPP | Sprague Dawley | cortico sterone | = | 140 |
| | mCPP | 10 | 15 | mCPP | Sprague Dawley | prolactin | = | 140 |
| | mCPP | 0.08 | 3 | mCPP | human | prolactin | ↓ | 148 |
| TCA | desipramine | 5 | 21 | MK-212 | Sprague Dawley | prolactin | ↓ | 155 |
| SSRI | fluoxetine | 10 | 21 | MK-212 | Sprague Dawley | prolactin | ↓ | 155 |
| | paroxetine | 20–30 | 21 | mCPP | human | prolactin | ↓ | 149 |

to depression and anxiety. It might be speculated that the 5-$HT_{2C}$-receptor-induced hyperthermia shares some characteristics with the stress-induced hyperthermia or that the 5-$HT_{2C}$-receptor-induced hypolocomotion is a 'surrogate' model for psychomotor retardation, which characterizes some depressed patients [9]. However, evidence supporting the latter interpretation is limited and not convincing in view of the behavioral studies (using the FST) in which 5-$HT_{2C}$ receptor agonists actually increased motor activity [26, 27]. Overall, there are not enough studies that have directly assessed tolerance on the basis of behavioral measures of anxiety/depression and the occurrence of putative desensitization of 5-$HT_{2C}$ receptors after chronic antidepressant treatment. Nonetheless, the antidepressant-like effect of acute 5-$HT_{2C}$ receptor agonist administration was found unaltered after repeated treatment with such a drug in the bulbectomy model of depression (table 5), suggesting that the therapeutic effect of 5-$HT_{2C}$ receptor activation is fast acting and does not develop tolerance under chronic treatment conditions. However, antidepressant drugs usually require chronic administration to be active in this model [110]. Therefore, the rapid inhibitory effect of 5-$HT_{2C}$ receptor agonists on hyperactivity measured in the bulbectomy model may reflect more a reduction of impulsivity than a 'classical' antidepressant-like effect. In particular, 5-$HT_{2C}$ receptors in the nucleus accumbens have been found to play an important role in the control of impulsivity [111]. Finally,

**Table 5.** Effects of acute versus chronic treatments with antidepressants and 5-$HT_{2C}$ receptor ligands in models of anxiety and depression

| Class | Treatment | Days | Acute effect | Drug challenge | Models | After treatment | Reference |
|---|---|---|---|---|---|---|---|
| Agonist | mCPP | 2–3 | anxiogenic | none | patients | anxiolytic | 148 |
| | WAY-63909 | 5–21 | antidepressant-like | none | bulbectomy | antidepressant-like | 27 |
| | Ro 600175 | 19 | no effect | none | anhedonia | antidepressant-like | 156 |
| Antagonist | S32006 | 20 | anxiolytic-like | S32006 | SI | anxiolytic-like | 25 |
| | mianserin | 14 | anxiogenic | none | EPM | anxiolytic-like | 113 |
| | ritanserin | 14 | no effect | none | EPM | anxiolytic-like | 157 |
| SSRI | fluoxetine | 6 | anxiogenic | mCPP | SI | anxiogenic | 35 |
| | fluoxetine | 4–14 | anxiogenic | fluoxetine | SI | anxiogenic | 35 |
| | fluoxetine | 28 | anxiogenic | fluoxetine | SI | anxiolytic-like | 35 |
| | sertraline | 14 | not tested | mCPP | SI | anxiolytic-like | 146 |
| | paroxetine | 21 | not tested | mCPP | SI | anxiolytic-like | 37 |
| Inverse agonist | SB243213 | 14 | anxiolytic-like | SB43213 | SI | anxiolytic-like | 114 |

SI = Social interaction test; EPM = elevated plus maze.

Dekeyne et al. [25] reported an inhibitory effect of a novel 5-$HT_{2C}$ receptor antagonist, S32006, on immobility in the FST and found no tolerance to this antidepressant-like effect [25].

As discussed above, 5-$HT_{2C}$ receptor activation generally exerts an anxiogenic effect. Although acute administration of SSRIs triggers anxiety, long-term, but not subchronic, administration of these drugs prevents the anxiogenic-like effect of mCPP (table 5). This anxiolytic effect of long-term SSRIs could be mediated by a desensitization of 5-$HT_{2C}$ receptors in the hippocampus [37, 112]. In one human study, a relatively rapid tolerance to the anxiogenic effect of mCPP was observed after repeated administration of this drug. However, a 1-month – but not a 2-week – treatment with an SSRI is needed to exert a similar anxiolytic-like effect (i.e. to prevent the effect of mCPP) in rats (table 5). In some exceptional cases, as explained above, antidepressant drugs with 5-$HT_{2C}$ receptor antagonist activity can exert a paradoxical increase in anxiety after acute administration, but after chronic treatment, this

anxiogenic effect is reverted into an anxiolytic effect concomitantly with decreased 5-$HT_{2C}$ receptor binding in the amygdala [113]. Finally, no tolerance to the anxiolytic effect of a 5-$HT_{2C}$ receptor inverse agonist was observed following chronic administration [114].

To account for the behavioral and hormonal changes listed in tables 2–5, only few studies have been done so far to identify the brain areas where 5-$HT_{2C}$ receptor desensitization might occur. The only brain area where a 5-$HT_{2C}$-receptor-mediated signaling can consistently be measured in vivo is the choroid plexus, where 5-$HT_{2C}$ receptors are sufficiently dense. However, tolerance to the anxiogenic effect of mCPP following repeated 5-$HT_{2C}$ receptor activation was not correlated with any changes at the level of the 5-$HT_{2C}$ receptors mediating PI hydrolysis in the choroid plexus [43]. Also, there is presently no reliable biochemical assay available to directly assess 5-$HT_{2C}$ receptor coupling with its G protein in brain tissues. Indeed, 5-$HT_2$ receptor agonists causing [$^{35}$S]GTPγS binding to Gαq proteins in rat cortex [115] or mouse striatum [C.B.P.M. and R.M., unpubl. obs.] involve exclusively 5-$HT_{2A}$ receptors, which are present at a much greater density than 5-$HT_{2C}$ receptors. Clearly, new tools have to be developed to assess, at the molecular level, whether or not 5-$HT_{2C}$ receptor desensitization occurs upon chronic antidepressant treatment.

*Assessing 5-$HT_{2C}$ Receptor Function in Selected Brain Areas*

A well-known assay to assess changes in central nervous system neuronal activity uses expression of the immediate early gene protein c-Fos, which is induced by an elevation of intracellular $Ca^{2+}$. This may appear as an ideal tool to investigate the functional state of 5-$HT_{2C}$ receptors as their activation mobilizes cellular $Ca^{2+}$ via PLC-induced inositol triphosphate signaling. However, as far as we know, no studies investigated the effect of long-term antidepressant treatment on 5-$HT_{2C}$-receptor-induced c-Fos expression. Two studies by Rowland et al. [116, 117] investigated the effect of repeated treatments with mCPP or the 5-HT releaser dexfenfluramine on c-Fos immunoreactivity in response to an mCPP challenge. They found a significant attenuation of Fos induction in several brain regions including the CeA, the PVN and the BNST in both rats and mice (fig. 3b). However, the dose of mCPP used in these studies was rather high and it is not clear to which extent the response was mediated by 5-$HT_{2C}$ receptors. Furthermore, as mentioned above, mCPP induces a stress response, and it is well known that repeated exposure to a homotypic stressor generally results in attenuated activation of the molecular cascade leading to the induction of immediate early genes such as c-Fos [118]. Therefore, further studies investigating the effects of chronic drug treatments on brain 5-$HT_{2C}$ receptors, using drugs different from the 5-$HT_{2C}$ receptor ligand used to trigger c-Fos expression, are eagerly awaited.

5-$HT_{2C}$ receptor function can also be assessed indirectly by measuring the effect of 5-$HT_{2C}$ receptor ligands on the release or the turnover of monoamines. Indeed, 5-$HT_{2C}$ receptor activation has been shown to have a predominant inhibitory effect on the release of catecholamines, but not of 5-HT, under basal conditions [74, 119],

and to reduce stress-induced increase in 5-HT turnover and extracellular 5-HT levels [37]. Using Flinders sensitive line rats, as a model of depression, Dremencov et al. [21] reported an attenuation of the inhibitory effect of 5-$HT_{2C}$ receptor activation on accumbal dopamine release following chronic treatment with nefazodone and desipramine. However, another study suggests that the 5-$HT_{2C}$ receptors that indirectly inhibit the firing rate of dopamine neurons in the ventral tegmental area do not desensitize following chronic SSRI treatments [120]. In contrast, the 5-$HT_{2C}$ receptors that inhibit the stress-induced increase in 5-HT turnover, most likely located in postsynaptic areas rather than in the raphe [23], appeared functionally desensitized in the hippocampus, the ventral tegmental area/substantia nigra and the accumbens following long-term SSRI (fig. 3b). This 5-$HT_{2C}$-receptor-mediated effect was more resistant to desensitization in the frontal cortex [37]. Mutant mice lacking the 5-HT transporter (5-$HTT^{-/-}$) also displayed functional desensitization of this 5-$HT_{2C}$-receptor-mediated inhibition of 5-HT turnover during stress in the same brain areas [121].

*The Effect of Antidepressant or 5-$HT_{2C}$ Agonist Treatments on 5-$HT_{2C}$ Receptor mRNA Editing*

Another indirect measure of the functional state of 5-$HT_{2C}$ receptors in various brain areas might become available through the measurement of 5-$HT_{2C}$ receptor pre-mRNA editing. This editing process, which leads to the substitution of amino acid residues in the second intracellular loop of the receptor via the conversion of adenosine into inosine by adenosine deaminases acting on pre-mRNA (at sites denominated A-E), is unique to the 5-$HT_{2C}$ receptor type among all 5-HT receptors. In vitro studies indicated that editing decreases the coupling of the receptor with its downstream signaling system [122], while clinical studies reported increased 5-$HT_{2C}$ receptor mRNA editing in the frontal cortex of depressed suicide victims [123–125]. On the other hand, Gurevich et al. [126] found that repeated treatment with the 5-$HT_{2A/2C}$ agonist DOI increased editing at the C′ site of the 5-$HT_{2C}$ receptor in the frontal cortex of 129SV mice. Furthermore, BALB/c mice did show significant editing at several sites in the cortex, albeit not at the C′ site following chronic fluoxetine [127], whereas C57BL/6 mice did not display any significant changes in editing under the same treatment conditions. Finally, a recent study [128] reported small increases of editing only at the A and B sites in the striatum and in the hippocampus, but not in the cortex, of C57BL/6 mice chronically treated with antidepressant drugs (amitriptyline, fluoxetine) or with a selective 5-$HT_{2C}$ antagonist (SB 206553). Obviously, much research is needed to ascertain the effects of antidepressant drugs on 5-$HT_{2C}$ receptor mRNA editing especially because these effects apparently vary not only between strains but also from one brain area to another. Recent studies [129, 130] on transgenic mice expressing only the fully edited 5-$HT_{2C}$ receptors (VGV) showed that these mice have increased 5-$HT_{2C}$-receptor-mediated behavioral responses (related to locomotion and feeding) and increased 5-$HT_{2C}$ receptor binding compared to wild-type mice and mice expressing only the nonedited isoforms. These transgenic mice presented a large

increased cell surface expression of the edited isoforms, which is likely to be an overcompensation for the blunted coupling of the VGV isoform with its G-protein transduction system [129, 130]. This shed doubts on the initial assumption that increased 5-$HT_{2C}$ receptor mRNA editing would be associated with decreased 5-$HT_{2C}$ receptor function in vivo.

## Overview and Perspectives

There is a great interest in the development of new 5-$HT_{2C}$ receptor ligands as evidence indicates alterations of 5-$HT_{2C}$-receptor-mediated functions in depressed patients [15, 108]. However, these dysfunctions need to be ascertained [131, 132] and it is not clear yet which pharmacological strategy would be the most appropriate – that is, the development of compounds with agonist, antagonist or inverse agonist properties. Some pharmaceutical companies have developed compounds such as agomelatine and S32006 which are endowed with 5-$HT_{2C}$ receptor antagonistic properties [12, 25], while others favor a 5-$HT_{2C}$ receptor agonistic action for the treatment of depression with WAY-163909 [27]. The latter strategy claims a rapid antidepressant action of selective 5-$HT_{2C}$ receptor agonists and lack of attenuation of many 5-$HT_{2C}$-receptor-mediated effects with repeated stimulation. As emphasized above, compounds that would act as 5-$HT_{2C}$ receptor agonists at the midbrain level might have beneficial effects against anxiety and panic. Therefore, 5-$HT_{2C}$ receptor desensitization after antidepressant treatment would not be especially helpful in some key brain areas such as the PAG involved in defense behavior. Other important sites for a potential side effect include the hypothalamic (ventromedial or dorsomedial) nuclei involved in feeding [133], even though selective blockade of 5-$HT_{2C}$ receptors with SB 242084 does not appear to induce obesity as much as the genetic deletion of 5-$HT_{2C}$ receptors [33]. In contrast, a reduction of 5-$HT_{2C}$-receptor-mediated transmission would be beneficial in other areas such as the PVN considering the known HPA axis hyperactivity of depressed patients and the prominent stimulatory action of 5-$HT_{2C}$ receptors on CRH neurons [58].

Although a majority of authors claim that antidepressant drugs do desensitize 5-$HT_{2C}$ receptors, this notion is based mainly on results obtained on cellular systems in vitro or in studies using either the hypolocomotion or the hyperthermia assays (tables 2, 3). Besides what has been found in relation with hypothalamic functions, there is a scarcity of data addressing the occurrence of 5-$HT_{2C}$ receptor desensitization in specific brain areas following long-term antidepressant treatment. Nonetheless, functional desensitization of 5-$HT_{2C}$ receptors modulating monoaminergic neurotransmission has been reported in some brain areas such as in the nucleus accumbens following long-term SSRI [21, 37].

Are the prominent effects of 5-$HT_{2C}$ receptor ligands on stress, defense and anxiety and the rapid desensitization/resensitization with agonists, antagonists and inverse

agonists in vitro really relevant to the antidepressant-like effects observed with these compounds in models such as the rat FST? All these questions, among others, need to be answered in order to rationally develop new therapeutic strategies aimed at changing 5-$HT_{2C}$-receptor-mediated transmission. In view of the central role played by 5-$HT_{2C}$ receptors in stress and anxiety, one valuable strategy in treating depression might be to reduce these comorbidities by targeting 5-$HT_{2C}$ receptors as an adjuvant to other antidepressant drugs [12, 24]. Resolving the controversies about the role of 5-$HT_{2C}$ receptors in depression is a key issue toward possible development of novel therapeutic strategies focused on this promising molecular target.

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# Chromatin-Based Treatments for Affective Disorders – Insight or Utopia

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**Abstract**

This chapter provides a brief overview of chromatin-related mechanisms and summarizes recent leads in neurobiology and biological psychiatry suggesting that some of these mechanisms may provide promising targets for future pharmacological treatments of affective disorders. We acknowledge the fact that experimental approaches in this area are still in their infancy, and point to some of the difficulties to overcome before epigenetic mechanisms can be considered bona fide therapeutic targets in psychiatry. A growing body of preclinical and translational research supports the view that patterns of DNA methylation, as well as histone posttranslational modifications, in the brain, are critically shaped during early postnatal periods of development, and remain relatively dynamic throughout adulthood. These developmental windows may offer opportunities for therapeutic interventions. Initial studies in preclinical models of affective disorders, with histone deacetylase inhibitors, the currently best characterized class of pharmacological chromatin modulators, suggest that gene transcriptional 'derepression' promoted by these drugs may bear antidepressant-like activity. These results are encouraging although many questions remain open regarding the exact mechanisms targeted by these compounds. Much needs to be uncovered also regarding the regional, cellular and developmental patterns of expression of chromatin-related enzymes in the brain, and how the function of these proteins may best be exploited to generate circuit-targeted psychopharmacological effects.



Our understanding of gene transcriptional mechanisms that couple synaptic activity to modifications in the structure and function of neural circuits has progressed dramatically over the past 20 years [1]. These important breakthroughs, both in cell biology and basic neuroscience, have helped to shape the current 'neuroplastic' psychopharmacological model. Under this view, the therapeutic activity of antidepressants is interpreted as an emergent consequence of molecular and cellular adaptations that result from repeated monoaminergic activation. Advances in this area of neuroscience have also allowed for the development of a molecular pharmacopoeia to directly manipulate gene transcription in neurons and glia. As compounds in this

category are being evaluated for their psychopharmacological properties in animal models, one hope is that direct targeting of transcriptional mechanisms may allow to shortcut the slow neuroplastic changes to accelerate antidepressant responses. Exploration of this class of mechanisms may also have significant heuristic value in promoting the emergence of novel etiological models for affective disorders, a disease category where morbidity rates remain largely unaccounted for by traditional gene × environment models [2]. The present review is primarily focused on the exceptional effort currently employed in the domain of 'epipharmaceutics', where agents that specifically target chromatin-related mechanisms of gene regulation are being developed and evaluated [3]. In this chapter, we provide a brief overview of these epigenetic mechanisms and summarize promising clinical and preclinical leads in the area of neurobiology. After a close examination of this literature, we suggest that there may be a future for chromatin-based approaches in the treatment of affective disorders. We also point to some of the difficulties that need to be overcome before chromatin-related mechanisms can be considered bona fide druggable psychopharmacological targets.

## An Introduction to Epigenetics and Chromatin Modifications

The concept of epigenetics centers around an ensemble of biological mechanisms that promote stable, and in some cases heritable, changes in gene expression or phenotype, independently of changes in DNA sequence [4–6]. In most biological fields, including certain areas of neurobiology, where experimental models primarily involve tissues or cell populations undergoing mitotic or meiotic divisions, epigenetic mechanisms examined are often intimately linked to DNA replication cycles or reproductive process [7, 8]. However, for psychopharmacologists interested primarily in functions mediated by fully differentiated neurons, the concept of epigenetics generally drops its accent on 'heritability' to designate stable alterations in transcription coupled to changes in chromatin status [6]. In this context, there are indications that epigenetic mechanisms may function as an archival system for records of past activity, and provide a mechanistic basis for durable functional alterations [6]. Given recent evidence that some neurons can reenter the cell cycle in specific pathological contexts, and that a portion of cells in the adult brain retain proliferative capability, it remains unclear to what extent epigenetic alterations reported from diseased brain homogenates reflect mechanisms that are independent of cell cycle [7]. This question will remain open until neuroscientists can gain access to epigenetic information in a cell-type-specific manner [9].

Chromatin, the basic constituent of chromosomes, is a nuclear complex that combines genomic DNA, histones and nonhistone proteins. The fundamental motif of chromatin, the nucleosome core particle, consists of 147 bp of DNA organized in approximately 2 superhelical turns of DNA wrapped around an octamer of core

histone proteins (2 copies each of canonical histones H2A, H2B, H3, and H4 or their variants) [6, 10, 11]. Various mechanisms that introduce variations into nucleosome structures, through attachment of chemical moieties to DNA or histones, can dynamically modulate chromatin folding. These mechanisms contribute to defining the 2 archetypal functional states of chromatin (i.e., euchromatin vs. heterochromatin) that broadly correspond to an open and locked state, respectively, and often but not systematically, align with actively transcribed versus silent genes. Two major processes have been described whereby chromatin conformation influences gene transcription. First, certain chemical alterations of chromatin or DNA can directly modulate the spatial organization of the DNA polymer (either by a change in electrostatic charge or through internucleosomal spacing) thus facilitating or restricting the physical access of DNA-binding proteins (such as transcription factors) to regulatory sequences. Second, the attachment of chemical moieties onto DNA or a nucleosome surface acts as a recruitment signal promoting the association of chromatin-binding enzymes involved in transcriptional activation and silencing [11]. In the last 10 years, several families of evolutionarily conserved chromatin-modifying enzymes have been discovered. The rapid rise in the number of new enzymes identified has required the establishment of a novel and more systematic nomenclature for the genes encoding chromatin-related enzymes [12]. Chromatin biologists have also proposed several simplified operational models which help the broader community navigate through the overwhelming complexity of epigenetic mechanisms [5]. A notable classification regroups enzymes according to their ability to write, read or erase epigenetic marks on chromatin [8]. *Writers* include, but are not limited to, kinases, methyltransferases and acetyltransferases which are able to add chemical moieties to DNA or histones, whereas *erasers* (phosphatase, deacetylases and demethylases) catalyze reciprocal reactions. *Readers* comprise a wide category of proteins harboring domains that are able to bind specifically to certain classes of epigenetic marks on histones or DNA. These classes of proteins include bromo-, chromo- or CXXC domains, which interact selectively with acetylated lysines, methylated lysines and methylated DNA, respectively (for reviews, see Berger [11] and Jaenisch and Bird [13]). A second operational classification, illustrated in figure 1, distinguishes 3 types of signals (namely *epigenators, initiators* and *maintainers*) corresponding to 3 sequential steps critical to the establishment of epigenetic marks [5].

Most studies in translational and basic neuroscience, thus far, have been limited to the examination of 2 main classes of epigenetic modifications involving DNA methylation and histone posttranslational modifications (PTMs). These 2 mechanisms have also been the targets of the majority of clinical trials conducted in the cancer field with the first generation of FDA-approved epigenetic drugs [i.e., the histone deacetylase (HDAC) inhibitors and DNA methyltransferase inhibitors]. It is therefore likely that these drugs will become the first to be tested clinically for the treatment of neuropsychiatric diseases [3]. While the scope of this review will focus largely upon these 2 specific mechanisms, it is important to keep in mind that these processes represent

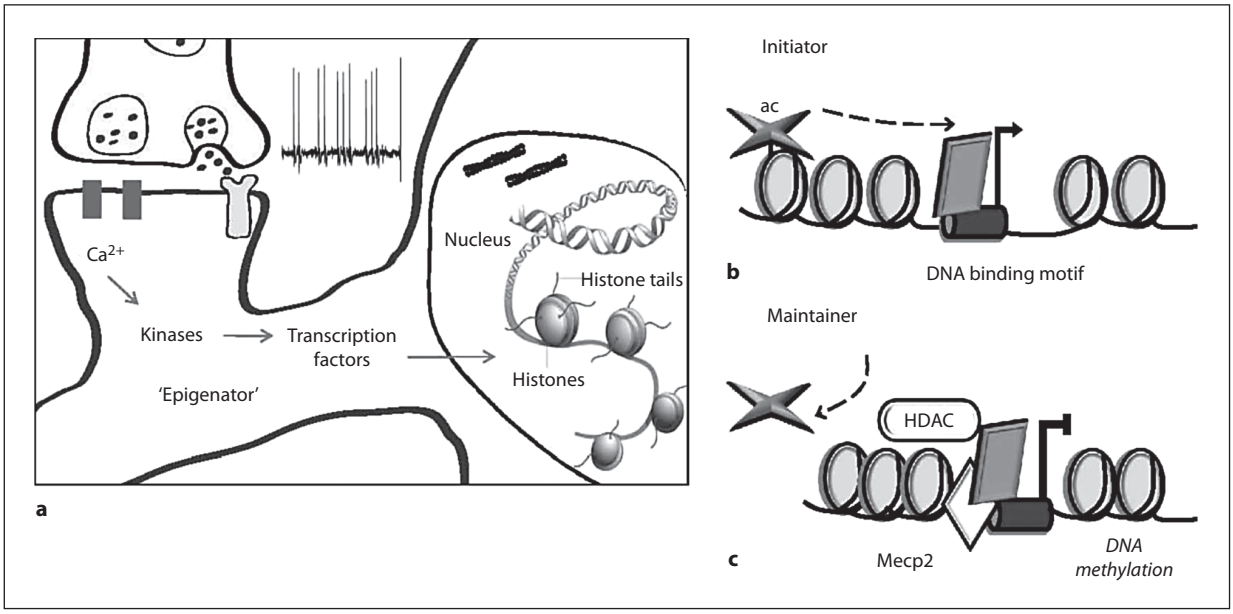


**Fig. 1.** The neuronal epigenetic pathway. Schematic view of the sequence of events leading to epigenetic changes in neurons, based on the operational definition proposed by Berger et al. [5]. In this example, the sequence of epigenetic events depicted in **a**, **b** and **c** leads to stable transcriptional silencing of an active promoter. Chromatin in a neuronal nucleus is depicted as a DNA string wrapped around nucleosomes (beads). **a** Changes in the environment, extracellular and intracellular signals occurring upstream of the first epigenetic event on chromosomes are referred to as 'epigenators'. The epigenator signal is transient and remains in the cell only long enough to trigger the epigenetic phenotype. In the context of neuronal transmission, calcium-dependent signals, which ultimately engage phosphorylation-dependent transcriptional activators or repressors, fit this definition. The participation of the epigenator pathway culminates in the establishment of a local chromatin context, at a precise location on the genome. **b** An active gene promoter with a relaxed chromatin conformation characterized by acetylated histone tails at H3 and H4. The modification in this chromatin conformation leading to transcriptional repression in response to epigenator signal involves a second category of players, referred to as 'initiators'. A key feature of proteins in the initiator category is their DNA sequence recognition capability which is necessary to determine the location of chromatin to be modified. Classical examples of epigenetic initiators in neurons include proteins harboring DNA binding domains such as the leucine zipper found in Fos/Jun family members, or zinc finger domains found in several immediate early genes including ZIF268, as well as many members in the nuclear receptor family, but also in the CREB coactivator CBP/P300, and the neuron-restrictive silencing factor REST. **c** The same gene promoter where gene transcription is now repressed. Note compacted chromatin conformation, deacetylated histone tails, and methylated DNA and histones. Contrary to the epigenators, initiators do not dissipate after their action, but persist through positive feedback mechanisms involving a 3rd category of players referred to as 'maintainers'. Epigenetic maintainers are a category of proteins able to sustain an epigenetic chromatin state but unable to initiate it. Maintainers facilitate many different pathways, including DNA methylation, histone modifications, histone variants and nucleosome positioning. Examples of maintainers here include HDAC-containing repressive complex, methylated DNA binding proteins discussed in subsequent sections.

only a fraction of the many known nonmutagenic mechanisms that can affect gene transcription in a heritable or stable manner. Other physiologically prevalent chromatin mechanisms include ATP-dependent nucleosome remodeling, histone variant deposition (e.g. H3.3, macroH2A, H2AZ, H2AX) as well as noncoding RNA-mediated regulation.

## DNA Methylation and Affective Disorders

DNA methylation is the most widely studied epigenetic mechanism. It consists of covalently added methyl groups to the position 5 of cytosines, and it is generally associated with gene silencing [18]. In vertebrates, methylation of DNA occurs at cytosine residues usually followed by guanines (i.e., CpG dinucleotides). Symmetrically methylated CpG sites are distributed throughout mammalian genomes at an average frequency of 1 methyl-CpG every 150 bp. Hypomethylated regions coincide with CG-rich stretches of DNA (average size of approx. 1 kb in humans) called CpG islands. Gene promoters, as well as noncoding RNA promoters found in intergenic regions, are often rich in CpG islands. Close to 60% of genes in the human genome contain CpG islands within close proximity to their transcriptional start site and these are usually unmethylated [13, 14]. DNA methylation is catalyzed by enzymes that belong to the DNA methyltransferase (Dnmt) family, composed of 4 members in eukaryotes: Dnmt1, Dnmt3a, Dnmt3b, and Dnmt3l. Dnmt1 is the most abundant isoform in mammalian cells and is coined a 'maintenance methyltransferase' because of its 20-fold higher affinity for hemimethylated over symmetrically methylated double-stranded DNA. Dnmt1 is responsible for reproducing DNA methylation patterns on newly synthesized strands of DNA during semiconservative replication [13]. In contrast, other Dnmt family members (i.e., de novo DNA methyltransferases) can effectively methylate CpG dinucleotides which are symmetrically unmethylated. De novo methylation occurs in a restricted set of instances during development after DNA has become completely demethylated, such as following fertilization, during gametogenesis and during early postnatal development [7]. Based on a classic view of the role of Dnmts, an organism's patterns of DNA methylation are established early on in the embryo (during the morula stage) and are reproduced across generations of cells throughout embryo development and into adulthood. Genome-wide analyses of methylated DNA in humans have demonstrated that within a specific cell type, patterns of DNA methylation are highly conserved between individuals, while there is a large variation between somatic cell types within the same individual [14, 15].

Classic examples supporting the physiological functions of DNA methylation in gene regulation are derived from studies of X chromosome inactivation, as well as gene imprinting in which 1 of 2 alleles is silenced in a progenitor-of-origin manner. Silencing of gene expression by DNA methylation has been shown to involve at least 2 classes of mechanisms. First, CpG methylation directly interferes with transcription

factor binding to DNA. This molecular event has been well illustrated in early reports on the transcription factor CTCF [13], and more recently by studies examining ZIF268 (NGFIA) during the silencing of the glucocorticoid receptor (GR) gene in the brain [16, 17]. A second class of mechanisms involves the recruitment of methylated DNA 'readers' with high affinity for methylated CpG sites, such as methyl-CpG-binding protein 2 (MeCP2) and its relatives, the methyl-CpG-binding domain proteins MBD1–MBD4 [13]. In most cases, these proteins partner with large multimeric repressor complexes, which also include several classes of histone-modifying enzymes discussed in the next section of this chapter, and thus promote a repressive form of nucleosomal remodeling. However, there are instances where MeCP2 has been found to act individually as a direct repressor of transcription [18, 19].

Initial insight for establishing a role for DNA methylation in central nervous system disorders has come from the observation that genetic mutations interfering with the proper establishment, or reading, of DNA methylation patterns lead to neurodevelopmental syndromes with dramatic behavioral components, such as Rett and fragile X syndrome [15]. A vital role of DNA methylation in brain development, particularly during periods that precede neuronal differentiation, has been well substantiated by studies with animal models and tissue culture systems. Both classes of DNA methyltransferases have been shown to participate in various stages of neural fate. For instance, Dnmt1 functions downstream of the JAK-STAT signaling pathway to control the timing of astrogliogenesis, a critical developmental step during which precursors switch from neurogenesis to gliogenesis [20]. Animals harboring timed conditional knockouts of Dnmt1 corroborate the critical role of DNA methylation during this developmental period. In mice with conditional Dnmt1 deletion, a 20–30% increase in hypomethylated excitatory neurons can be observed across early development and the adult life span, an effect that leads to severe cortical and hippocampal neuronal degeneration as well as neurobehavioral defects in learning and memory. Contrasting with the drastic consequences of early Dnmt1 mutations, knockouts carried out in later stages of development do not develop obvious phenotypes, suggesting that this gene may no longer be required to maintain global DNA methylation patterns after neuronal differentiation [20]. Nonetheless, behavioral studies discussed below indicate that subtle changes in DNA methylation levels may serve a predisposing role in some psychiatric diseases, including depression.

Since DNA methylation patterns are thought to be fixed in nondividing cells, this epigenetic mark has long been considered an unlikely mechanism for dynamic regulation of gene expression in postmitotic neurons. However, such a view has been hard to reconcile with the observation that postmitotic neurons (but not oligodendrocytes or astrocytes) maintain relatively high constitutive levels of Dnmt expression in adulthood [9, 19]. Furthermore, inhibitors of Dnmts applied to neuronal cell lines or primary neuron cultures have now been shown to inhibit synaptic transmission and to stimulate the transcription of several genes involved in psychiatric diseases such as reelin and glutamic acid decarboxylase. There have also been reports of activity-

dependent demethylation of genomic DNA in neurons. These results, if corroborated, could have dramatic theoretical implications; however, the search for enzymes that actively demethylate DNA has long been a controversial line of investigation and such mechanisms remain unclear [20]. Candidate enzymes involved in activity-dependent demethylation of specific gene promoters and regulation of neurogenesis include growth arrest and DNA-damage-inducible, beta (Gadd45b) a member of the Gadd45 family of proteins previously reported to be involved in DNA repair and DNA 5-methylcytosine excision [19]. Likewise, neuronal depolarization has been shown to promote the release of methyl-binding protein MeCP2 from several gene promoters, thereby allowing activity-dependent derepression of brain-derived neurotrophic factor (BDNF) and the norepinephrine transporter [9], 2 genes also regulated by chronic antidepressant treatments. It is currently unclear as to whether or not classical antidepressants can directly promote demethylation of these genes and if such changes are involved in their therapeutic effects.

Further indications that global DNA methylation landscapes are more unstable than originally thought are derived from studies that reveal this process can evolve with aging [9, 15], in response to specific environmental events during postnatal development or in adult life [16]. This form of epigenetic metastability is increasingly considered as a potential source for the high rates of monozygotic twin discordance reported in certain psychiatric disorders, including depression, where the explanatory value of identified nonshared environments has been poor. Of note, methylation differences between monozygotic twins have been reported for CpG sites in a number of genes associated with psychiatric illness such as dopamine $D_2$ receptor and catechol-O-methyltransferase genes [16]. Several other lines of evidence reviewed below, linking DNA methylation to the regulation of hypothalamic-pituitary-adrenal axis function, neurotrophic factor signaling, and several neurotransmitter systems, both in humans and animals, provide clues about how this epigenetic mechanism could play a role in the emergence and alleviation of depression.

A series of recent postmortem studies using direct sequencing and bisulfite mapping in brain tissues from suicide completers have uncovered the occurrence of DNA hypermethylation in regulatory regions of several genes found to be strongly repressed in this population – namely, the GR [17], $GABA_A$ receptor $\alpha_1$ subunit [48] and the truncated variant of the neurotrophin receptor TrkB T1. In the case of the $GABA_A$ receptor subunit, for instance, transcript abundance in cortical areas was negatively correlated with levels of *Dnmt3b* mRNA. These results suggest that alterations in the regulation of this de novo DNA methyltransferase could drive cortical repression of $GABA_A$ receptor gene. Although these are among the first reports of changes in DNA methylation at GABAergic genes in depression, it is important to note that several previous reports have demonstrated epigenetic regulation of GABAeric genes in the context of other psychiatric diseases, such as schizophrenia and bipolar disorders [19, 47]. These results invite the question of whether DNA methylation changes in adults with depression reflect pathological 'states' occurring at the time of tissue

collection or 'trait' alterations that precede the occurrence of the psychiatric symptoms. Experimental data by several groups, illustrated partly in the next paragraph, suggest that critical developmental windows may exist at which these stable modifications could be established in responses to environmental factors such as diet or social interactions.

Meaney and colleagues [16] have shown in an impressive line of experimental work developed over the last 2 decades that the quality of maternal care received during early postnatal life epigenetically determines components of adult emotional behavior. This maternal influence appears to exert itself, at least in part, by programming how efficiently the hypothalamic-pituitary-adrenal axis terminates the neuroendocrine stress response. Normalization of plasma adrenal steroid levels following stress exposure involves a negative feedback loop mediated primarily by the centrally expressed GR. Hypoactivity of this negative feedback loop is a well characterized endocrine feature of several affective disorders, including major depression, where this trait has been linked to GR downregulation. In a series of experiments comparing pups raised by mothers with diverging rearing styles, Meaney and colleagues have identified DNA methylation events at the GR gene that bridge the influence of a postnatal environment to the stable changes in the transcription and function of this gene. This maternal influence is captured within the first 2 weeks of life in the rodent (i.e., the critical period for corticosteroid developmental influences) during which many genes, including the GR, undergo rapid de novo methylation followed by progressive demethylation. When deficits in mother-pup interactions occur during this period, a disruption to the normal level of demethylation on a CpG island located within the exon $1_7$ of the GR gene is observed, and this effect results in a permanent state of repression for the GR. Importantly, the hypermethylated DNA region of the GR is highly localized and embedded within the promoter that drives brain-specific expression of this gene. This promoter region overlaps with a consensus binding site for the transcription factor NGFIA. In hippocampal neurons, expression and binding of NGFIA to the GR gene promoter is directly triggered by maternal contact and involves serotonin-dependent synaptic transmission. Hypermethylation of the 5′ CpG island at the NGFIA consensus site can be mimicked artificially in tissue culture systems, and has thus been found sufficient to impede binding of this transcription factor, and to silence GR expression. Reciprocally, NGFIA binding to the GR seems to be required for the normal developmental occurrence of GR demethylation.

A number of studies conducted by independent research groups support the view that alterations in DNA methylation, caused by environmental perturbations during early development, are likely to be widespread and encompass multiple additional gene promoters (such as the arginine vasopressin gene or estrogen receptor gene) also contributing to define a neuroendocrine liability towards affective disorders. This line of results has started providing a long-sought mechanistic blueprint to explain the critical contribution of early social environments in programming vulnerability to affective diseases. The first wave of translational studies that have examined the

methylation status of the GR promoter in depressed populations indicated that the model proposed by Meaney and colleagues (based on rodent data) may be most relevant to subpopulations of patients that experienced early abuse, or children born from mothers that suffer from postpartum depression [16].

How does new knowledge about the role of DNA methylation in the brain lend itself to the advent of improved therapeutic interventions? The good news is that in rodents both GR hypermethylation and long-term behavioral and neuroendocrine consequences of deficient mother-pup interactions can be rescued by restoring adequate maternal environment through fostering conditions. A similar rescue can be observed after directly infusing the HDAC inhibitor trichostatin A into the hippocampus. In contrast, supplementing diet of highly nurturing mothers with methyl donors that promote DNA hypermethylation antagonizes the beneficial effect of maternal care. The latter result suggests that repression of GR gene expression, as driven by deficiencies in mother-pup interactions, likely involves the recruitment of HDACs containing repressive complexes to hypermethylated DNA at the GR gene promoter. The exact composition of the repressor complexes involved in reduced GR expression, and the identity of a demethylase (if any) responsible for active GR demethylation during development continue to be investigated [16].

### Covalent Modifications of Histones and Affective Disorders

Covalent modifications of histones reflect the repertoire of PTMs also encountered in nonhistone proteins (e.g. acetylation, methylation, ubiquitination, sumoylation, and ADP ribosylation of lysine residues; methylation of arginine residues, and phosphorylation of serines and threonines). Compared to epigenetic modifications targeting DNA, histone marks are more labile. There is currently little evidence that these PTMs are heritable in dividing mammalian cells. The currently accepted view is that histone modifications act dynamically, in a combinatorial or sequential fashion defining the so-called 'histone code', to alter nucleosome conformation and modulate gene transcription. The vast majority of known histone covalent modifications occur at the amino termini portions of core histones H3 and H4, which encode their protruding tail domains. From a functional perspective, acetylation and methylation of lysine residues are currently the best understood modifications. Both of these epigenetic marks have been the focus of a small number of studies in postmortem tissues and animal models of depression, suggesting that they may provide future targets [3, 6, 21]. As the function of other types of histone modifications such as ubiquitylation and sumoylation becomes more clear, it is likely that some of these larger chemical moieties, which compete with acetyl and methyl groups for the same lysine residues, may also be found to influence brain function and behavior. As mentioned in the introduction of this chapter, histone PTMs involve several types of histone-modifying enzymes, which are devoid, in most cases, of intrinsic DNA binding activity and

therefore recruited via interactions with initiator complexes (fig. 1) that also include transcriptional activators or repressors. Several excellent reviews published recently provide exhaustive descriptions of these enzyme families, their sites of activity and their functions [6, 11, 21].

*Histone Acetylation*

At present, knowledge about the functional role of histone acetylation is greater than of any other histone modification. Histone acetylation, at lysine residues on H3 (lysine positions 9, 14, 18, and 23) and H4 (lysine positions 5, 8, 12, and 16), strongly correlates with gene transcription and appears to be required for gene activity. Acetylation and deacetylation of nucleosomes at gene promoters are catalyzed by 2 antagonistic enzyme families, namely histone acetyltransferases (HATs) and HDACs. HATs transfer acetyl moieties from acetyl-coenzyme A to the ε-amino group of lysine side chains on histone tails. This addition neutralizes positive charges on lysine residues and reduces internucleosomal contacts, thus promoting a relaxed chromatin conformation that allows physical access of transcriptional machinery to promoter regions [11]. Acetylation marks on nucleosomes also provide signals to recruit certain classes of 'acetylation readers' involved in gene activation, such as the bromodomains containing proteins of the SWI/SNF family. In contrast, HDACs remove the acetyl groups from histone tails, using zinc as a cofactor. By doing so, HDACs promote a closed, repressive state of chromatin as well as the further recruitment of transcriptional corepressors [6, 11].

There are a total of 18 HDAC isoforms in the mammalian genome. These enzymes are usually divided into 4 classes (I, II, III, and IV), based on sequence homology to their yeast homologues. Class I, II, and IV HDACs are the zinc-dependent hydrolases, while class III HDACs (also called sirtuins; SIRT1–7) are NAD+-dependent enzymes that are not going to be discussed here. Class I HDACs (that include HDAC1, 2, 3, and 8) exert deacetylase activity on histones as well as nonhistone substrates and localize exclusively to the cell nucleus. Class II HDACs can be divided into class IIa members, which include HDAC4, 5, 7, and 9, and class IIb members, which include HDAC6 and 10. HDAC4 and HDAC5 have been shown to undergo nucleocytoplasmic shuttling in response to neural activity, while class IIb family members, HDAC6 and 10, are localized mainly to the cytoplasm and are involved essentially in chromatin-independent mechanisms. HDAC6 is unique in the family, due to its possession of 2 deacetylase domains, and because of its role as an α-tubulin deacetylase [3].

Analysis of the expression levels and distribution of HDAC1–11 in rodents using the *Allen Brain Atlas* (http://www.brain-map.org) or based on published mapping studies reveals that most HDAC isoforms are expressed in the brain with some variations in levels and cell type specificity [3, 22]. Recent studies have shown that HDAC1 is expressed predominantly in glia and neural progenitor cells. In contrast, HDAC2

appears more highly expressed in mature neurons and, to a lesser extent, in differentiated glial cells. This pattern is coherent with the observation that HDAC1 exerts a repressive influence on the number and function of excitatory synapses in immature hippocampal cultures, which is lost once neurons have matured. In contrast, HDAC2 seems to promote excitatory neurotransmission in mature neurons [23]. Together, these findings suggest important roles for class I HDACs in the development and maintenance of the nervous system. Of note, we have reported that the cytoplasmic class IIb HDAC6 is also expressed in the brain and is found highly concentrated in the midbrain raphe nuclei, where it is expressed almost exclusively by serotonergic neurons [24].

Drug discovery efforts have led to the identification of a significant number of compounds that modulate HATs and HDACs. The pharmacology of HDACs is currently much better understood than that of HATs, and HDAC chemistry has generated multiple families of inhibitors [e.g. *hydroxamic acids* including suberoylanilide hydroxamic acid (SAHA) and trichostatin A, *carboxylic acids* such as sodium butyrate and sodium valproate, and *benzamides* including MS-275 (N-(2-aminophenyl)-4-[N-(pyridine-3-yl-methoxycarbonyl)aminomethyl]-benzamide)], which are all competitive inhibitors that chelate zinc. Several leads in these categories have proven very successful in experimental models of cancer, whereupon their administration represses oncogene expression and promotes cell differentiation. In fact, SAHA (vorinostat) was approved by the FDA 4 years ago for the treatment of cutaneous T-cell lymphoma, and several other HDAC inhibitors have passed safety and tolerability trials and are currently tested in phase II or III for different types of cancer [3, 22].

When given systemically or locally into the brain, or applied in neuronal tissue culture, most HDAC inhibitors have been shown to significantly raise global levels of histone acetylation, and to alter constitutive patterns of gene expression [3, 6, 25]. In contrast to their antiproliferative activity in cancer cell lines, HDAC inhibitors reportedly exert antiapoptotic and neuroprotective effects in neuronal tissue culture models, although it remains unclear whether these effects are directly and solely related to chromatin-dependent activity of HDAC inhibitors [22]. A significant number of studies have examined HDAC inhibitor activity in animal models in vivo. However, most of these studies have focused primarily on models of memory and neurodegenerative diseases, and only a handful of studies have examined the activity of HDAC inhibitors in rodent models of depression and antidepressant response. Published data suggest that increasing histone acetylation may promote antidepressant-like effects in rodents [25, 26], although, as detailed below, such effects may be contingent upon the brain area affected by HDAC inhibition as well as environmental conditions and drug administration variables.

At least 2 studies have examined the effects of systemically administered NaB and reported a modest antidepressant-like effect of this compound. However, a later study did not confirm antidepressant-like effects of a systemic HDAC inhibitor administration protocol despite an increase in bulk H3 acetylation in the brain.

The first generation of carboxylic acids, such as NaB, is notorious for having poor brain bioavailability and pharmacokinetic profiles, which may explain variations in some behavioral results, particularly in the case of systemic administration studies. Furthermore, some of the drugs used in the studies mentioned above are considered 'pan-inhibitors' based on their broad affinity for several subclasses of HDACs that could possibly exert opposite effects on behavior. Current efforts in drug discovery have the goal to optimize brain permeability and increase subclass specificity [27, 28]. As an example of a need for more HDAC selective compounds, there are indications for the participation of HDAC5 as a potential mechanism by which an antidepressant effect is obtained in the hippocampus via increasing histone acetylation at certain BDNF gene promoters, as shown after chronic imipramine [25], as well as fluoxetine treatment [29]. Interestingly, the direct infusion of class I (MS-275) or class II (SAHA) selective HDAC inhibitors into the nucleus accumbens has potent antidepressant-like actions in rodents and reverses stress-induced patterns of gene expression in a similar manner to patterns of gene expression driven by the conventional antidepressant fluoxetine [30]. Further studies incorporating broad platform gene arrays may help provide further insight into particular gene targets, by identifying those that are regulated after stimuli such as stress, and oppositely regulated by administration of an HDAC inhibitor [30]. Further genetic analysis using conditional ablation should help decipher which specific HDAC isoforms and which brain areas and cell types are mediating antidepressant-like effects of nonspecific HDAC inhibitors. As a side note, cognitive endophenotypes present in several affective disorders may also be tied to patterns of histone acetylation in the brain [31, 32]. Notable increases in histone acetylation are observed in the hippocampus and cortex after learning new information, and the acquisition of learned responses can be enhanced via HDAC inhibition [33, 34].

In contrast to other psychiatric diseases, such as schizophrenia or bipolar disorder, there is a dearth of information available on histone acetylation from postmortem tissues in the context of depression. In leukocytes of major depressive disorder, expression of HDAC2 and HDAC5 mRNA was increased in patients that were in a depressive state, but not in patients experiencing remissive state, compared to controls. HDAC6 and HDAC8 were decreased in both depressive and remissive states compared to controls. Recent results from our group indicate that changes observed in blood cells may not be representative of regulations occurring in the brain. Indeed, our observations indicate that HDAC2 is downregulated in the nucleus accumbens of depressed patients, both at the mRNA and protein level. Furthermore, this change is associated with an increase in bulk levels of H3K14 acetylation. Interestingly, both changes in HDAC2 levels and histone acetylation can be mimicked in animals by exposing mice to repeated social defeat stress. Changes in H3K14 acetylation are minimal at the end of the stress period but reach a peak approximately 2 weeks after the end of the social stress exposure, an observation suggesting that this change may reflect adaptative responses. The use of this animal model will be helpful for determining whether

changes in acetylation at the level of bulk chromatin result in functional alterations at specific gene promoters. Along this line, a promising approach will be to incorporate chromatin immunoprecipitation studies that utilize postmortem human brain tissue. Although there certainly are technical difficulties to overcome before such studies can be routinely conducted [2], recent results reported for other neurological and psychiatric disorders are encouraging and indicate that these types of measurements are technically feasible [35]. Thus, histone acetylation data at specific gene promoters in clinical depression should start becoming available in the near future.

### *Histone Methylation*

Unlike histone acetylation, histone methylation is a multivalent process whereby lysines may become mono-, di-, or trimethylated. Methylation states can have divergent activational or repressive transcriptional effects depending on the specific position of the lysine residue on histone tails and on the valence of methylation [36, 37]. For example, trimethylation at histone H3K4 is correlated with high rates of transcription, whereas mono- and dimethylation of H3K4 are most often associated with gene inactivation. Recent experimental studies conducted from postmortem human brain tissue have begun to unravel the role of histone methylation in the brain, and highlight methylation marks possibly involved in the promulgation of depressive-like behavior and other psychiatric diseases. As it is the case with DNA methylation, global patterns of histone methylation in the brain vary across life span with most changes occurring during the first postnatal year in humans.

Histone methylation is controlled by the complex interplay between histone methyltransferases and demethylases. A substantially larger number of enzymes is involved in regulating histone methylation versus acetylation and only a handful of these will be discussed here as they may pertain more specifically to neuronal function and possibly to gene regulation processes during affective behaviors. Two states of histone methylation are tightly correlated with gene promoters undergoing active transcription: first, H3K4me3, which is coordinated by the methyltransferase Set1, and second, H3K36me3, which is initiated by the methyltransferase Set2 and its association with the elongating form of RNA polymerase II. In contrast, mono- and dimethylation at H3K36 in higher eukaryotes are known for having an opposing influence on gene transcription, and are mediated by Set2 homologues.

Su(var)3–9 was the first histone methyltransferase to be described [38]. This enzyme is responsible for the trimethylated (transcriptionally repressive) state of H3K9, a mark for constitutive heterochromatin. Heterochromatin protein 1 further associates with this modification to allow for Su(var)4–20 to facilitate H3K20me3 and stabilize heterochomatic domains [39]. H3K9me2 is methylated by G9a. As discussed below, recent evidence shows that decreased G9a expression with a corresponding decrease in H3K9me2 may be an important component of transcriptional

'sensitization' that occurs in response to repeated monoaminergic stimulation such as produced by drugs of abuse, a process that instigates aberrant forms of plasticity and behavior [40]. Another well-characterized repressive mark that is not associated with heterochromatic formation, H3K27me3, is prompted by the polycomb repressive complex 2 by the enhancer of zeste homolog 2 subunit. An example of the importance of H3K27me3 repression is the attenuation of HOX gene expression in embryonic stem cells, and possibly during adaptative responses in postmitotic cells [41].

Histone methylation is a reversible and dynamic process and another important enzyme regulating demethylation of histone tails is lysine-specific demethylase 1 (LSD1 or KDM1A). LSD1 associates with other transcriptional repressors, such as HDAC1/2, and REST corepressor, which together are important for repressing neuronal genes in nonneuronal cells [42]. Of note, LSD1 belongs to the global family of monoamine oxidases and as such is inhibited by several antidepressants from the monoamine oxidase inhibitor class, the most potent of which to inhibit LSD1 is tranylcypromine. Also important to note here is that repressive and active forms of trimethylated states are not mutually exclusive processes. For example, both H3K27me3 and H3K4me3 are concomitantly present at gene loci, at least during early stages of development.

There are at least 3 sites of histone methylation on lysine residues that have been found regulated in animal models of depression and chronic stress, as well as in postmortem tissues. Initially it was reported that dimethylation of histone H3K27 was robustly elevated at the promoter regions of 2 *bdnf* variants in response to chronic social stress [25]. This repressive mark at *bdnf* promoter regions may explain how significant reductions in *bdnf* mRNA expression in the hippocampus occur after chronic stress. Interestingly, administration of an HDAC inhibitor reversed the impact of stress on hypermethylation of BDNF promoters, *bdnf* mRNA expression, and depressive-like behavior [25], while viral overexpression of the HDAC5 gene, locally in the hippocampus, impaired the ability of imipramine treatment to alleviate depressive-like behaviors. In the orbitofrontal cortex, TrkB receptor expression is decreased in major depression [43], and increased dimethylation of histone H3K27 at the promoters of TrkB receptors has been observed in suicide victims [44, 49]. These results indicate that repressive forms of histone methylation, like acetylation, are dynamic processes that can be potentially modulated in the brain using pharmacological approaches. Indeed, studies utilizing chronic stress as a model of depression found that broad-scale stress-induced patterns of gene expression, which are controlled by this repressive histone modification, are essentially reversed by prolonged treatment with the conventional antidepressant imipramine [45]. Second, the repressive histone methylation mark dimethyl H3K9 is substantially decreased in the nucleus accumbens after repeated cocaine administrations [40]. This deficit in a transcriptional repressive mark may explain the aberrant patterns of gene activation that occur after chronic cocaine exposure. The decrease in H3K9 dimethylation is linked to significant reductions in the histone H3K9 methyltransferase G9a, which can be pharmacologically

inhibited by BIX01294 [40]. Thus, it is possible to begin experimental assessments on the function of this enzyme in depression models [46]. High rates of comorbidity between depression and addiction argue positively for such studies. Finally, human postmortem brain samples collected from the prefrontal cortex of schizophrenic patients have revealed deficits in the activational mark H3K4 trimethylation in this clinical population [47]. Decreases in trimethyl H3K4 are correlated with deficits in neuronal proteins that are important for maintaining both excitatory and inhibitory connectivity. Thus, research programs that follow a similar course of investigation in depression models will likely provide much needed insight regarding the stability of cognitive disruptions that occur with depression.

## Conclusion

Efforts to understand the role of epigenetic mechanisms in affective disorders are in their infancy, yet, we anticipate that advances in this area will be steady as they are fueled by the considerable excitement surrounding the initial studies as well as by ambitious funding initiatives both in the US and Europe. Notable successes in establishing experimental methods to assess chromatin modifications at specific promoters in postmortem brain will likely have a determining influence on the field. It is expected that important breakthroughs will be derived from combining such current approaches with emerging high-throughput sequencing methods. A comprehensive view of the role of chromatin-related mechanisms in affective regulation and disease will necessitate development of equally innovative approaches to assess chromatin changes in a circuit or cell-type-selective manner. A frontier that currently remains unexplored in this context is the role played by numerous ATP-dependent nucleosomal remodeling complexes that were not directly discussed in this chapter. On the clinical side, psychological evaluations conducted in cohorts of cancer patients currently treated with the first generation of FDA-approved epigenetic drugs (as treatments) should soon provide insights about their impact on mood and cognition.

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# Neurotrophic Factors and Antidepressant Action: Recent Advances

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**Abstract**

It is now commonly accepted that neuronal plasticity plays a central role in depression and antidepressant drug action. Accumulating evidence from studies in both humans and animals suggests that depression is associated with alterations in the cellular architecture of specific brain regions that are important for the regulation of mood. Moreover, many of these changes are attenuated or reversed by chronic antidepressant treatment. Since neurotrophic factors regulate many features of neuronal plasticity including the proliferation and structure of neurons, there is a rapidly growing interest in determining whether growth factor signalling might contribute to the pathophysiology and treatment of depression. Several families of neurotrophic factors are found in the adult brain including the neurotrophins, fibroblast growth factors, insulin-like growth factors, transforming growth factors, neuropoietic cytokines as well as various other growth factors such as vascular endothelial growth factor. Of all the neurotrophic factors, brain-derived neurotrophic factor (BDNF) has been the most intensively investigated in the depression and antidepressant research field, and there is convincing supporting evidence of a role for BDNF in the pathophysiology and treatment of depression. Additional evidence suggests that other neurotrophic factors, such as fibroblast growth factor, vascular endothelial growth factor and insulin-like growth factor 1, might also contribute to the mechanism of antidepressant drug action, although more exhaustive investigations are required. The present chapter reviews both human and animal studies investigating the roles of neurotrophic factors in the pathophysiology and treatment of depression. 

Over the last decade, it has become clear that neuronal plasticity plays a central role in depression and antidepressant drug action. Antidepressant treatments increase the expression of several neurotrophic factors that are important regulators of neuronal growth and survival as well as various facets of neuronal plasticity including neurogenesis and synapse formation [1–5]. Post-mortem and neuroimaging studies suggest that depression is associated with reduced volumes of specific brain regions that are important for the regulation of mood, including the hippocampus and prefrontal cortex [6]. Parallel studies in animals demonstrate that chronic stress, which

**Table 1.** Neurotrophic factors which are thought to play a role in the pathophysiology and treatment of depression

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|---|
| Neurotrophins |
| Nerve growth factor (NGF) |
| Brain-derived neurotrophic factor (BDNF) |
| Neurotrophin 3 (NT-3) |
| Neurotrophin 4/5 (NT-4/5) |
| Fibroblast growth factors (FGF) |
| Insulin-like growth factors (IGF) |
| Vascular endothelial growth factor (VEGF) |
| Transforming growth factors |
| Transforming growth factor β′ |
| Glial cell-line-derived neurotrophic factor (GDNF) |
| Neurturin |
| Persephin |
| Artemin |
| Neuropoietic cytokines |
| Leukaemia inhibitory factor (LIF) |
| Ciliary neurotrophic factor (CNTF) |

is a precipitating factor for depression, can induce atrophy in both of these brain regions and can also result in functional behavioural changes that are reminiscent of clinical depression [7]. Moreover, antidepressant treatments can attenuate the hippocampal volume loss observed in depression as well as the stress-induced changes in both animal behaviour and the cellular architecture of the hippocampus [7, 8]. Taken together, there is unmistakable evidence that alterations in neuronal structure and function (i.e. neuronal plasticity) are fundamental components of the mechanism of antidepressant drug action.

Since neurotrophic factors regulate many features of neuronal plasticity, there has been a rapidly growing interest in determining whether growth factor signalling might contribute to the pathophysiology and treatment of depression. Neurotrophic factors were initially identified as proteins that are required for neuronal survival and differentiation during development [9, 10]. However, many of these factors are also actively expressed during adulthood and can be found in tissues other than the brain [10]. Neurotrophic factors can be classified into several different families including the neurotrophins, fibroblast growth factors (FGFs), insulin-like growth factors (IGF), transforming growth factors, neuropoietic cytokines as well as various other growth factors including vascular endothelial growth factor (VEGF) (table 1). Of all the neurotrophic factors, brain-derived neurotrophic factor (BDNF) has been the

most intensively in the depression and antidepressant research field. Nevertheless, in the present chapter, we also outline how the other neurotrophic factors might contribute to the mechanism of antidepressant drug action.

## Neurotrophins: Brain-Derived Neurotrophic Factor, Nerve Growth Factor, Neurotrophin 3 and Neurotrophin 4/5

### *Brain-Derived Neurotrophic Factor*

BDNF was initially described as a secreted protein that supports the survival of a subset of peripheral neurons during development [10]. However, it is now recognised that BDNF is an important mediator of several activity-dependent plasticity processes both in the adult and developing brain [3]. The function of BDNF is mediated through 2 different receptors, TrkB and $p75^{NTR}$. Binding of mature BDNF to TrkB is thought to mediate most of the plasticity-enhancing effects of BDNF, while binding of its precursor protein, Pro-BDNF, to $p75^{NTR}$ has been linked to apoptosis and synaptic depression [11]. In this way, BDNF-mediated signalling pathways can regulate dynamic neuronal networks by creating and maintaining active neuronal connections which are appropriate for information processing, while eliminating the inactive connections which mediate random noise. For this reason, the role of BDNF in depression and antidepressant action has been intensively investigated.

*Clinical Evidence for a Role of BDNF in Depression and Antidepressant Drug Action*
Several studies including 2 meta-analyses have demonstrated that serum BDNF levels are reduced in depressed patients [12–16]. Moreover, serum BDNF levels are normalised in patients that successfully respond to antidepressant treatments including chemical antidepressants, electroconvulsive therapy, sleep deprivation therapy and repetitive transcranial magnetic stimulation [14, 15, 17–21]. Even though several negative findings have also been reported, meta-analysis studies strongly suggest that serum BDNF levels are reduced in depression and are increased by antidepressant treatments [15, 16]. Although serum levels of BDNF are clearly altered by antidepressant treatments, it is currently unclear whether such changes reflect BDNF levels in the brain. However, post-mortem studies have reported that BDNF protein levels are increased in specific areas of the hippocampus of medicated depressed patients [22].

The role of BDNF in the pathophysiology and treatment of depression has also been investigated using genetic association studies. A number of studies have investigated whether the common Val66Met polymorphism as well as other single nucleotide polymorphisms (SNPs) in the *bdnf* gene are associated with risk for depression or the efficacy of antidepressants. Generally, these studies report a lack of association of *bdnf* SNPs with both the clinical response to antidepressants and the risk for depression [23–28]. Given the limited number of studies investigating

the contribution of *bdnf* gene variants to the antidepressant response, it is clear that significantly more clinical research is warranted before any firm conclusions can be drawn.

*Chronic Stress in Animals Decreases BDNF Expression in the Hippocampus in an Antidepressant-Reversible Manner*

Exposing animals to chronic stress induces both cellular changes in the hippocampus and alterations in behaviour that are reminiscent of depression. It has been consistently reported that exposure to different types of chronic stress decreases mRNA levels of BDNF in the hippocampus of both the rat and the mouse [29–34]. These stress-induced reductions in BDNF mRNA levels have been observed in most subregions of the hippocampus including the dentate gyrus, CA1 and CA3 areas [35, 36]. Moreover, this effect can be reversed or prevented by various classes of antidepressant drugs including monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, norepinephrine reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, as well as other antidepressant treatments including buproprion, electroconvulsive shock (ECS), subconvulsive electrical stimulation and voluntary exercise [29, 30, 33, 35–38]. Similarly, both pharmacological and genetic animal models of physiological responses to stress, such as chronic corticosterone treatment or antisense-induced downregulation of the glucocorticoid receptor, reduce mRNA levels of BDNF in the hippocampus in an antidepressant-reversible manner [39, 40]. Several studies have also reported stress-induced downregulation of BDNF protein in the hippocampus, an effect that can also be reversed by chronic treatment with the antidepressant venlafaxine [29, 37, 41, 42]. Finally, there is emerging evidence that stress-induced behavioural changes related to depression may be dependent upon BDNF signalling in the hippocampus. Acute administration of BDNF directly into the mouse hippocampus reverses corticosterone-induced increases in depression-like behaviour in the forced swimming test (FST) [43]. Taken together, there is unequivocal evidence that chronic stress decreases BDNF expression in the hippocampus in an antidepressant-reversible manner and that alterations in BDNF signalling might also mediate stress-induced changes in behaviour.

*Antidepressant Treatments Alter BDNF Expression in the Brain*

Many animal studies have reported that chronic administration of different types of antidepressants including chemical antidepressants, ECS, repetitive transcranial magnetic stimulation, vagus nerve stimulation as well as atypical antidepressants increase both mRNA and protein levels of BDNF in the hippocampus [33, 40, 44–57]. However, a significant number of other studies have also reported that BDNF expression in the hippocampus is not affected by chronic antidepressant treatments [45, 46, 51, 53, 58–62]. Similarly, there are also conflicting reports on whether chronic antidepressant treatment increases BDNF mRNA and protein levels in the frontal cortex [40, 45, 46, 51, 58–60, 63].

Although there is a general consensus that antidepressant treatments increase the expression of BDNF mRNA in the hippocampus and the frontal cortex, conflicting changes have also been reported and there is a generally poor correlation between antidepressant-induced changes in mRNA levels and protein levels of BDNF. Discrepancies between studies may be due to variations in the dose, type and time period of antidepressant treatment, the methodology used for BDNF analysis as well as a number of other factors. First of all, antidepressant treatments might induce a biphasic regulation of BDNF gene expression. For example, while 4 days of fluoxetine treatment decreased BDNF mRNA levels in the hippocampus, 14 days of treatment increased expression in the same brain area [64]. Similarly, it was reported that chronic fluoxetine treatment increased BDNF mRNA expression in the dentate gyrus of the rat hippocampus when it was measured 24 h following the last injection, but it decreased BDNF mRNA levels when measured 4 h following the last injection [62]. A second factor which may contribute to conflicting results is that the techniques used to measure BDNF protein levels may vary in sensitivity. For example, although it was reported that 21 days of fluoxetine treatment increased the number of BDNF-positive cells in the CA1 and CA3 regions of the hippocampus, the same study failed to detect this change when BDNF levels in whole hippocampus extracts were measured using ELISA [64]. Another variable is that the effects of antidepressant treatments on BDNF protein levels in the mouse brain may be strain dependent. For instance, chronic treatment with fluoxetine increased BDNF protein levels in the hippocampus of MRL/MpJ mice but decreased BDNF protein levels in the hippocampus of C57BL/6 mice. Similarly, while chronic desipramine treatment increased protein levels of BDNF in the frontal cortex of MRL/MpJ mice, it was without effect in C57BL/6 mice [65]. Finally, another possibility is that antidepressant treatments do not increase the synthesis of BDNF per se, but may induce regional or subcellular changes in BDNF protein levels through active translocation of the protein. For example, while chronic treatment with duloxetine decreased protein levels of mature BDNF in the cytosol fraction of the rat frontal cortex, BDNF protein levels were increased in the synaptosomal fraction, thus suggesting that duloxetine may have induced the translocation of BDNF from the cell body to the synapses [60].

As aforementioned, there is generally a poor correlation between antidepressant-induced changes in mRNA and protein levels of BDNF. In an effort to address this issue, Musazzi et al. [66] designed an experiment examining the temporal profile of antidepressant-induced changes in both mRNA and protein levels of BDNF in the rat hippocampus and frontal cortex. While 1–2 weeks of antidepressant treatment (fluoxetine or desipramine) was sufficient to increase protein levels of mature BDNF in these brain regions, 3 weeks of treatment was required to increase mRNA levels [66]. Taken together, these findings suggest that BDNF protein levels might be increased by antidepressant treatment initially through post-transcriptional mechanisms or through transport from other brain areas, followed by a much slower induction of BDNF mRNA synthesis.

The gene encoding BDNF has a particularly complex structure and this genomic complexity might also underlie some of the discrepancies reported in antidepressant-induced changes in BDNF mRNA levels. Initially, it was reported that the *bdnf* gene of the rat had 4 promoters each of which drives 1 of 4 short 5′ untranslated exons that are alternatively spliced to a common 3′ translated exon that encodes the mature BDNF protein [67]. The structure of both the rat and mouse *bdnf* gene has since been revised and it is now thought that both the mouse and rat *bdnf* genes consist of at least eight 5′ untranslated exons which are alternatively spliced to the protein-coding 3′ exon [68]. Thus, transcription of the rat or mouse *bdnf* gene results in mRNA transcripts encoding 1 of the 8 exons spliced to the coding 3′ exon, as well as a transcript containing the 5′ extended protein-coding exon [68]. Similarly, the human *bdnf* gene also has a complex genomic structure with 11 exons and 9 functional promoters [69]. Importantly, the expression of individual BDNF mRNA transcripts in the brain is region and cell type dependent and is also differentially regulated within different subcellular compartments of the neuron [68–70]. Furthermore, these promoters are differentially regulated by stress and antidepressant treatments. Different antidepressants enhance BDNF expression in the hippocampus and frontal cortex using different combinations of these promoters [40, 71–73]. Similarly, chronic stress or chronic treatment with the stress hormone, corticosterone, decreases BDNF mRNA in the hippocampus and frontal cortex through specific decreases in BDNF transcript IV plus transcript II or III [40, 73]. Moreover, these promoters are subject to epigenetic regulation, a process that leads to long-lasting changes in gene transcription, and both stress and antidepressant treatment can induce epigenetic regulation of various BDNF promoters in different brain regions including the hippocampus [73, 74]. For example, chronic social defeat stress in mice produces long-lasting suppression of BDNF mRNA transcripts III and IV by increasing dimethylation of histone H3 on the chromatin of the respective promoters. However, chronic treatment with the antidepressant imipramine prevents stress-induced reductions of transcript III by inducing upregulation of its transcription through acetylation of histone H3 on the chromatin of its promoter [73]. Taken together, it is clear that future studies examining the effects of antidepressant treatments on BDNF mRNA expression should also consider the complex gene structure of the *bdnf* gene.

*Antidepressants Rapidly Activate TrkB*

One potential explanation for the discrepancy between the BDNF mRNA and protein levels is the fact that BDNF protein is quite stable in the brain. BDNF protein is not degraded upon release or receptor binding, but instead undergoes reuptake and recycling. Therefore, total brain BDNF protein levels may not reflect the dynamic processes of BDNF release and receptor activation, which are processes that are more functionally relevant. Since it is difficult to measure extracellular levels of BDNF in the brain, several groups have used the phosphorylation status of TrkB receptors as an indicator of BDNF release in vivo. Interestingly, it has been observed that acute

treatment with different classes of antidepressant drugs induces TrkB autophosphorylation and TrkB-mediated activation of the phospholipase Cγ signalling pathway as well as CREB phosphorylation in both the hippocampus and anterior cingulate cortex of the mouse 30–60 min following drug administration [75, 76]. These observations raise the possibility that antidepressants rapidly (within 1 h) induce BDNF release in the hippocampus and cortex and activate TrkB-dependent signalling without producing any net change in BDNF protein levels. Over time, BDNF recycling needs to be complemented with increased synthesis, which leads to elevated BDNF mRNA levels. Therefore, dramatic changes in BDNF release and signalling may take place in the absence of any net changes in BDNF protein levels and enhanced mRNA levels might only represent a homeostatic response to BDNF release. Such effects may explain the apparent discrepancy between antidepressant-induced changes in BDNF mRNA and protein levels. Moreover, it is important to note that according to this scenario, the effects of antidepressants on BDNF would occur soon after initial drug administration and not weeks later when BDNF mRNA levels are increased. This hypothesis is consistent with the observation that the acute behavioural effects of antidepressants in the FST are lost when BDNF or TrkB levels are reduced [76], thus further supporting a role for rapid BDNF release and TrkB activation in the mechanism of antidepressant drug action.

*BDNF Can Induce Antidepressant-Like Behavioural Effects*

Accumulating evidence from animal studies suggests that BDNF itself may have antidepressant-like properties. A single infusion of BDNF directly into the midbrain, lateral ventricles, dorsal hippocampus, or the dentate gyrus or CA3 regions of the hippocampus induces antidepressant-like behaviours in both the rat FST and the learned helplessness paradigm [77–80]. These antidepressant-like behavioural effects of a single infusion of BDNF are long-lasting and can persist for at least 6 days [78, 79]. In addition, mice overexpressing BDNF or TrkB in the forebrain exhibit an antidepressant-like behavioural phenotype in the FST [81, 82]. In contrast, however, mice with reduced forebrain levels of BDNF ($BDNF^{+/-}$ mice) or reduced activation of the full-length TrkB receptor do not display a depression-like behavioural phenotype [76, 83]. However, it has been reported that female (but not male) conditional BDNF knockout mice demonstrate depression-like behaviour in the FST [84]. Moreover, lentiviral-mediated knockdown of BDNF in the dentate gyrus (but not the CA3 region) of the rat hippocampus induces anhedonic-like behaviour as well as depression-like behaviour in the FST [85]. Taken together, it appears that increasing BDNF levels in the hippocampus is sufficient to induce antidepressant-like behavioural effects, while reductions in BDNF in the dentate gyrus of the hippocampus may induce depression-like behaviours.

Recent studies characterising the antidepressant effects of BDNF suggest that the antidepressant-like behavioural effects of BDNF may be region specific. While intrahippocampal infusion of BDNF produces antidepressant-like behavioural effects,

infusion of BDNF into the mesolimbic dopamine pathway (the brain's reward circuit), exerts a prodepressive-like behavioural phenotype. Infusion of BDNF into the ventral tegmental area (VTA) induces a depression-like behavioural phenotype in the rat FST, while viral-mediated knockdown of BDNF in the VTA produces an antidepressant-like effect in a mouse social defeat stress model [74, 86]. Moreover, viral-mediated overexpression of the truncated TrkB receptor, TrkB.T1 (a dominant-negative receptor of TrkB), into the nucleus accumbens has an antidepressant-like effect in the rat FST [86]. Taken together, these data suggest that in contrast to its action in the hippocampus, BDNF activity in the VTA → nucleus accumbens pathway may contribute to the development of a depression-like phenotype.

*Antidepressant-Induced Changes in Behaviour and Neuronal Plasticity Are BDNF Dependent*

Not only is BDNF sufficient to induce antidepressant-like behaviours, but it may also be a critical mediator of the behavioural effects of both acute and chronic antidepressant drug treatment. $BDNF^{+/-}$ mice and TrkB.T1-overexpressing mice do not respond to acute treatment with the antidepressants fluoxetine or imipramine in the FST [76]. In addition, conditional knockout of forebrain BDNF prevents the behavioural effects of subchronic desipramine treatment in the FST [84, 87]. Moreover, BDNF in the dentate gyrus but not the CA1 region of the hippocampus is required for the behavioural effects of subchronic antidepressant treatments including desipramine and citalopram [88]. Recently, knock-in mice were generated that express a common SNP found in the human *bdnf* gene. This SNP results in a valine to methionine substitution at position 66 and while these $BDNF^{met/met}$ mice have normal levels of BDNF protein in the brain, they exhibit inefficient activity-dependent release of BDNF. $BDNF^{met/met}$ mice fail to respond to chronic treatment with the antidepressant fluoxetine, thus suggesting that activity-dependent release of BDNF is an important mediator of antidepressant action [89]. Taken together with the data from Adachi et al. [88], it is likely that activity-dependent release of BDNF, specifically in the dentate gyrus of the hippocampus, can mediate the behavioural effects of antidepressants.

BDNF signalling not only mediates the behavioural effects of antidepressant drugs but is also thought to play a significant role in antidepressant-induced enhancement of various forms of neuronal plasticity in the adult brain including hippocampal neurogenesis, synapse formation and network connectivity. Moreover, some of these processes, such as hippocampal neurogenesis, are necessary for at least some of the behavioural effects of antidepressant drugs [90]. Antidepressant-induced increases in the survival of newly born neurons in the adult hippocampus are prevented in both $BDNF^{+/-}$ mice and TrkB.T1-overexpressing mice [91]. Moreover, mice lacking the TrkB receptor on neural progenitor cells in the dentate gyrus do not demonstrate antidepressant-induced increases in hippocampal neurogenesis and are insensitive to the behavioural effects of chronic antidepressant treatment [92]. In contrast, mice lacking the TrkB receptor only in differentiated neurons of the dentate gyrus display

normal behavioural and neurogenic responses to chronic antidepressant treatment [92]. Taken together, these data suggest that BDNF signalling in neural progenitor cells of the dentate gyrus is an important mediator of antidepressant-induced increases in hippocampal neurogenesis as well as antidepressant-induced changes in behaviour. Moreover, we recently demonstrated that BDNF signalling is a mediator of antidepressant-induced increases in proteins associated with synapse formation [93] and that BDNF might also contribute to antidepressant-induced reactivation of developmental-like neuronal plasticity and changes in neuronal network connectivity [94], processes which are thought to contribute to maladaptive information processing in depression [95].

Taken together, a vast body of evidence supports the hypothesis that BDNF is a critical mediator of antidepressant drug action and likely exerts its effects through alterations in neuronal plasticity.

### *Nerve Growth Factor*

Nerve growth factor (NGF) was the first neurotrophin to be described and is critical for the maintenance and survival of sympathetic neurons, subsets of sensory neurons as well as cholinergic neurons of the basal forebrain [96, 97]. Various animal studies also suggest that NGF might play a role in the pathophysiology and treatment of depression. Exposure to acute or chronic stress decreases NGF mRNA and protein levels in the hippocampus of the mouse, rat and tree shrew [31, 98, 99], and in some cases this effect is reversed by chronic antidepressant treatment [31]. Acute stress has also been reported to decrease NGF protein levels in the frontal cortex and amygdala of the rat [99]. Regional alterations in NGF expression have also been reported in various animal models of depression or antidepressant-like activity. Learned helplessness decreases NGF protein levels in the mouse frontal cortex [100] and NGF mRNA is decreased in the hippocampus of the olfactory bulbectomized rat model of depression [101]. Regional alterations in NGF protein levels have also been described in a genetic model of depression, the Flinders sensitive line of rats [102], and systemic administration of NGF reverses the depression-like behavioural phenotype of these rats when tested in the FST [103]. Taken together, it is clear that stress can reduce NGF levels in the hippocampus and frontal cortex and in at least some instances this effect is reversed by antidepressant treatment.

Despite relatively strong evidence for the modulation of NGF by stress, few studies have investigated whether antidepressant treatments alter NGF levels in the brain. Nevertheless, it has been reported that ECS increases NGF protein levels in the rat hippocampus, although this effect is strain dependent [104, 105]. ECS also increases NGF protein levels in other regions of the rat brain including the frontal cortex and the striatum [104, 105]. In contrast to ECS, however, the chemical antidepressant escitalopram did not alter NGF protein levels in the rat hippocampus or frontal

cortex [59]. Finally, the mood stabiliser lithium, which is used as adjunct therapy for refractory depression, increases NGF protein levels in both the hippocampus and the frontal cortex of the rat [104, 106]. Although ECS increases NGF levels in the hippocampus, NGF itself does not induce antidepressant-like behavioural effects when it is directly administered into the rat hippocampus [79].

A limited number of clinical studies have investigated whether NGF levels are associated with stress, depression or antidepressant action. Neither paroxetine nor amitriptyline treatment altered serum levels of NGF in depressed patients [107], but depression has been associated with elevated serum levels of NGF [108] and lower hippocampal mRNA levels [109]. These clinical findings are generally inconsistent with those from the animal studies described above but given the limited number of clinical investigations, it is currently difficult to speculate upon the role of NGF in the therapeutic effects of antidepressant drugs.

### *Neurotrophin 3*

Neurotrophin 3 (NT-3) is an important survival factor of proprioceptive and mechanoreceptive sensory neurons and exerts its biological effects through TrkB, TrkC and $p75^{NTR}$ receptors [110–113]. Importantly, NT-3 is expressed in the dentate gyrus of the hippocampus where it regulates the differentiation of newly born neurons [4]. Since NT-3 is a regulating factor of neurogenesis, and hippocampal neurogenesis is thought to play a role in the mechanism of antidepressant drug action [90], some studies have also investigated the contribution of this neurotrophin to the mechanism of action of antidepressant drugs.

Direct administration of NT-3 into the dentate gyrus of the rat hippocampus exerts antidepressant-like effects in 2 different models of antidepressant-like activity including the FST and the learned helplessness paradigm [79]. However, few studies have investigated whether antidepressant treatments alter the expression of this neurotrophin in the brain [114, 115]. Nevertheless, studies using $NT\text{-}3^{+/-}$ mice suggest that NT-3 does not mediate the acute behavioural effects of antidepressants [76].

The effects of stress on regional mRNA and protein levels of NT-3 have been investigated in both the mouse and rat brain but conflicting results have been described with either increases, decreases or no changes being reported in both the hippocampus and the frontal cortex [29, 116, 117]. In contrast to animal models, clinical studies have revealed a consistent association of reduced hippocampal levels of NT-3 in suicide victims [109, 118]. Studies of peripheral NT-3 levels have also revealed reduced NT-3 mRNA levels in the blood [119] but increased levels of NT-3 protein in the cerebrospinal fluid of depressed individuals [120]. Finally, various haplotypes of the TrkC receptor, of which NT-3 is a ligand, have been associated with childhood onset mood disorders [121]. Taken together, it appears that alterations in NT-3 might also make an important contribution to the pathophysiology and treatment of depression.

*Neurotrophin 4/5*

Neurotrophin 4/5 (NT-4/5) exerts its cell survival effects through activation of the TrkB receptor [122]. Few studies have investigated whether NT-4/5 itself is directly involved in depression and its treatment. However, just recently it was reported that serum levels of NT-4/5 protein are increased in patients with bipolar disorder irrespective of their mood state [123].

### Fibroblast Growth Factors

The fibroblast growth factor (FGF) family in humans is comprised of 22 ligands and 5 receptors [124]. Dysregulation of the FGF system has been reported in major depression, and animal studies suggest that these growth factors may also play an important role in the mechanism of action of antidepressant drugs [124]. Of all the FGF ligands and their receptors, FGF2 and the receptor FGFR1 have been the most intensively investigated in the depression and antidepressant research field because they are important regulators of neurogenesis as well as the differentiation and maturation of progenitor cells [1, 125].

Chronic treatment with different classes of chemical antidepressants or vagus nerve stimulation increases both mRNA and protein levels of FGF2 in the rat hippocampus as well as in cortical areas [126–129]. Moreover, intracerebroventricular administration of FGF2 exerts antidepressant-like effects in the rat FST [130]. Taken together, there is compelling preliminary evidence that FGF2 plays a role in the neurobiological and behavioural effects of antidepressant drugs.

A role for the FGF system in the pathophysiology and treatment of depression is further bolstered by findings from clinical studies. Dysregulation of the FGF system in both the hippocampus and the frontal cortex is associated with major depression [131, 132]. The pattern of change in the cortex is complex with some components of the system being upregulated while others are downregulated [124, 132]. Nevertheless, reduced density of FGF2-positive cells has been reported in post-mortem samples of the hippocampus from depressed patients, while its receptor, FGFR1, was upregulated [131]. Increased serum levels of FGF2 have also been reported in major depression [133] and several SNPs of FGF2 have been associated with the clinical efficacy of selective serotonin reuptake inhibitor antidepressant drugs [134].

### Insulin-Like Growth Factor 1

Insulin-like growth factor 1 (IGF-1) is a polypeptide secreted primarily by the liver under the control of growth hormone. Although peripheral IGF-1 can cross the

blood-brain barrier, it is also produced locally in the brain including the hippocampus, where it promotes neurogenesis [135–137].

Intracerebroventricular administration of IGF-1 into either the mouse or the rat brain exerts antidepressant-like behavioural effects [78, 138]. More recently, it was demonstrated that chronic peripheral administration of IGF-1 also exerts antidepressant-like behavioural effects in several behavioural models of antidepressant-like activity in mice [139]. Interestingly, the antidepressant-like effects of a single intracerebroventricular infusion of IGF-1 are long-lasting, persisting for 6 days following administration. Moreover, the antidepressant effects of a single intracerebroventricular infusion of IGF-1 only become apparent 3 days following administration, thus suggesting that IGF-1 exerts its effects through the long-term activation of downstream signalling pathways [140]. It has been suggested that these pathways may involve the monoamine neurotransmitter serotonin because depletion of endogenous serotonin prevents the antidepressant-like effects of IGF-1 [140]. Moreover, the same study demonstrated that a single intracerebroventricular infusion of IGF-1 also increased basal serotonin levels in the hippocampus 3 days but not 1 day following IGF-1 administration. Taken together, it is clear that IGF-1 can exert antidepressant-like changes in behaviour and some of these effects might be a result of enhanced serotonergic signalling.

Despite ample evidence that IGF-1 can exert antidepressant-like behavioural effects, few studies have examined whether IGF-1 expression in the brain is altered by stress or antidepressant treatment. Chronic treatment of rats with fluoxetine increased both mRNA and protein levels of IGF-1 in the frontal cortex but decreased expression in the hippocampus [141]. In contrast, chronic fluoxetine treatment had no effect on IGF-1 protein levels in the hippocampus of female mice [142]. Nevertheless, it has been demonstrated that peripheral IGF-1 is necessary for the antidepressant-like behavioural effects of exercise in the mouse FST [139]. Whether IGF-1 is a mediator of the behavioural effects of chemical antidepressants has yet to be investigated.

In contrast to animal studies, results from clinical studies investigating the utility of IGF-1 as a potential biomarker for depression and antidepressant efficacy are largely inconsistent. While several studies have reported that plasma levels of IGF-1 are increased in depressed patients [143–145], others have reported no change [146, 147]. Similarly, serum IGF-1 levels have been reported to be either decreased or remain unaltered following antidepressant treatment [146, 148]. Although animal studies suggest that IGF-1 may be involved in antidepressant action, it is clear that more clinical studies are required before any firm conclusions on the role of IGF-1 in antidepressant drug action can be made.

### Vascular Endothelial Growth Factor

VEGF was initially described as a modulator of vascular permeability, but it is also an endothelial cell mitogen and survival factor that stimulates the growth of new blood

vessels [5, 149, 150]. There are 6 members of the VEGF family and these proteins exert their biological effects through 2 receptor tyrosine kinases, fetal liver kinase 1 (Flk-1) and fms-like tyrosine kinase 1, as well as through a family of coreceptors, termed neuropilins [5]. There has been growing interest in the potential role of VEGF signalling in both the pathophysiology and treatment of depression because Flk-1 is highly expressed in neuronal progenitor cells as well as in mature neurons and endothelial cells of the rodent hippocampus [5]. Moreover, VEGF stimulates neurogenesis in the adult hippocampus [151–153] and antidepressant-induced increases in hippocampal neurogenesis are mediated by Flk-1 [151, 154].

VEGF induces antidepressant-like behavioural effects in animal models. Mice overexpressing VEGF in the forebrain exhibit reduced immobility in the FST [152] and intracerebroventricular administration of VEGF into the rat brain has antidepressant-like effects in several behavioural tests including the FST, learned helplessness and novelty-suppressed feeding test [154]. Moreover, there is some evidence to suggest that VEGF might exert its antidepressant effects through the modulation of the serotonergic system [154, 155]. For example, intracerebroventricular administration of VEGF increases swimming behaviour in the rat FST, a behaviour that is modulated by endogenous serotonin [154, 156], and it was recently reported that the 5-$HT_{1A}$ receptor mediates fluoxetine-induced increases in VEGF levels in the rat hippocampus [155].

Chronic treatment with different classes of chemical antidepressant drugs, including selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors and tricyclic antidepressants, increases both mRNA and protein levels of VEGF in the rat hippocampus [154, 155]. ECS also increases VEGF in the rat hippocampus [157, 158]. Moreover, the behavioural and neurogenic effects of chemical antidepressants and ECS are mediated by Flk-1 [151, 154, 155, 159]. Conversely, chronic stress decreases both mRNA and protein levels of VEGF and Flk-1 in the rat hippocampus [160, 161].

Clinical studies have also investigated whether VEGF is a biomarker of depression and antidepressant drug efficacy. Genetic association studies have generally reported a lack of association with depression or the antidepressant response although the number of published studies is currently too small to form a general consensus [162, 163]. It was recently reported that blood mRNA levels of Flk-1 are lower in depressed patients compared to controls and that these patients also demonstrate a reduced number of Flk-1-positive endothelial progenitor cells in peripheral blood [164]. Increased VEGF levels have been reported in the serum and peripheral leucocytes of depressed patients and clinical improvement has been associated with a normalisation of these levels [133, 163]. Finally, 1 study investigating the effects of chronic escitalopram treatment on serum levels of VEGF reported no change, but it is important to note that this was a small study and more investigations are warranted [165]. Although the direction of change in VEGF levels in animal studies of stress is somewhat opposing to that of clinical studies, the data still suggest that VEGF is implicated in some way in depression and the antidepressant response.

## Transforming Growth Factor Family

### *Transforming Growth Factor β*

Transforming growth factor β (TGF-β) is an anti-inflammatory cytokine thought to confer neuroprotection against a variety of insults that can lead to cell death [166]. Several clinical studies suggest that both the pathophysiology and treatment of depression may include alterations of TGF-β signalling but conflicting findings have been reported. While some studies report that plasma and serum concentrations of TGF-β are reduced in depression and are increased by antidepressant treatment [167–169], others have reported no change [133, 169, 170]. To date, no animal studies have reported whether antidepressant treatment alters TGF-β expression in the brain.

### *Glial Cell-Line-Derived Neurotrophic Factor Family Ligands*

Glial cell-line-derived neurotrophic factor (GDNF) family ligands (GFLs) include GDNF, neurturin, artemin and persephin. All GFLs signal through the RET receptor tyrosine kinase, which is activated only if the GFL is first bound to a class of proteins called GDNF family receptor α (GFR-α). Four different GFR-α receptors have been described, GFR-$\alpha_{1-4}$, and these receptors determine the ligand specificity of the GFR-α-RET complex. GDNF binds to GFR-$\alpha_1$, neurturin to GFR-$\alpha_2$, artemin to GFR-$\alpha_3$ and persephin to GFR-$\alpha_4$. The GFL-GFR-α complex then binds to and activates RET [171]. The following section summarizes the contribution of GFLs and their receptors to the pathophysiology and treatment of unipolar depression.

#### *Glial Cell-Line-Derived Neurotrophic Factor*

GDNF was initially discovered as a potent survival factor for dopaminergic neurons but has since been found to also be an important trophic factor for several other neuronal populations [171]. Considering that GDNF protects dopaminergic neurons and that the mesolimbic dopamine system is a key circuit in the brain's reward system which is thought to be dysregulated in depression [172], it is important to consider whether GDNF may play a role in both the pathophysiology and treatment of depression. For the most part, clinical studies have reported that serum and peripheral blood cell levels of GDNF are reduced in major depression [119, 170, 173] and that this effect is reversed by antidepressant treatment [173, 174].

Despite clinical evidence that low circulating levels of GDNF may be a biomarker of depression, few preclinical studies have examined whether stress or antidepressant treatments alter GDNF expression in the brain. Several cell culture studies have reported that different classes of chemical antidepressants increase GDNF mRNA levels as well as the cellular release of GDNF into the culture medium [175, 176] and an in vivo study demonstrated that chronic ECS increases mRNA levels of GFR-$\alpha_1$

in the dentate gyrus of the rat [177]. However, chronic treatment with the chemical antidepressants fluoxetine, desipramine, or tranylcypromine did not alter mRNA levels of GDNF or that of its receptors, GFR-$\alpha_1$ or RET, in the rat hippocampus [177]. The possibility that chemical antidepressants could alter GDNF signalling in other brain regions should be considered, because lithium, which is used as an augmentation strategy in the treatment of depression, increased GDNF protein levels in both the frontal and occipital cortex of the Flinders resistant line of rats [106]. Moreover, albeit a small study, alterations in protein levels of GDNF have been reported in postmortem samples of the frontal cortex from depressed subjects [178]. Finally, given the role of the mesolimbic dopamine system in depression and the protective effects of GDNF on dopaminergic neurons, it will be important to determine whether changes in GDNF function in this circuit could modulate the behavioural effects of stress and antidepressant drugs in rodent models.

*Neurturin, Persephin and Artemin*

Like GDNF, neurturin, perspehin and artemin are also potent survival factors for dopaminergic neurons [171, 179, 180]. Few studies have investigated a role for these proteins in the pathophysiology and treatment of depression. However, chronic treatment with ECS (but not chemical antidepressants) increases mRNA levels of neurturin's receptor, GFR-$\alpha_2$, in the dentate gyrus of the rat [177]. More recently, a clinical study reported a lack of association between major depression and the expression of neurturin or perspsephin in peripheral blood cells, but it did report an association with reduced levels of artemin [119].

## Neuropoietic Cytokines

The neuropoietic cytokine family of neurotrophic factors includes leukaemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF). LIF is a member of the common IL-6 family, a cytokine known to be dysregulated in depression [181]. Although LIF knockout mice demonstrate an antidepressant-like behavioural phenotype in the FST [182], serum levels of LIF are not altered in depression [183].

Even though CNTF is an important regulator of adult hippocampal neurogenesis [184], as of yet no preclinical studies have investigated whether it plays a role in the mechanism of antidepressant action. Recently, a clinical trial investigating the neuroprotective effects of CNTF in Huntington's disease reported that depression occurred in 3 out of 6 subjects when the CNTF protein-releasing capsule was removed from the lateral ventricle [185]. In contrast, however, genetic association studies have reported a lack of association of the CNTF null allele with unipolar depression [186, 187].

Given the limited number of studies investigating the role of neuropoietic cytokines in the pathophysiology and treatment of depression, it is too early to speculate

on whether these growth factors play a major role in the mechanism of antidepressant action.

## Conclusions

It is clear that a variety of neurotrophic factors play important roles in the mechanism of antidepressant drug action. However, of all the neurotrophic factors, BDNF has been the most intensively investigated in the antidepressant research field. Both clinical and preclinical studies strongly support a role for this neurotrophin in both the pathophysiology and treatment of depression. Peripheral and central levels of BDNF are reduced in clinical depression as well as in animal models of stress. Conversely, antidepressant treatments can increase BDNF levels and restore them to normal levels in both depressed patients and stressed animals, effects which may be a consequence of epigenetic regulation of the *bdnf* gene. Moreover, increasing BDNF levels in the hippocampus has antidepressant-like effects and endogenous BDNF in the hippocampus is required for the behavioural effects of antidepressant drugs. Finally, BDNF also mediates antidepressant-induced changes in various forms of neuronal plasticity which may be important in the regulation of neuronal networks that control mood.

There is some evidence to suggest that other neurotrophic factors including FGF, IGF-1 and VEGF might also be involved in the mechanism of action of antidepressant drugs. However, most of this evidence is indirect and more rigorous evaluations are required if we are to draw any firm conclusions. Nevertheless, it is clear that a variety of neurotrophic factors are important targets of antidepressant treatments and that antidepressants can activate neurotrophin signalling in the brain. The next challenge will be the rational optimisation of combinations of antidepressants and new neurotrophin-activating drugs together with psychotherapy or other forms of rehabilitation that would optimally train and shape the neuronal networks rendered plastic by the drug treatment. Such optimised combination therapies are likely to reduce the number of drug-resistant patients and improve drug efficacy.

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# Neurogenic Basis of Antidepressant Action: Recent Advances

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**Abstract**

A wide range of evidence links impairments of brain plasticity to major depressive disorder (MDD). Given the role of the hippocampus in both cognitive and emotional processing, and that MDD includes alterations in these processes, hippocampal-related plasticity, and more specifically hippocampal neurogenesis, has been suggested to be an endpoint of MDD and of antidepressant action. The observation that neurogenesis is increased by antidepressants and that factors involved in the etiology of MDD such as chronic stress can lead to a decrease in neurogenesis and in functional integrity of newborn neurons has led to the hypothesis that neurogenesis might play an important role in both the etiology of MDD and the mechanism of action of antidepressant drugs. Experimental data first argued in favor of this hypothesis by showing that all treatments having antidepressant-like effects induce increased hippocampal neurogenesis while suppression of neurogenesis prevents the efficacy of the antidepressant. Recent data, however, challenge these observations, as suppression of neurogenesis does not induce a depressive-like phenotype, since the contribution of neurogenesis in the antidepressant effects seems important in some particular conditions and for some specific phenotypes, and not for others. The discovery of the function of hippocampal neurogenesis will help in elucidating these issues.


The search for the biological basis underlying the therapeutic effects of the currently used antidepressant drugs has focused on various targets. First, as all currently used antidepressants act on the monoaminergic system (they either inhibit the serotonin and/or the noradrenalin transporter, inhibit some enzymes involved in monoamine degradation or act on some monoaminergic receptors such as for example the 5-$HT_{2C}$ receptors), it was proposed that the therapeutic effects of these molecules might be related to their aptitude to raise serotonin and noradrenalin levels in specific brain areas. However, this hypothesis met several limits, particularly because the dynamic of the therapeutic effects of antidepressants was different from their effects on monoaminergic neurotransmission (the first one occurring after several weeks of treatment, while the second one initiates immediately after the beginning of the treatment) and

because deficit in these neurotransmission systems does per se not cause depression symptoms, neither in humans [1], nor in animal models [2]. Thus, the hypothesis on the mechanisms underlying the effects of antidepressant drugs shifted toward explanations that may account for the late onset of the therapeutic action of these drugs that is seen in the clinic. Indeed, this observation suggests that antidepressants, together with their immediate action on neurotransmission, might also produce some effects related to longer time windows.

## Hippocampal Dysfunction in Depression

Interestingly, major depressive disorder (MDD) and/or animal models of depression are associated with several brain modifications, particularly morphological alterations or functional dysregulation of cortical areas including the prefrontal cortex and the subgenual cingulate cortex (Cg25) as well as of some subcortical areas, such as the hippocampus, the amygdala and the nucleus accumbens (for a review, see Ressler and Mayberg [3]) and even midbrain regions such as the periaqueductal gray [4]. Particularly, the involvement of the hippocampus in MDD was suggested by neuroimaging studies as well as by postmortem data showing a reduction of its volume paralleling the duration of disorder [5–8]; this might be related to atrophy or neuronal loss within this area [5, 9]. Interestingly, most of the MDD-related brain changes are abolished in remittent patients, indicating that these changes are state markers. For example, paroxetine treatment reverses the hippocampal changes seen in MDD patients [10]. Convergent data can be found from animal studies: using c-*fos* immunostaining, it has been shown that chronic fluoxetine reverses the pattern of activation induced by the novelty-suppressed feeding (NSF) test in the cingulate cortex and the hippocampus [11].

## Hippocampal Neurogenesis: Background

If antidepressant therapy is able to restore functional and morphological MDD-related brain alterations to a control level, it can be that these drugs may target some particular mechanisms involved in brain plasticity, particularly at the morphological level. Morphological plasticity can be related to several causes, including changes in the number of dendritic spines or number of new hippocampal cells. Recently, research on the mechanisms underlying the antidepressant's action mainly focused on one of these processes: hippocampal neurogenesis. Indeed, in some areas of the brain of adult mammals such as rodents, neural precursor cells can differentiate to all types of neural cells, including neurons, astrocytes, and oligodendrocytes [12]. These new neurons integrate into the existing circuitry, receive functional input and exhibit electrophysiological activity. Adult neural stem cells can be found in many areas of the

adult mammal brain, but differentiation in new neurons (neurogenesis) has only been consistently described in two regions: the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampus that provide new neurons to the olfactory bulbs and to the hippocampus, respectively. It is thus hypothesized that specific factors in the microenvironments of the SGZ and SVZ, termed as the neurogenic niche, may be permissive for the differentiation and integration of new neurons, and that these factors may be absent in nonneurogenic brain regions. Proliferating cells in both SGZ and SVZ are closely associated with the vasculature, which suggests that factors released from the blood vessels might be involved in neurogenesis [13, 14]. The new hippocampal neurons show typical features of mature granule cells at 4 weeks of age, even if they continue to change both physiologically and morphologically after that time period [15–17]. About 50% of newborn neurons die within 4 weeks after birth.

## Stress, Depression and Neurogenesis: Common Regulation Factors

Neural precursor cell proliferation, as well as differentiation and survival of new hippocampal neurons are regulated by several factors including stress, aging, physical exercise, neurotrophic factors and neurotransmission. Interestingly, most of these factors are also associated with MDD remission and/or antidepressant effects. More precisely, factors that induce a decrease in hippocampal neurogenesis, such as stress or aging or some diseases associated with increased risk to exhibit MDD (for example diabetes), are also associated with increased risk for MDD, while factors that promote hippocampal neurogenesis, such as physical exercise, neurotrophic factors, some neurotransmitters and antidepressant therapy, seem to prevent MDD. This is summarized in figure 1.

### *Stress, Depression and Neurogenesis*

Indeed, stress is thought to precipitate and exacerbate MDD and a decrease in cell proliferation within the SGZ and/or hippocampal neurogenesis has been observed following exposure to various types of stressors. Such effects have been observed in different species (tree shrews [18]; monkeys [19]; rodents [20, 21]) and using different types of stressors including psychosocial [18, 22] and physical stressors [23–25]. Further, these effects of stress on neurogenesis or cell proliferation have been found both after acute and chronic stress [18, 22, 26]. Among the psychosocial stressors, several protocols have been employed that all induce a suppressive effect on hippocampal neurogenesis including repeated restraint stress [24], inescapable stress [23], or unpredictable chronic mild stress (UCMS) [20, 21]. These observations might have relevance for some stress-related pathologies such as MDD, as in some cases, effects of stress on hippocampal neurogenesis were specific to type of stressors or protocols that

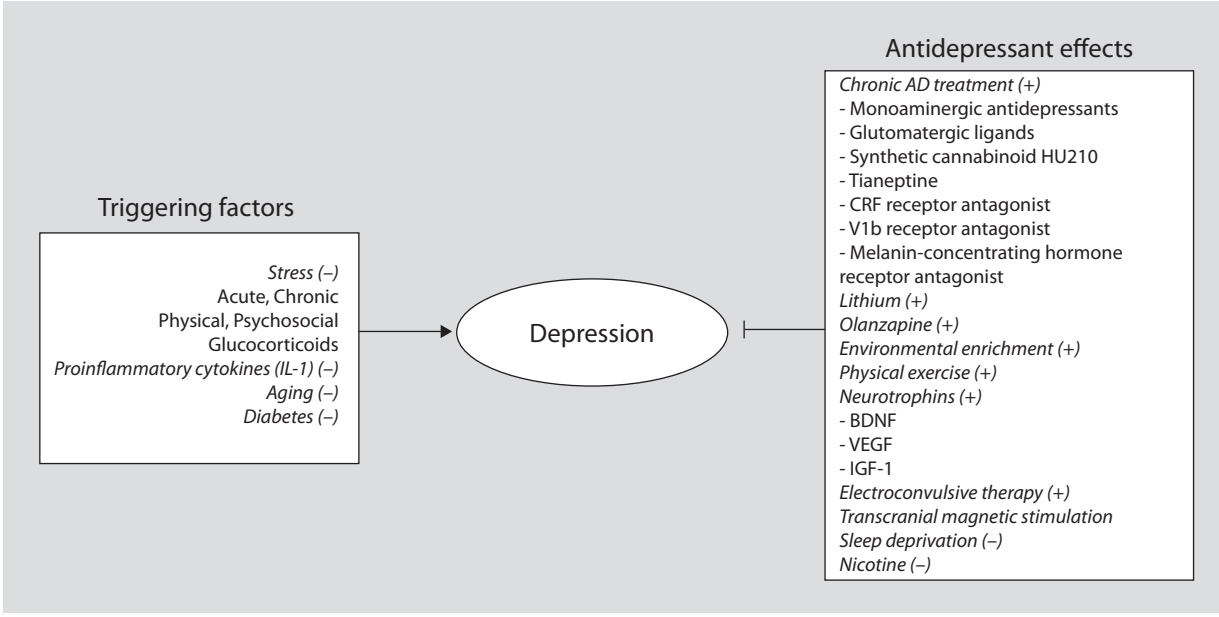


**Fig. 1.** Correlational link between regulation of neurogenesis and depression. Triggering factors on the left usually share the same antineurogenic properties (–). Compounds or manipulations that have antidepressant effects are displayed in the right box, the major part of them exerting a proneurogenic effect (+), while only few exert either no effect on neurogenesis or have an antineurogenic profile (–). AD = Antidepressant.

are involved in the etiology of MDD. For example, the learned helplessness protocol, a widely used rodent model of depression [27], reported, using male rats, that controllable stress (animals were trained to escape electric shock) causes less reduction in SGZ cell proliferation than uncontrollable stress (animals received the same amount of shocks than the ones of the first group, but in a yoked manner, with no possibility to escape). This suggests that it is not the stress that causes the proliferation decrease, but the way the stressor is perceived by the experimental subject. Interestingly, MDD is associated with uncontrollable stress, rather than controllable stress. Finally, it is also to be noted that the effects of stress on neurogenesis concern all the aspects of the process leading to newborn neurons. Indeed, stress interferes both with cell proliferation in the hippocampus and with neuronal survival [22, 28–31].

The above-mentioned effects of stress on neurogenesis might be related to the fact that hormones that are released by stressors, such as glucocorticoids, induce a robust decrease in cell proliferation and differentiation into neurons as well as in survival of the new hippocampal neurons [31–34]. Two other candidates have been suggested to be the key factors explaining the stress-induced decrease in neurogenesis: the neurotransmitter glutamate [18, 35] and the proinflammatory cytokine interleukin 1β (IL-1β) [36]. For example, blockade of the IL-1β receptor, IL-1RI, by using

either an inhibitor or IL-1RI null mice, blocks the antineurogenic effect of stress [36]. Interestingly, these two factors are also involved in the pathophysiology of MDD and in the effects of antidepressant drugs. Indeed, high levels of IL-1 have been found in patients with several forms of depression, and those levels were correlated with the severity of depression, the duration of the current depressive episode, and the age of disease onset [37–39]. Furthermore, polymorphisms in IL-1 family genes have been associated with severity of depression and responsiveness to antidepressant treatment [40]. Regarding glutamate, its role is rather complex as it depends upon the receptor that is involved [N-methyl-D-aspartate (NMDA) versus α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) or metabotropic receptor] and upon the process (proliferation, differentiation, survival) that is targeted. Indeed, on the one hand, activation of AMPA receptors as well as blockade of mGluR2/3 receptors stimulate proliferation [41]. On the other hand, in vitro stimulation of the precursors via NMDA receptors increases neuronal differentiation [42] and NMDA receptor stimulation is essential to the survival of immature neurons [43]. This should be paralleled with the fact that antidepressants induce a reduced depolarization-stimulated glutamate release and overflow in the hippocampus as well as a reduced expression or function of NMDA receptors [44] and an altered expression and activation of AMPA receptors in the hippocampus. Further, several compounds targeting the glutamatergic system, such as the glutamate release inhibitor riluzole, the partial NMDA receptor agonist D-cycloserine, an NR2B-specific NMDA antagonist, CP-101 606, or mGluR2/mGluR3 antagonists (for example, LY 341495 and MSG0039), all elicit antidepressant-like effects.

Aging, another factor that is associated with increased risk to exhibit MDD, is also associated with decreased cell proliferation and/or neurogenesis in the hippocampal region [45–48]. For example, in rodents, the number of proliferating cells in the dentate gyrus decreases about 30-fold between the age of 2 and 18 months [46]. Similar data are observed in primates. Indeed, hippocampal neurogenesis in the dentate gyrus decreases linearly with age in macaques and this decrease begins in midlife before the onset of old age [49]. Finally, neural precursor cells decline from preadolescence (8–10 years old) to adulthood (30–35 years old) in humans [50].

A decline in the survival of new hippocampal neurons has also been shown in several rodent models of diabetes [51–53], inducing a general decrease in the number of new hippocampal neurons, even if associated with increased hippocampal cell proliferation. Again, diabetes is associated with comorbid MDD [54–57] further indicating a correlation between risk for MDD and decreased neurogenesis within the hippocampus.

### *Antidepressants and Regulation of Neurogenesis*

Other factors that are related to depression, such as monoaminergic neurotransmission, alter cell proliferation and/or neurogenesis. Indeed, serotonin levels are

positively correlated with hippocampal neurogenesis [58–62] and stimulation of serotoninergic receptors (5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_{2C}$ and 5-$HT_4$ receptors) induces an increase in proliferation and, depending upon the molecule, in differentiation and survival of new hippocampal neurons [59–61, 63–65]. Further, cell proliferation in the SGZ is decreased by noradrenergic depletion while noradrenergic stimulation has opposite effects [65, 66].

All these data suggest that monoaminergic antidepressants may increase hippocampal neurogenesis. This has indeed been observed. In the early 2000s, several teams reported that monoaminergic antidepressants induce an increase in the number of new neurons within the hippocampus [67, 68]. These effects were observed after chronic administration of antidepressants, but not after acute injections: the effects of antidepressants on neurogenesis thus parallel their therapeutic effects. Interestingly, 2–3 years later, other teams reported similar effects with putative antidepressant-like compounds acting via other, nonmonoaminergic, pathways such as glutamatergic ligands [41], synthetic cannabinoid HU210 [69], tianeptine [28, 70], compounds acting on the stress axis such as corticotrophin-releasing factor 1 ($CRF_1$) or vasopressin V1b receptor antagonists [21] or a melanin-concentrating hormone (MCH) antagonist [71]. This stimulating effect of antidepressant-like treatments on hippocampal neurogenesis was also observed with nonpharmacological therapy, such as electroconvulsive therapy [67]. In some cases, neurogenic effects were observed in normal animals [67] and in other cases antidepressants rather acted to restore a decrease in neurogenesis induced by a model of depression [21]. The effects of antidepressant-like drugs on neurogenesis have also been assessed in 2 studies in human subjects, but discrepant results were observed. Indeed, in 1 study, depression and/or antidepressant therapy were not associated with a modified number of neural progenitor cells [72], while in a more recent study, MDD patients treated with chronic serotonin transporter inhibitors showed an increase in the number of neural progenitor cells, a similar effect being observed after treatment with tricyclics [73].

Do these effects of chronic antidepressants on hippocampal neurogenesis occur via an increase in proliferation, differentiation or survival of new cells? Recent studies have begun to shed light on this issue, mainly focusing on the effects of fluoxetine in rodents. First, in 2006, Encinas et al. [74] showed that fluoxetine targeted a very precise cell population as treatment with this drug did not affect the division of the stem-like cells in the SGZ or the number of neuroblasts or of immature neurons but it stimulated the proliferation of neural progenitor cells. This suggested that the effects of fluoxetine may involve a very precise step in the formation of new hippocampal neurons. However, a more recent study showed that chronic fluoxetine caused a decline in the number of immature neurons and an increase in mature neurons, suggesting that it accelerated the maturation of new immature neurons [75]. The ability of chronic antidepressant treatments to enhance the survival of newly born neurons is rather a matter of debate. A first study showed that 2 weeks of fluoxetine did not affect cell survival [67], but a later study showed that 4 weeks of fluoxetine was effective in

stimulating cell survival [76]. Further, fluoxetine also acts on the functional properties of the new hippocampal cells. Indeed, chronic fluoxetine stimulates a form of synaptic plasticity, the neurogenesis-dependent long-term potentiation in the dentate gyrus, and this effect of fluoxetine is suppressed after irradiation of the hippocampus [75]. It is still unclear whether antidepressants from other pharmacological classes work through similar mechanisms.

Interestingly, the ability of compounds to stimulate hippocampal neurogenesis is specific to drugs having antidepressant-like effects. For example, lithium and olanzapine (an atypical antipsychotic that elicits some antidepressant-like effects) share this ability to stimulate neurogenesis [77, 78], while other psychoactive drugs that do not elicit antidepressant-like effects, such as haloperidol or benzodiazepines, do not elicit such effects [64, 67, 79]. This again suggests a correlation between antidepressant effects and increased hippocampal neurogenesis. However, the picture is not always as idyllic. For example, sleep deprivation, a process known to have antidepressant-like properties, depresses hippocampal neurogenesis [80, 81]. A similar profile is found with nicotine, that both elicits antidepressant-like effects [82–84] and decreases cell proliferation [85, 86]. Finally, repetitive transcranial magnetic stimulation, a treatment eliciting antidepressant effects, only mildly counteracts the stress-induced decrement in hippocampal cell proliferation, while it suppresses the survival rate of proliferating cells [22].

Other, nonpharmacological treatments that increase neurogenesis are also associated with antidepressant-like effects. For example, environmental enrichment, a regimen that elicits antidepressant-like effects in rodents [87–90], is also associated with increased hippocampal neurogenesis [91–93]. Similarly, voluntary physical exercise stimulates neurogenesis [94] and elicits antidepressant-like effects [95, 96].

### *Neurogenesis, Neurotrophins and Depression*

In the last few years, much popularity has been generated by the 'neurotrophin hypothesis of depression', implying that neurotrophins such as brain-derived neurotrophic factor (BDNF), neural growth factor, vascular endothelial growth factor (VEGF) or insulin-like growth factor 1 are involved in MDD. Indeed, administration of these factors generally promotes recovery in animal models of MDD [97, 98]. Interestingly, these factors also promote the generation of new hippocampal neurons, while a decrease in BDNF is associated with decreased neurogenesis. Indeed, mice deficient in BDNF (BDNF +/– mice) show a decrease in the survival of new neurons [99, 100]. However, no effects are observed on differentiation of the new cells [99, 100], while a controversy is found as to the effects of this mutation on cell proliferation, one study [100] showing a decrease and the other study [99] an increase in proliferation induced by the genetic invalidation. Further, intrahippocampal injection of BDNF promotes survival of new hippocampal cells [101] and mice deficient in p75,

one of the BDNF receptors, exhibit increased cell proliferation and decreased survival [99]. On the other hand, intracerebroventricular administration of neural growth factor in rats stimulates cell survival, while it elicits no effects on differentiation or survival [102]. Administration of VEGF stimulates cell proliferation in SGZ and elicits antidepressant-like effects in several tests. Interestingly, signalization associated with this neurotrophin and its receptor Flk1 seems necessary for the neurogenic and behavioral effects of antidepressant drugs [103]. Finally, infusion of insulin-like growth factor 1 also induces antidepressant-like effects as well as neurogenic effects [104].

### Are Antidepressants Required for Antidepressant Efficacy?

The causal relationship between antidepressant efficacy and neurogenesis was first addressed by Santarelli et al. [64], who used focal X-ray irradiation to specifically abolish hippocampal neurogenesis in mice before testing antidepressant efficacy in 2 paradigms, the NSF test and the UCMS model of depression. The NSF test consists of exposing food-deprived mice to a novel environment (typically an open field) in which a food pellet is positioned in the center under bright light, and the latency to feed is recorded. This test induces a conflicting motivation between the drive to eat and the fear to venture into the center of the arena, where a higher latency reflects a more anxious/depressive profile. While this test has also been used to screen for anxiety-like behaviors, it is among the few that can reliably demonstrate a behavioral change in response to chronic but not acute treatment with antidepressants and has therefore been used to assess antidepressant efficacy. Whereas control mice in their study displayed a reduced latency to feed in the NSF test after chronic fluoxetine or imipramine treatment, irradiated mice failed to respond to both treatments. However, assessing antidepressant efficacy in naive/control/wild-type mice is questionable. As the neurobiological mechanisms involved in antidepressant effects might not be similar under pathological and standard condition, they further confirmed these results by addressing the requirement of neurogenesis for the behavioral effects of fluoxetine by using the UCMS, which is a well-validated model of depression [105]. This model is based on chronic and unpredictable exposure to various stressors of mild intensity for several weeks and elicits a wide range of antidepressant-sensitive physiological and behavioral alterations, partly analogous to some of the symptoms of depression, including anhedonia, hypothalamic-pituitary-adrenal (HPA) axis dysregulations, or decreased neurogenesis [105, 106]. One of the core behavioral changes induced by UCMS is a progressive degradation of the coat state of the mice, which has been used as a reliable index of depressive-like state. While chronic fluoxetine treatment in their study was able to reverse the coat state degradation induced by UCMS in nonirradiated mice, hippocampal irradiation completely abolished the effects of fluoxetine. It is noteworthy that localized irradiation of the SVZ, which is another neurogenic niche, had no

**Table 1.** Effects of suppression of neurogenesis on the behavioral effects of antidepressants

| Method of suppression | Species | Strain | Model of depression | Antidepressant treatment | Behavioral assessment | Effect of suppression | References |
|---|---|---|---|---|---|---|---|
| Irradiation | mouse | 129/SvEv | WT | fluoxetine (28 days) imipramine (28 days) | NSF | prevents AD effects | 64 |
| Irradiation | mouse | Balb/c | UCMS (5 weeks) | fluoxetine (28 days) imipramine (28 days) | fur state; splash test | prevents AD effects | 64 |
| Irradiation | rat | Long-Evans | WT | CB1 agonist (HU210)(10 days) | NSF; FST | prevents AD effects | 139 |
| Irradiation | mouse | Balb/cJ | WT | fluoxetine (30 days) | NIH; FST | no effect | 125 |
| Irradiation | mouse | 129/SvEvTac | WT | MCH1 antagonist (SNAP 94847) (28 days) | NSF | no effect | 71 |
| Irradiation | rat | Fisher 344 | WT | fluoxetine (7 days) | FST | prevents AD effects | 138 |
| Irradiation | mouse | Balb/c | UCMS (5 weeks) | fluoxetine (28 days) imipramine (28 days) | fur state; NSF; splash test | prevents AD effects | 107 |
| Irradiation | mouse | Balb/c | UCMS (5 weeks) | $CRF_1$ antagonist (28 days) V1b antagonist (28 days) | fur state; splash test | no effect | 107 |
| Irradiation | mouse | Balb/c | UCMS (5 weeks) | $CRF_1$ antagonist V1b antagonist (28 days) | NSF | prevents AD effects | 107 |
| Irradiation | mouse | SvEv129 | WT | fluoxetine (28 days) | NSF | prevents AD effects | 75 |
| MAM | rats | Wistar | UCMS | fluoxetine imipramine CP 156526 SSR 149415 (14 days) | FST; SCT | no effect | 110 |
| MAM | rats | Wistar | UCMS | fluoxetine imipramine CP 156526 SSR 149415 (14 days) | NSF | attenuates AD effects? | 110 |

**Table 1.** Continued

| Method of suppression | Species | Strain | Model of depression | Antidepressant treatment | Behavioral assessment | Effect of suppression | References |
|---|---|---|---|---|---|---|---|
| MAM | mouse | ICR | WT | rolipram (14–23 days) | EPM; FST; TST; NSF | attenuates AD effects | 140 |
| Irradiation | mouse | C57BL/6Ntac | chronic corticosterone treatment | fluoxetine (21 days) | NSF | prevents AD effects | 108 |
| Irradiation | mouse | C57BL/6Ntac | chronic corticosterone treatment | fluoxetine (21 days) | FST; OF | no effect | 136 |

FST = Forced swimming test; SCT = sucrose consumption test; EPM = elevated plus maze; TST = tail suspension test; OF = open field; MAM = methylazoxymethanol acetate; WT = wild type; AD = antidepressant; NIH = novelty-induced hypophagia.

effect on antidepressant efficacy. This was the first evidence that hippocampal newborn neurons might directly be involved in the behavioral effects of antidepressants.

Much attention has been given to the causal role of hippocampal neurogenesis in the effects of chronic antidepressants since then and several studies have now addressed this question with conflicting results (table 1). Data come predominantly from irradiation studies and to a lesser extent from mutant models or systemic treatment with antimitotic drugs, such as methylazoxymethanol acetate, a cytostatic agent that does only partially reduce neurogenesis, which makes the comparisons somehow difficult. The heterogeneity of the results shows that the requirement of neurogenesis for the behavioral effects of antidepressants might be influenced by multiple factors such as the type of behavioral paradigms used to assess antidepressant efficacy, the type of antidepressant, the species and strain of animals used.

## Is Neurogenesis Crucial for All Aspects of the Antidepressant Action?

Two recent studies highlighted the fact that the question whether or not neurogenesis is required for the behavioral effects of antidepressants might be irrelevant, but rather antidepressants might exert their effects via both neurogenesis-dependent and independent pathways. In a first study, irradiation was used to arrest neurogenesis and the ability of chronic $CRF_1$ antagonist treatment to reverse the behavioral changes induced by UCMS was assessed. While hippocampal irradiation prevented the effects of the $CRF_1$ antagonist in the NSF test, further confirming previous results, the same animals still responded to treatment in the coat state and splash test [107]. Similarly, another study showed that suppression of neurogenesis by

irradiation prevented the behavioral effects of fluoxetine in the NSF test, but not in the forced swimming test or the open field [108]. It is therefore possible that these paradigms reflect different aspects of the pathology and involve different pathways/structures in which neurogenesis, and more generally the hippocampus might play a different role. It has been argued for instance that neurogenesis might be particularly involved in anxiety-like behaviors [109] and that performance in the NSF test is more reflective of anxiety-related behavior and therefore irrelevant to depression. It is however noteworthy that chronic fluoxetine treatment had a significant effect in the open field test in this last study, and if requirement of neurogenesis was specific to the relief of anxiety-like behavior, one would expect that antidepressant efficacy was suppressed in the open field following irradiation, which was not the case [108].

Also supporting the view that antidepressants might exert their therapeutic action via both neurogenesis-dependent and independent mechanisms is the fact that so far evidence indicates that neurogenesis does not seem to be required for the improving effects of antidepressants in measures of hedonia [110, 111].

Involvement of neurogenesis in the efficacy of antidepressants also seems dependent on the nature of the drugs used. Whereas fluoxetine, $CRF_1$ antagonist, or the CB1 agonist HU210 have shown to depend on neurogenesis to exert their behavioral effects in the NSF test [64, 69, 107], it does not seem to be the case with an MCH1 antagonist [71]. Neurogenesis can therefore unlikely be considered as the critical process by which antidepressants exert their behavioral effects and achieve remission. A possible explanation would be that these different classes of antidepressants could act via distinct and possibly compensating mechanisms, some of which being linked to neurogenesis while others not. These different classes could however also act by targeting the same common endpoint, some of these compounds requiring the recruitment of newborn hippocampal neurons to do so, while others having more direct means to modify this endpoint. For example, the hippocampus is known to be involved in the glucocorticoid receptor-mediated negative feedback inhibition of the HPA axis [112]. Attenuation of this negative feedback inhibition has been reported in human depression and following chronic stress in animals [113–115], and monoaminergic drugs have been shown to partially reverse this attenuation by increasing glucocorticoid receptor levels or function thus leading to an enhanced negative feedback inhibition over the HPA axis [116, 117]. It is possible that hippocampal neurogenesis might play an important role in this inhibition. Indeed, a recent study showed that suppression of neurogenesis led to increased release of glucocorticoids following exposure to a mild stressor, suggesting an impaired negative feedback inhibition [118]. It is therefore conceivable that monoaminergic drugs, which do not directly target the HPA axis, might exert their actions by stimulating neurogenesis and restoring a proper hippocampal function, notably the hippocampus-driven inhibition of the HPA axis. Drugs acting directly via HPA axis-related functions, such as the $CRF_1$ antagonist, could therefore exert antidepressant effects independently from their neurogenic

effects. However, these hypothetical views are not exclusive, as neurogenesis is also required for the behavioral effects of $CRF_1$ antagonist in the NSF test [107].

### Key Factors Involved in the Neurogenic Effects of Antidepressants

Finally, some studies tried to identify the molecular targets required for the effects of fluoxetine on hippocampal neurogenesis. They found that ablation of VEGF [119], of the BDNF receptor TrkB [120, 121], or of aquaporin 4 (a key molecule for maintaining water homeostasis in the brain [122]), or dysfunction of glycogen synthase kinase 3 [123] disrupt the stimulating effects of antidepressants on neurogenesis, suggesting that these molecules may be involved in the molecular pathway by which the proneurogenic action occurs.

Other experimental data may help in elucidating the function of the hippocampal neurogenesis in the effects of antidepressant drugs. Indeed, the stimulating effect of antidepressants on hippocampal neurogenesis is not found in some specific conditions, and a careful analysis of these failures could provide some additional tools to disentangle the role of this process in the therapeutic effects of antidepressant-like treatments. Indeed, the stimulating effect of fluoxetine on hippocampal neurogenesis and/or cell proliferation depends upon the strain [124–126] and the age of the animals, as it can be found in adult rodents, but neither in peripubescent animals [127] nor in aged ones [128]. Discrepant results have been found regarding the gender, as some found gender differences in the ability of antidepressant drugs to stimulate neurogenesis [127, 129], while others did not replicate these findings [130]. The stimulating effect of fluoxetine on neurogenesis or cell proliferation can also be suppressed by some treatments. For example, it is abolished by the anxiolytic diazepam [131], by manipulation of the hormonal stress axis leading to abolished circadian rhythm of corticosterone [132], by lesion of the basolateral nucleus of the amygdala leading to decreased anxiety-like behavior in the elevated plus maze [133] or by environmental manipulations such as early-life stress [134]. Other treatments act to augment the effects of antidepressants on neurogenesis, including Glu2/3 receptor agonists and DHEA.

### Neurogenesis as an Etiological Factor of Depression

These overall conflicting results do not allow a clear understanding regarding the potential implications of neurogenesis in the pathophysiology of depression and the mechanisms of actions of antidepressants. It is, however, important to dissociate these two aspects. Indeed, impairment of most of the investigated pathways currently known to be involved in antidepressant efficacy rarely leads to a depressive phenotype. For example, several polymorphisms have been described that alter antidepressant

response including polymorphisms of the serotonin transporter gene, of some serotonin receptors, or of the BDNF gene (for a review, see Kato and Serretti [135]), but no evidence indicates that these abnormalities increase the occurrence of depression. This is also true for neurogenesis, since in all of the mentioned studies arrest of neurogenesis was not sufficient to induce a depressive-like phenotype or to increase vulnerability [64, 107, 136]. This lack of effect on basal behavior has also been observed on other hippocampus-related functions. Indeed, results are also rather conflicting regarding the role of neurogenesis in memory- and learning-related processes (for a review, see Leuner et al. [137]).

## Conclusion

Whether these discrepancies are due to heterogeneous experimental approaches (e.g. differences in species, strains, or behavioral paradigms used) or whether neurogenesis might be solely an epiphenomenon is hard to say. Nonetheless, functional integration of newborn hippocampal neurons has been clearly demonstrated, and recent studies suggest that arrest of neurogenesis could alter the local network properties of the hippocampus [75, 138], thus potentially modifying hippocampal output and optimal functioning. Recovery of a proper hippocampal function in return could help in alleviating specific symptoms of depression. Refinement of the paradigms used to assess behavior in animals could help in this way by giving a better idea about the precise cognitive processes in which neurogenesis might be involved.

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# The Nature and Treatment of Therapy-Resistant Depression

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**Abstract**

Therapy-resistant depression (TRD) is highly prevalent and has major health and economic implications. Although it is associated with psychiatric and medical comorbidity, there does not appear to be any common underlying biological substrate: more likely TRD represents a final pathway for various subpopulations of major depressive disorder patients. Sequential treatment studies indicate that over 40% of patients have not achieved remission after 2 adequate trials. Atypical antipsychotics and neurostimulation therapies are the most recently applied approaches to TRD, each with considerable success. Nevertheless, there is an urgent need to characterize the subtypes of TRD (at neurotransmitter, neural circuitry or neuroanatomical levels) with the aim of providing a personalized medicine approach to treatment.



Major depressive disorder (MDD) is recognized as a prevalent and disabling disorder worldwide [1, 2]. According to the global burden of depression study, depression also profoundly affects occupational function, both through presenteeism and absenteeism, [3, 4], and exerts a huge economic burden on society [5]. A large percentage of this burden can be attributed to therapy-resistant depression (TRD) [6].

## Defining Therapy-Resistant Depression

There is a lack of consensus on what constitutes TRD, although several approaches have been proposed. Thase and Rush [7] suggested a staging definition, based on failure to respond to different classes of antidepressant treatment, including selective serotonin reuptake inhibitor, mixed reuptake inhibitor, tricyclic and monoamine oxidase inhibitor antidepressants, as well as electroconvulsive therapy (ECT). Failure to respond to 2 or more adequate trials of different classes of antidepressants is the

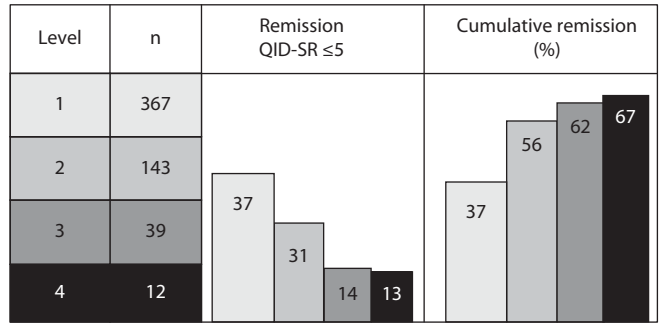


**Fig. 1.** Remission rates from STAR*D. Modified from Warden et al. [16]. QID-SR = Quick Inventory of Depressive Symptomatology-Self-Report.

minimum requirement for treatment resistance [8], while the term 'pseudoresistance' has been used to describe patients with nonresponse to inadequate treatment [9]. The Massachusetts General Hospital Staging method assigns differential weighting to the number of treatments and to different modalities, with ECT receiving a higher score [10]. A multidimensional approach to evaluating TRD has also been proposed involving severity and duration of illness, as well as antidepressant, augmentation, and ECT failures [11]. However, none of these definitions reflects current patterns of sequential and combination approaches to treatment.

All of these definitions rely on accurate capture of past and recent treatment history, which is frequently unreliable. One way to improve the historical accuracy of such information is to use a structured instrument to assess past treatments such as the Antidepressant Treatment History Form [12].

## Prevalence and Risk Factors for Therapy-Resistant Depression

Without consensus on the definition of TRD, it is difficult to provide reliable estimates for prevalence. However, sequential rates of treatment nonresponse can be derived from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D). Patients who did not achieve remission, defined as a score of 7 or less on the Hamilton Rating Scale for Depression [13] after the first stage, were offered alternative or combination treatments with antidepressants or cognitive therapy during the second level of therapy [14, 15]. Additional treatment alternatives were offered during the third and fourth levels [16, 17].

After initial treatment with citalopram, approximately 30% of depressed patients achieved remission [14]. Based on failure to remit after 2 adequate trials involving different strategies, 44% of patients in the STAR*D trial met criteria for TRD. The cumulative remission rates declined progressively with subsequent interventions, leaving 33% of patients in the TRD category by the end of the trial [16] (fig. 1). However, some treatments that would typically be used today in the TRD sample were not included in this study (e.g. atypical antipsychotics or ECT).

Several authors have explored TRD in the context of unique clinical characteristics, biological and environmental factors, concluding that there is likely no unique underlying biological substrate [18]. Although no major depressive episode subtype (e.g. atypical or melancholic) appears to be overrepresented in TRD populations, the most consistent finding across clinical trials is evidence that comorbid anxiety disorders are associated with worse outcomes [19, 20] and with treatment resistance [21]. Other risk factors associated with TRD are first onset under 20 years; undetected hypomanic symptoms; more than 3 prior episodes, and failure to achieve remission after previous depressive episode [22].

## Current State of Pharmacotherapy for Therapy-Resistant Depression

In 2010, psychiatrists, family doctors and other prescribing practitioners have access to various national and international guidelines for the treatment of MDD (for a comparative review, see Davidson [23]). While some guidelines confine their content to pharmacotherapy [24], others extend the evidence-based recommendations to include psychotherapies, nonpharmaceutical preparations and neurostimulation therapies [25]. Much less attention has been devoted to treatment resistance in guidelines, although various antidepressants and their combinations may help to alleviate depressive symptoms in this population.

### *Atypical Antipsychotics*

Atypical antipsychotics have emerged as monotherapy and combination therapy options for TRD [26]. Three of the 4 first-line combination strategies recommended in the CANMAT guidelines [27] for treatment resistance include atypical antipsychotics. These recommendations reflect the large body of evidence from meta-analyses [28] and from individual placebo-controlled trials involving olanzapine [29], quetiapine [30] and aripiprazole [31].

Based on a series of studies, the combination of olanzapine and fluoxetine demonstrated superiority over either agent alone for TRD [29]. This evidence is limited to the combination of olanzapine with fluoxetine, although olanzapine may be equally effective when combined with other antidepressants.

Quetiapine has not only demonstrated efficacy as an antidepressant monotherapy in the treatment of MDD [32, 33], but has also been shown to enhance antidepressant outcomes when combined with a range of antidepressants in previously treatment-resistant patients [30, 34, 35]. These positive results complement findings in bipolar depression involving quetiapine monotherapy [36, 37].

Aripiprazole has also displayed efficacy as an add-on strategy in MDD in several studies [31, 38]. In these trials, response and remission rates were approximately

doubled in the aripiprazole 'add-on' group compared to those who received add-on placebo. Efficacy has also been demonstrated in TRD populations [39]. However, aripiprazole did not demonstrate superiority over placebo in 2 identically designed add-on trials for bipolar depression [40]. To date, there is no evidence to support the role of ziprasidone as an add-on strategy in the treatment of MDD [41].

Although atypical antipsychotics provide an additional treatment avenue for TRD, the lower tolerability profile compared to first-line antidepressants and their potential to cause serious side effects including weight gain, dyslipidemia, hyperglycemia, extrapyramidal symptoms and QTc interval prolongation restrict their use to second-line treatment in MDD or to TRD.

### *Antidepressant Combinations*

Combining 2 or more antidepressants to enhance therapeutic outcomes in previously unresponsive patients is common practice in many countries [42, 43]. Nevertheless, there is limited evidence to support this practice. Both mianserin [44] and mirtazapine [45] produced significant benefits when added to the first antidepressant. However, there was no benefit in a large placebo-controlled trial when mianserin was combined with sertraline compared to sertraline monotherapy [46].

Most combination trials follow a sequential process, whereby nonremitters to a previous monotherapy are then eligible to receive sequential combined therapy. An alternative method involves a concurrent combination approach. In a small randomized controlled trial, Blier et al. [47] examined the effects of initiating concurrent mirtazapine with fluoxetine, venlafaxine or bupropion compared to fluoxetine monotherapy. The combination of mirtazapine and venlafaxine was statistically superior to fluoxetine alone after 8 weeks [47]. Unfortunately, increasing the dose of fluoxetine in the monotherapy arm was not an option in the study and it remains unclear whether this strategy would have been as effective as the combination. Nevertheless, this study highlights the need to examine concurrent combined therapies, particularly in previously treatment-resistant patients.

### *Other Pharmacological Strategies*

Dopamine agonists are frequently prescribed to TRD patients, which supports the role of dopamine in depression [48]. Pramipexole, an antiparkinsonian agent, in particular, may have efficacy in TRD based on small randomized controlled pilot studies [49, 50]. Methylphenidate or dextroamphetamine are also frequently combined with serotonin reuptake inhibitors and other antidepressants for TRD, despite limited evidence to support this strategy [51–53].

**Table 1.** Recommendations for neurostimulation therapy

| Neurostimulation | Overall recommendation | Acute efficacy | Relapse prevention | Safety and tolerability |
|---|---|---|---|---|
| Electroconvulsive therapy | First-line under specific circumstances; second-line for otherwise treatment-resistant or medication-intolerant populations | Level 1 | Level 1 | Level 1 |
| Repetitive transcranial magnetic stimulation | Second-line | Level 1 | Level 3 | Level 1 |
| Vagus nerve stimulation | Third-line | Level 3 | Level 2 | Level 2 |
| Deep brain stimulation | Investigational | Level 3 | Level 3 | Level 3 |

From Kennedy et al. [25]. Level 1 and 2 evidence refers specifically to treatment studies in which randomized comparisons are available. Level 3 evidence includes open-label trials and case series.

The benefits of augmentation with pindolol in TRD patients are inconclusive, with both positive [54] and negative [55, 56] results. At the third level of STAR*D, lithium or triiodothyronine ($T_3$) was added to existing therapies and both produced modest increases in efficacy [57].

## Advances in Neurostimulation Therapies

Given the lack of effective pharmacotherapy options for TRD, there is an increased focus on emerging neurostimulation therapies. These treatments deliver a physical intervention through electric current or magnetic field to specific or generalized brain regions. They have been facilitated by advances in neuroimaging and growing recognition of neural circuitry changes in depression.

### *Electroconvulsive Therapy*

ECT is the prototypic neurostimulation and has been used since the 1930s. Several meta-analyses have confirmed the superior efficacy of ECT compared to both sham ECT and antidepressant medications [58, 59], thus asserting that the induction of a seizure with ECT is an active element in the therapeutic effects of ECT.

The strongest predictor of the acute antidepressant efficacy of ECT is a prior history of resistance to antidepressant medication. While the evidence to support the use

of ECT as a first-line treatment is limited, response rates in the 80–90% range have been reported in patients who received inadequate trials of antidepressant medication [60, 61]. ECT response rates for patients who have failed to respond to 1 or more adequate antidepressant medication trials are around 50–60% [60]. Although results from the recent Consortium for Research in Electroconvulsive Therapy study suggest that a history of resistance to antidepressant medication does not predict acute remission status with ECT [62], baseline medication resistance is associated with increased rates of relapse following ECT [63].

While ECT has a robust evidence base for efficacy, its use is limited by patient perceptions, device and anesthetist availability, lack of target specificity, tolerability, and sustainability of treatment effect [64].

On the balance of evidence for the antidepressant properties and side effect profile of ECT, the 2009 CANMAT clinical guidelines for the management of MDD recommend that ECT be considered a first-line treatment for MDD characterized by prominent suicidal ideation or psychotic features, while reserved as a second-line treatment for patients with medication resistance [25] (table 1).

### *Repetitive Transcranial Magnetic Stimulation*

Repetitive transcranial magnetic stimulation (rTMS) involves repeated subconvulsive magnetic stimulation to the brain. Compared to ECT, where much of the electrical stimulus is prevented from exerting its effects on the brain due to impedance from the skull and scalp, the magnetic fields generated by rTMS are able to directly stimulate superficial cortical areas of the brain. This offers the promise of greater control over the location and extent of cortical stimulation than can be achieved with ECT. The frequency of stimulation with TMS determines its effects, with slow (defined as a frequency ≤5 Hz) TMS decreasing neuronal firing rates and fast (defined as a frequency ≥5 Hz) TMS exerting the opposite effects.

A meta-analysis of rTMS for TRD reported response and remission rates of 25 and 17%, respectively, for active treatment compared to 9 and 6%, respectively, for sham treatment [65]. Since then, further studies have been published supporting the role of rTMS for TRD [66, 67]. A history of antidepressant medication resistance has been shown to be a negative predictor of acute response to rTMS [68]. Based on a paucity of long-term relapse prevention data and modest, but significant, acute antidepressant effects, rTMS has been recommended as a second-line treatment for MDD [25] (table 1).

### *Vagus Nerve Stimulation*

Treatment with vagus nerve stimulation (VNS) involves an implantable pulse generator, which provides intermittent electrical stimulation through a lead connected to

the left vagus nerve. In a randomized sham-controlled 10-week trial, there was no significant difference between active and sham treatments [69], although improved results were reported during extension phases [70]. The degree of previous resistance to antidepressant pharmacotherapy is inversely correlated with the likelihood of responding to VNS [71].

Despite approval for TRD in the United States, this treatment awaits further publications to demonstrate efficacy. VNS is recommended as a third-line treatment for MDD [25] (table 1).

### *Deep Brain Stimulation*

This is the most invasive and investigational form of neurostimulation and involves the implantation of bilateral electrodes under stereotactic guidance to a specified neuroanatomical target. The use of deep brain stimulation (DBS) for patients with MDD is based on the premise that TRD may represent a subset of depressed individuals for whom more anatomically targeted, focal brain stimulation, such as DBS, may be required to enlist the neurocircuitry necessary for antidepressant response [72].

The electrodes are connected to a pulse generator implanted subclavicularly, which delivers continuous electrical stimulation. To date, the subcallosal cingulate gyrus [73, 74], the nucleus accumbens [75], and the ventral capsule/ventral striatum region [76] are the neuroanatomical target sites under investigation for TRD. Each of these research groups has published a promising open-label report of efficacy in about 50% of TRD patients, but results of active versus sham trials are required to confirm these findings for acute and maintenance therapy. At the present time, given the absence of data from sham-controlled trials regarding its acute efficacy, relapse prevention properties and side effect profile, DBS is considered an investigational approach for treating MDD [25] (table 1).

## Conclusions

This chapter highlights the complexity of defining and treating TRD. Without universally accepted criteria, it is difficult to ascertain the prevalence of TRD, and estimates range from 20 to 30% of patients in large clinical samples. There is also evidence that patients with comorbid psychiatric and medical conditions are more likely to become treatment resistant and these patients require increased vigilance, potentially benefitting from initial combination therapy. Evolving from ECT, several forms of localized neurostimulation offer promising alternatives for TRD, but are still in the early phases of evaluation. Future studies should be directed at identifying biomarkers to define unique depression subtypes that would preferentially respond to selected treatments.

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# Optimizing Antidepressant Management of Depression: Current Status and Future Perspectives

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**Abstract**

Since the 1950s, clinicians have targeted the same 3 monoamine neurotransmitters to treat depression and have held the antidepressants to the standard of achieving 50% symptom reduction in an average amount of patients on a per study basis. As such, researchers have developed newer antidepressants that are more tolerable, but they have not developed drugs that are better in efficacy. A greater number of patients are now treated with safer and more tolerable drugs which has allowed a decrease in the stigma of mental illness and has allowed primary care clinicians to become more comfortable in treating the mentally ill. These treatments are reasonable, but often not curative. This paper will attempt to provide an overview of ideas so that an adept, well-rounded clinician might be able to obtain better outcomes despite using *old* monoamine strategies. Also, an overview of new agents that utilize old monoamines but with newer pharmacodynamic mechanisms of action will be covered. Finally, novel pharmacologic agents in the potential future armamentarium in the treatment of major depressive disorder will be discussed. 

## Making Good with What Is Available…

Basically, there are a limited number of ways to obtain remission in major depressive disorder patients: using psychotherapy or pharmacotherapy. There have been advancements in both modalities, but the focus of this paper is largely towards that of antidepressant pharmacotherapy. Basically, clinicians have been using the same pharmacodynamic approaches since the 1950s to boost monoamine [serotonin (5-HT), norepinephrine (NE), dopamine (DA)] activity with the tendency to obtain similar results for patients across the decades. Facts are facts. An average number of patients will likely achieve an average amount of symptom reduction [1, 2]. The mammoth STAR*D trial suggests that remission occurs one third of the time with initial

monotherapy and each subsequent antidepressant treatment yields less favorable outcomes as treatment-resistant depression increases [3]. This independent, collaborative trial started with all patients receiving adequately dosed citalopram and then allowed for successive medication changes for patients who failed to respond to this initial antidepressant. Linear and systematic use of other agents, such as bupropion, venlafaxine, sertraline, buspirone, lithium, mirtazapine, and nortriptyline, was tried in sequence to determine if any medication regimen was more effective and also to better determine outcomes of patients who had treatment-resistant depression noted over time. After 4 successive, adequately dosed, and full-duration antidepressant treatments, only about two thirds of patients finally remitted in this trial. This left one third of depressed patients still with significant symptoms and by definition as having treatment-resistant depression after failing 1 year of aggressive treatment with 4 antidepressant treatment strategies. If all antidepressant research stopped today, or if researchers' ability to discover another viable set of neurotransmitters to manipulate failed, what would clinicians do? How could clinicians better use what they currently have to get better outcomes in major depressive disorder? The next pages will suggest options for better psychopharmacological management of depression.

## Basic Psychopharmacology Principles

### *Dosing*

Every psychotropic has a simple dose-response curve, especially for side effects. One must dose higher than the minimum effective dose, or the drug will not work. However, most psychotropics often carry official wording that 'there is no additional benefit' from dosing higher than this minimal effective dose. When large groups of psychopharmacologists gather, it often seems to be a unanimous conclusion that higher-dose monotherapies work better, but also come with a greater side effect burden, poorer compliance, and greater expense. One possibility why randomized controlled trials fail to show a distinct dose-response curve for antidepressant efficacy could be that antidepressant treatments are modestly effective and rating scales in trials may be lowered 50% after initial dosing of the drug, leaving little room for improvement as doses are further escalated. There are fewer patients remaining in the study with significant symptoms where 50% more improvement can even occur. Leaders in the field of depression research suggest that studies with 1,000–2,000 subjects may be needed to truly show that dose escalation statistically works [4]. Perhaps if studies were run with greater statistical power and were actually designed to show better response at higher doses, then the evidence base might match what clinical findings in practice suggest. The bottom line is that in the absence of adequate antidepressant efficacy, the use of the full dose range of any antidepressant is warranted unless adverse effects limit dose escalation.

*Duration*

What is a good antidepressant treatment duration? Some authors suggest that if there is no partial response within the first 2 weeks then remission is doubtful [5]. Others, and the NIMH-sponsored STAR*D trial, suggest that 12 weeks at moderate to high dosing is really needed as some patients respond early and some later [6]. The antidepressants change transmitter levels almost immediately but remission is not seen within hours. After 4–6 weeks of treatment, clinicians can actually see a solid clinical response at the same time when researchers can see receptor downregulation or a buildup in neurotrophic factors. Antidepressants may also work by changing neuronal gene activity to facilitate the production of new proteins (neurotrophic factors), which takes 4–6 weeks as well [7]. So pharmacodynamically, this time frame is a good duration for an adequate clinical trial in clinical practice.

*Switching*

Once adequate antidepressant dose and duration are applied, if there is no response, then the suggestion is to switch to a new treatment. Certain authors also suggest that if an initial antidepressant allows only a partial response with an effectiveness of 0–30%, it should likely be abandoned and a switch to a new antidepressant treatment should occur [8]. The new drug should affect a different monoamine dynamic mechanism. Switching from selective serotonin reuptake inhibitor (SSRI) to SSRI to SSRI makes little pharmacodynamic, and clinical, sense. If the response is greater than 30%, then an augmentation/combination approach should occur [8]. There is almost no conclusive data to support multiple switches or multiple combinations, so oftentimes this is at the discretion of the clinician. Clinically, if a drug is not working over many weeks, it likely will not start to work months later, so switching agents is often the prudent choice.

*Augmenting and Combining Antidepressant Treatments*

Augmenting refers to adding a nonapproved agent to boost clinical antidepressant effectiveness (e.g. thyroid hormone added to tricyclic antidepressant for depression), and combining refers to adding 2 approved agents (e.g. bupropion added to sertraline for depression) together instead. This polypharmacy is utilized in order to obtain better outcomes. One survey asked a large audience of psychiatrists how many drugs their usual depressed patient was prescribed. The answer was 'two or more', routinely [9]. This polypharmacy approach seems to be the standard, especially as treatment-resistant depression evolves. Clinicians tend to sequentially add medications that utilize different monoamine mechanisms to move patients towards remission. There are few approvals for combined medications like: amitriptyline-perphenazine for major

depressive disorder and olanzapine-fluoxetine for treatment-resistant depression, but most of the time clinicians are prescribing 'off-label' where there is little to no evidence base with statistically powered analysis to support the combination/augmentation strategies. A bit a more aggressive, but a likely warranted, approach is to utilize combinations of antidepressants right away at the initiation of treatment, which will be discussed later in this paper.

### *Rational Polypharmacy*

In 2002, one of the authors of this paper proposed some simple rules for treating patients. They are reviewed below and some new rules are now proposed. The idea behind these rules is to use the available but modestly effective antidepressant treatments, but to get more out of them [10]. When prescribing and combining medications consider the following *old rules:*

1 aim for full clinical remission of symptoms;
2 use and combine medications that utilize different therapeutic mechanisms of action;
3 continue one's clinical education and push the envelope to learn new techniques;
4 monitor for side effects and establish treatment intolerance versus resistance;
5 create 'win-win' situations when combining drugs for better effects but also to cancel each other's side effects;
6 diagnose and treat all comorbidities;
7 'be the student of a patient's life'; take a full history, be thorough and make accurate assessment per DSM, dynamics, etc.

Now let's consider some *new rules.*

### *The Placebo Effect*

The placebo effect should be used to clinical advantage. In randomized controlled trials, this effect is now escalating above 30–40% [11]. To enhance this effect:

a build rapport with patients;
b use psychotropics that the clinician believes in;
c use psychotropics the patient believes in;
d be book smart and know the facts. Patients will ask about the epidemiology and etiology of their illness and how the medications may help. If a clinician knows his/her facts and answers their questions directly, there will likely be a better placebo response and treatment adherence;
e utilize informed consent. Really explain to patients that the drug is approved and studied for their condition. Be honest and thorough about side effects. Learn how to describe the drug's mechanism of action and what it may be doing to help

the depressed, anxious, or manic brain. Giving patients a better rationale and reason for the prescription will likely build rapport and improve compliance.

*Understand and Recognize Personality Disorder*

If a standard of the 15-min 'med management' session continues to be the usual and depressed patients are seen only every several weeks, clinicians will lose their skills and opportunities to detect axis II traits. Clinicians will continue to detect DSM-IV axis I conditions correctly in these short, sparse encounters, but might take a few years to see axis II disorders [12]. An axis-I-based major depressive disorder is classic or clinical depression. This is the disease state that most clinical trials study in order to determine antidepressant effectiveness. Axis II conditions tend to be long-standing personality disorders, such as borderline personality disorder. This latter condition may include the symptom of depression which usually has an empty quality to it. Patients with personality disorders often have poor, maladaptive coping skills which often lead to stressful life events and onset of comorbid major depressive disorder. Clinicians need to be realistic about axis II outcomes from axis I medications in that response rates likely plummet and sustaining remission may be impossible pharmacologically without intensive psychotherapy. The use of axis II rating scales and keeping psychodynamic skills sharp may help to better detect axis II features in depressed patients. A prompt referral of these patients for psychotherapy in addition to medication management should ensue [13, 14].

*Psychotherapy*

Remember that psychopharmacologists are 'always doing psychotherapy', even in shorter medication visits. It is often not practical to conduct manualized cognitive-behavioral therapy, dialectical-behavioral therapy, interpersonal psychotherapy or dynamic psychotherapy, but psychopharmacologists should always use the skill set of core psychotherapy techniques (motivation, empathy, openness, collaboration, warmth, positive regard, sincerity, providing corrective experience, catharsis, establishing goals, establishing a time-limited relationship, establishing that patient effort is needed to succeed) which are common to all psychotherapy styles and common amongst good psychotherapists [15]. Using these core skills should build rapport, medication compliance and boost the placebo effect discussed above.

*Outcome Measures*

Some data suggest that psychopharmacologists who use rating scales routinely in practice will obtain better outcomes in certain disorders [16]. Routine use of rating scales

allows the clinician to see that full remission is not present, and it forces more aggressive treatment by increasing dose, maximizing duration, adding other medication or psychotherapy until remission occurs. There are simple self-report scales for depression. These can be filled out in waiting rooms and scored by secretaries. In the age of the electronic record, patients can enter scales into kiosks or laptops in waiting rooms so that scores are at the psychopharmacologist's fingertips and automatically stored in databases that can be graphed later to show response, remission, or pending relapse. Scales can be mailed out as appointment reminders, completed early in the waiting room, or at home via a web-based service so as not to take up valuable appointment time. Oftentimes the data reported in the scales save time in the office visit and allows more time for treatment planning, informed consent, and rapport building.

### *Consider Combination at Initiation of Treatment*

The standard of care is to try one monotherapy antidepressant treatment at a time and switch when remission fails to occur or add one monotherapy to another sequentially to increase remission rates. If the average depressed patient is on a few medications at any given time due to sequential prescribing and the average remission rate is only 33% on a given monotherapy, why wouldn't clinicians hedge their bets and start major depressive disorder patients on a combination of antidepressant treatments all at once? Cost and adverse effects must be considered, but if a 'win-win' situation could be created where 2 agents that are effective and well tolerated could be combined, perhaps clinicians should think and act more aggressively and engage in combination at initiation of treatment in the more resistant patient. Reviews suggest that at least 6 trials exist using combination at initiation of treatment which show effectiveness of combining SSRI and desipramine, L-methylfolate, eszopiclone, or mirtazapine [17, 18].

The above sections are clinical ideas and rationales about using both psychopharmacology and psychotherapy skills to get better outcomes out of the available antidepressant treatment options. The second half of this paper will now focus on the future. What clinical techniques are cutting edge and more aggressive in the management of major depressive disorder? What new pharmacologic agents are in the pipeline or about to be approved? These are now to be discussed.

## Making Good on the Future…

### *Improving on Existing Medications and Mechanisms of Action*

Bupropion HBr has been approved and allows administration of single-pill doses up to 450 mg equivalency compared to the original bupropion HCl salt. This single,

easier dose could facilitate better compliance. A once-daily controlled-release formulation of trazodone is now just approved as well [19]. This may increase the utility of trazodone use in major depressive disorder, as the immediate-release formulation is often not tolerated due to excessive sedation [19]. Desvenlafaxine, the active metabolite of venlafaxine, was approved 2 years ago. It has clinical SNRI effectiveness at its starting dose of 50 mg/day. It bypasses the P450 2D6 system and is excreted by the renal system. It has minimal drug-drug interactions and is likely the lowest protein-bound antidepressant [20]. This drug is a controlled-release product which also improves its tolerability. It may also become available for vasomotor symptoms of menopause and neuropathic pain in the future [21, 22].

Initially atypical, second-generation antipsychotic drugs were approved to treat schizophrenia and then secondarily to treat bipolar mania. Over the last 1–2 years, researchers have become more aware of their pharmacodynamic properties that may lend to antidepressant potential and an invigorated focus on using these agents to treat depressive disorders. Several pharmacodynamic mechanisms may explain the antidepressant effects of the second-generation drugs [21]. These newer antipsychotics can increase the availability of 5-HT, DA, and NE. Specifically, actions at 5-$HT_{2A}$, 5-$HT_{2C}$, and 5-$HT_{1A}$ receptors indirectly lead to NE and DA disinhibition, which may improve mood and cognition. Mood may also be improved by increasing NE and 5-HT via actions at $\alpha_2$-receptors, by increasing NE via blockade of the NE transporter, and by increasing 5-HT via actions at 5-$HT_{1D}$ receptors and blockade of the 5-HT transporter. Antihistamine actions may improve insomnia or agitation associated with depression as well.

Each second-generation antipsychotic has a unique pharmacodynamic fingerprint that may contribute to its antidepressant action [23]. This may explain why these agents differ in their ability to treat the depression due to major depressive disorder or bipolar disorder. Quetiapine and quetiapine XR have the most evidence of efficacy as a monotherapy for bipolar depression, and have just been approved now for adjunctive treatment in unipolar depression [24]. The effective dose of quetiapine (XR) in bipolar depression is 300–600 mg/day, lower than that needed for saturation of the $D_2$ receptor (antipsychotic and antimanic effects) but sufficient to cause 5-$HT_{2C}$ antagonism, 5-$HT_{1A}$ agonism, and NE reuptake inhibition. Dosing as augmentation in unipolar illness is lower at 150–300 mg/day. These antidepressant actions may occur especially through the newly discovered pharmacologic actions of its active metabolite, norquetiapine [23, 25]. 5-$HT_{1A}$ agonism antidepressant effects are explained further below, but 5-$HT_{2C}$ antagonism antidepressant effects should be noted here. Blockade of this receptor in the brainstem ventral tegmental area and locus ceruleus removes GABA inhibitory tone with the net effect of allowing more DA and NE release to the dorsolateral prefrontal cortex allowing for potential antidepressant effects to occur.

The olanzapine-fluoxetine combination has recently been approved for treatment-resistant depression [26]. It was previously the first ever FDA approval for bipolar

depression as well. It utilizes 5-$HT_{2A/C}$ and $H_1$ antagonism from olanzapine and SSRI properties from fluoxetine to treat depression. Olanzapine monotherapy failed to treat depression and required the added serotonergic potential of fluoxetine to become effective. The olanzapine $D_2$ blockade component also helps to prevent escalation into bipolar mania.

Whether atypical antipsychotic drugs will be proven effective as monotherapies for unipolar depression given their higher adverse effect profile compared to SSRI and SNRI is still under intense investigation [27]. Aripiprazole was the first atypical antipsychotic approved, not as a monotherapy, but as an adjunct treatment for treatment-resistant major depressive disorder at doses of 2–10 mg/day, again lower than that used for schizophrenia or mania. Aripiprazole is primarily a partial $D_2$ and $D_3$ receptor agonist with only weak 5-$HT_{2A}$ receptor antagonist and partial 5-$HT_{1A}$ receptor agonist properties [28]. This agent carries many antidepressant pharmacodynamic properties through its $D_3$ agonism which increases *tonic* DA release to the cortex, $D_2$ agonism which enhances *phasic* cortical DA tone, and 5-$HT_{1A}$ agonism which ultimately allows increased NE and DA neurotransmission to the dorsolateral prefrontal cortex [21, 29]. As noted above, quetiapine (XR) has gained a recent antidepressant augmentation approval likely due to its NE reuptake inhibitor and 5-$HT_{2C}$ receptor antagonist properties. It also has current data under review at the FDA for use in generalized anxiety disorder [30].

Asenapine was just simultaneously approved for the treatment of schizophrenia and bipolar mania. This agent carries antidepressant properties of 5-$HT_{2C}$ antagonism, 5-$HT_{1A}$ agonism, $\alpha_{2A}$ antagonism and is structurally very similar to the FDA-approved antidepressant mirtazapine. Potential atypical antipsychotics, such as lurasidone and cariprazine, contain similar 5-$HT_{1A}$ receptor agonist/5-$HT_{2C}$ receptor antagonist antidepressant properties, and cariprazine also carries the aripiprazole-like $D_2/D_3$ partial agonism properties [31, 32]. Given the current approvals for depression treatment for some of the atypicals, and the fact that many current and future atypicals share even more antidepressant-like pharmacodynamic similarities, further studies should be conducted to better determine their ultimate clinical effectiveness.

*Triple Reuptake Inhibitors*

A newer line of investigation revolves around triple reuptake inhibitors. It is common practice in treating depression to take a partial SSRI responder and add an NE-DA reuptake inhibitor such as bupropion XL. This combined therapy now is a triple reuptake inhibition action and allows facilitation of all 3 monoamines simultaneously. Again, typically these drugs are added sequentially, not as a combination at initiation of treatment. The triple reuptake inhibitors would be a bit more aggressive and fully work in the combination at initiation of treatment model as a monotherapy. Several different triple reuptake inhibitors (or 5-HT-NE-DA reuptake inhibitors) are being investigated at phase I and II levels [21]. Some of these agents have additional pharmacological properties as well. In particular, LuAA24530, currently in clinical

trials, is not only a triple reuptake inhibitor, but also binds 5-$HT_{2C}$, 5-$HT_{2A}$, 5-$HT_3$, and $\alpha_{1A}$ receptors.

*Novel Serotonin Targets (Serotonin Receptor Agonists and Antagonists)*

A large number of novel 5-HT targets are in testing as well [21]. There again seems to be more of a focus on 5-$HT_{2C}$ receptor blockade. These antidepressant treatments could be called NE-DA disinhibitors.

A novel, possibly available antidepressant, agomelatine, combines this property of 5-$HT_{2C}$ receptor antagonism and receptor agonist actions at melatonin receptors ($MT_1$ and $MT_2$). $MT_1$ stimulation allows the brain to decrease arousal at night time and might facilitate the treatment of insomnia due to depression, and stimulation of $MT_2$ may help correct circadian rhythms also felt to be altered in certain depressed patients. Positive trials of agomelatine have been completed in the United States, and it is now approved in Europe with liver function monitoring required [33].

LuAA21004 is an SSRI and also a receptor antagonist at 5-$HT_3$ receptors, with additional receptor agonist actions at 5-$HT_{1A}$ receptors. This drug would offer the same as a typical SSRI plus a buspirone augmentation which is a common strategy in treating treatment-resistant depression patients. It would have the added benefit of the 5-$HT_3$ blockade which may dampen arousal and help insomnia secondarily [21].

*New Antidepressant Treatment (Nonmonoamine) Targets and Mechanisms*

L-5-Methyl-tetrahydrofolate has been approved in the US as a medical food supplement and is available by prescription in 7.5-mg tablets. It does not yet have stringent enough data to become a full medical prescription antidepressant treatment but trials are ongoing [34]. It is used as an adjunct treatment for major depressive disorder augmentation. L-5-Methyl-tetrahydrofolate is the active form of folate available in the brain and plays a critical role in allowing and increasing intraneuronal monoamine synthesis [34, 35]. Adding L-5-methyl-tetrahydrofolate might allow neurons to create and secrete more monoamines into the synaptic cleft. Secondarily, SSRI/SNRI would then have more monoamine to 'work with' and promote better antidepressant efficacy [21].

As major depressive disorder is a prevalent, often chronic and disabling illness, a large number of companies and researchers continue to look for nonmonoamine ways to help depressed patients to remission [21]. Many of these agents are low-molecular-weight drugs that target stress hormone release from the hypothalamic-pituitary-adrenal axis, while many others are receptor antagonists at neurokinin (NK) receptors. Some data suggest that $\beta_3$-receptors, located in the amygdala, may be related to depressed mood [35]. Hypofunctioning of these receptors could lead to hypoactivation of the amygdala as well as brain regions closely connected to the amygdala, such as the ventromedial prefrontal cortex. Receptor agonists (amibegron) here might be able to treat depression by restoring neuronal communication and activity between

dysfunctional brain regions. However, makers of this product have stopped conducting clinical trials (http://www.apmhealtheurope.com/story.php?numero=12308).

The glucocorticoid receptor antagonists, corticotrophin-releasing factor 1 receptor antagonists, and vasopressin 1B receptor antagonists are in testing not only for depression but also for various anxiety disorders. Unfortunately, numerous trials with several different corticotrophin-releasing factor 1 receptor antagonists in major depression have proven to be disappointing [36].

MIF-1 (also known as L-prolyl-L-leucyl-glycinamide, or PLG) is a tripeptide tail of oxytocin under investigation for treating major depressive disorder [37]. Similarly, nemifitide is a pentapeptide analog of endogenous MIF-1 but also of the tripeptide tail of vasopressin. This experimental agent has been shown to be active in animal models of major depressive disorder and is in testing in human models now as well [38, 39] Both of these agents are administered by injection and appear to have rapid onset of antidepressant action and are felt to facilitate DA, 5-HT, and enkephalin neurotransmission through a novel mechanism.

Another class of peptide receptor antagonists is the NK antagonists which belong to the family of peptides known as tachykinins. The NK-1 receptor antagonists (substance P receptor antagonists) in this class appear to be the most studied and have not yielded consistently positive results in studies of major depression. More recently, NK-2 and NK-3 receptor antagonists have begun to be evaluated. The theory that hyperactivity in the NK system is a precursor to depressive states suggests that these receptor antagonists might become effective antidepressants in the future. NK-2 receptors are located in the prefrontal cortex, hippocampus and in other limbic structures suggesting a role of the NK-2 system in the generation and treatment of major depressive disorder symptoms. Furthermore, NK-2 antagonism appears to dampen the hypothalamic-pituitary axis where excessive depressogenic cortisol activity is concerned [40]. Saredutant, an initial agent in this area, showed promise in acute trials, but failed to provide maintenance antidepressant effects and was discontinued. Similar agents, such as SAR 1022279, SR 144190, and GR 159897, are also being evaluated.

The glutamate and NMDA system is also being heavily investigated. NMDA receptors seem to play an important role throughout the brain but their role in depression is still not clear. Reports suggest that NMDA receptor density is decreased in the prefrontal cortex or hippocampus of depressive patients suggesting that hypofunctioning has occurred [41].

What is unclear is whether the NMDA underfunctioning is due to glutaminergic excitotoxicty and downregulation or if the NMDA receptor itself is hypoactive due to genetic or other inherent defects.

One antidepressant approach would be to dampen excessive glutamate activity. Ketamine, an NMDA receptor antagonist, has now been observed to have rapid onset of antidepressant effects in animal and human models of depression [42]. It also has effects on opioid transmission, DA and 5-HT reuptake inhibition [43]. A current shortcoming for ketamine is in the lack of long-term effectiveness data, its

nonselective nature, and its dosing may be problematic in that ketamine may decrease depression and anxiety, or contrarily even become anxiogenic and psychotomimetic.

A more selective way to treat major depressive disorder via the NMDA system would be to create drugs that are more specific. Specificity for amount of NMDA affinity and blockade is one option and specificity for neuroanatomical location is another. A class of NR2B NMDA receptor subunit antagonists may be useful in this area and offer a more specific NMDA dampening solution [44]. EVT 101, TXT-0300, and MK-0657 are currently under investigation. NR2B blocking agents are felt to be neuroprotective as well. NR2B animal studies suggest that this subunit is active in long-term potentiation and learning at a synaptic level [45]. This approach could improve executive function, memory, vigilance and alertness all of which falter in depression, some anxiety disorders, ADHD and schizophrenia.

Another glutamatergic approach would be to improve hypofunctioning NMDA receptors via a different NMDA modulating receptor called $\sigma_1$. In animal models, its underactivity has been implicated in depression. A $\sigma_1$ receptor agonist, SA 4503, has had positive effectiveness in treating agitation, loss of interest, and impaired cognition. This agent is a highly selective agonist of $\sigma_1$ receptors, with higher binding affinity than other prototypical $\sigma_1$ receptor agonists (i.e. SKF 10047) [46]. Similar $\sigma_1$ agents may also facilitate the release of acetylcholine or DA in addition to potentiating the functioning of NMDA receptors, all of which may help treat depressive symptoms [47–49].

Finally, nonpharmacological, device-based treatments for major depressive disorder seem to be on the rise. Transcranial magnetic stimulation was recently approved in the US for major depressive disorder patients who have failed to respond to an initial antidepressant monotherapy. Transcranial magnetic stimulation uses a repeated, high-strength magnet which causes rapid alternating electrical currents to be transmitted through skull into the dorsolateral prefrontal cortex [50]. Deep brain stimulation was approved for compassionate use in obsessive-compulsive disorder and has research ongoing in treatment-resistant depression now as well. Deep brain stimulation uses a battery-powered pulse generator (pacemaker) which is implanted in the chest wall with 1 or 2 leads tunneled directly into the brain, to the subgenual area of the ventromedial prefrontal cortex, to send brief repeated pulses there to treat depression [51]. Initial work involves another device called a cortical stimulator. About the size of a band-aid, this device is laid across the surface of the dorsolateral prefrontal cortex and is also activated by a pulse generator device for direct stimulation of the dorsolateral prefrontal cortex [52].

## Conclusion

In summary, there are many ways for clinicians to increase their clinical effectiveness and the effectiveness of current antidepressant treatments to obtain better outcomes for treatment-resistant patients. Some of these techniques include the use of psychotherapy

skills as well as more aggressive dosing, augmenting and combining of treatments. Psychopharmacologists may even consider more combination at initiation of treatment approaches in the future in their resistant and debilitated patients. Researchers should also continue to explore new and unique treatment mechanisms as it is certain that treatment-resistant depression will continue to exist and remain difficult to treat.


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