Mental depression represents the most common and proliferating health problem worldwide (Wong and Licino, 2001; 2004) and has an estimated lifetime prevalence of about 15-20% (Artigas et al., 1996; Greist et al., 2002; Kessler et al., 2003). From an epidemiologic point of view, the prevalence of depression has been estimated to vary from 2.6% to 5.5% in men and from 6.0% to 11.8% in women (Fava and Davidson, 1996). The burden on health systems around the world from depression is hard to overstate (Greenberg et al., 2003), but the World Health Organization (WHO) has predicted that unipolar depression will be the second most (ischemic heart diseases being the first) prevalent cause of illness-induced disability by 2020 (Murray and Lopez, 1997a). Individuals suffering from depression and related neurological disorders are not only faced with considerable disruption to their psychological well-being, but are at considerably greater risk for various somatic conditions including cardiovascular disorders as well as obesity (Sheps and Sheffield, 2001; Rumsfeld and Ho, 2005). In fact, disability from depression exceeds that of most chronic general medical conditions such as hypertension, diabetes, arthritis, and lung diseases (Wells et al., 1989) etc. The disorder is relatively common among patients with the diagnosis of dementia (Ballard et al., 1996; Stepaniuk et al., 2008) and may be a risk factor for developing dementia (Kokmen et al., 1996). The disease is both recurrent and widespread. The social environment has long been thought to play an integral role in depression. Alarmingly, suicide, which is invariably associated with emotional disturbance, is now the third largest cause of death among young adults not only in the western countries (Licino and Wong, 2005) but also in developing countries like India (Vijayakumar, 2005). Goa, one of the India’s most affluent and literate states has highlighted a high prevalence of depression and stress-related problems, affecting 5-10% of young adults (Chinai, 2007). Despite the devastating impact of depression, little is known about its exact etiology, or pathophysiology. Although, the currently available antidepressant drugs
are effective for many patients, however, approximately 60-70 % of the patients continue to experience residual symptoms despite appropriate treatment, and 30-40 % will not respond to drug treatment or will have only partial response (Fava and Davidson, 1996; Holtzheimer and Nemeroff, 2006a). Surprisingly, pediatric population is usually resistant to antidepressant treatment, except some may respond to selective serotonin reuptake inhibitors (SSRIs) (Malkesman et al., 2007). Unfortunately, even the treatment with various block-buster antidepressants such as SSRIs is known to increase the risk of suicidability as compared to placebo (Goren, 2008). Therefore, there is always a need to discover and develop newer antidepressant drugs with unique mechanism of action. This is possible only if we understand the exact pathophysiology of depression and the precise mechanism of action of already existing drugs.

Definition and etiology
Depression, or more specifically, major depressive disorder, is defined as a despairing mood and the loss of interest or pleasure in nearly all activities that were previously considered pleasurable (To et al., 2005) accompanied by at least several psychophysiological changes (Belmaker and Agam, 2008). The mood of an individual with major depression is often described as miserable, hopeless, or discouraged, and there are many physical symptoms associated with depression (Kelsey, 2001; To et al., 2005).

A clinician’s index of suspicion about the diagnosis of depression should be raised if a patient presents with a chief complaint of fatigue, pain, sleep disturbances, anxiety, irritability, or gastrointestinal problems (Walker et al., 1992; Richelson, 2001).

mentioned by World Health Organization in 1992 (Berton and 2006), are some of the widely used international classifications of disorders, which include the diagnostic criteria of depressive disorders defined by the presence of certain symptoms (Table 2) (Nestle 2002). According to the DSM classification, a major depression is characterized by at least five of the symptoms listed below (Table 2) (Refer to DSM IV, 2000; Schechter et al., 2005). DSM-IV states that at least 60% of the people who experience a single episode of major depressive disorders will probably have a second episode (TR, 2000).

In the ICD-10, major depression was replaced by the term ‘depressive episode’, which is diagnosed based on the presence of five of the mentioned symptoms (Table 2) and is further classified as mild, moderate, or severe, depending upon the severity of symptoms (WHO, 1992).  

Table 2: Diagnostic criteria for depression

- Depressed or irritable mood
- Decreased interest in pleasurable activities and ability to experience pleasure
- Significant weight gain or loss (> 5% change in a month)
- Insomnia or hypersomnia
- Psychomotor agitation or retardation
- Fatigue or loss of energy
- Feelings of worthlessness or excessive guilt
- Diminished ability to think or concentrate
- Recurrent thoughts of death or suicide

Adapted from Diagnostic Statistical Manual of Mental Disorders IV (DSM IV, 2000)

Recently, the American Psychiatric Association and the Health Organization have begun the process of revising DSM-IV to include the severity of the individual symptoms in these disorders (Andrews et al., 2008). As the present criterion of diagnosing
depression is too complex, therefore, it is expected that revised versions 
viz. DSM-V (See, DSM V) and ICD-11 would propose a reduced criterion 
set that can be remembered by clinicians (Andrews et al., 2008). These 
new guidelines are expected to be released by the year 2012.

Etiology of mental depression
Depression is caused by a complex array of factors including biology, 
genetics, and environmental factors (Frances et al., 1992). Heritability is a 
dependable variable in approximately 40 % of the cases (Uhl and Grow, 
2004) and a family history of affective disorders is amongst the strongest 
predictor of vulnerability of depression (Reinherz et al., 2003; Monk et al., 
2008). The pathophysiology of depression is not linked to alteration in any 
single or specific gene, rather the nature is multifactorial (multiple genes) 
which includes epigenetic factors such as early life experiences that may 
result in the expression or deletion of various genes (Charlie and Manji, 
2004). The relative risk (ratio of risks to first-degree relatives of major 
depressive disorder vs. the general population) is around 2 to 3 
(Weissman et al., 1984; Maier et al., 1992). However, some researchers 
have ruled out the genetic involvement in the depressive outcome of the 
patients (Foster and Macqueen, 2008). The high risk groups include: 
adolescent or middle-aged people experiencing stressful life events (such 
as the death of a loved one), people with chronic illnesses (such as 
cancer, heart disease, chronic headaches, hormone disorders), people 
with history of abuse (mental, physical or sexual), people with little social 
support, and those with a current or past alcohol or drug abuse. Patients 
with psychiatric disorders, such as schizophrenia (Zisook et al., 1999), 
anxiety disorders (Andrews et al., 2008), Alzheimer’s (Ballard et al., 1996), 
Huntington and Parkinson diseases (Robinson et al., 1999) may have 
increased chances of depression. Other disorders both central and 
peripheral such as tumors, infections of the brain, hypothyroidism, right 
hemisphere stroke, hyperparathyroidism, Cushing and Addison diseases 
may produce depression and related neuropsychiatric symptoms
In fact, endocrine abnormalities are one of the risk factors in the pathophysiology of depression (Goodyer et al., 2001). It is proposed that hypothyroidism and depression have overlapping symptoms, therefore, speculated to have common pathophysiology. Depression is often described as a stress-related disorder, and there is good evidence that episodes of depression often occur in the context of some form of stress. However, it is debated that stress per se is not sufficient to cause depression (Nestler et al., 2002). Most people do not become depressed after stressful experiences such as during combat, rape, or any other terrible stress, but instead suffer from post-traumatic stress disorder (Nestler et al., 2002).

**Classification of mental depression**

A biological basis for mood disorders was described as early as the 5th Century BC, when Hippocrates referred to “melancholia” meaning “black bile” as a severe depressive condition associated with “aversion to food, sadness, sleeplessness, irritability and restlessness” (Cryan and Slattery, 2007). Throughout history, the concept of depression has evolved from “melancholia” in the Greek period to “major depressive disorder” in the current DSM-IV-TR classification (Wada, 2007). Milder cases are classified as “dysthymia”, although there is no clear distinction between the two (Nestler et al., 2002). Depression should not be viewed as single disease, but a heterogeneous syndrome comprised of numerous diseases of distinct causes and pathophysiologies. Attempts have been made to establish subtypes of depression defined by certain set of symptoms (Table 3) (Akiskal, 2000; Blazer, 2000; Nestler et al., 2002). However, these subtypes are based solely on symptomatic differences.

**Neural Circuitry of mood**

Many brain regions have been implicated in regulating mood. Various imaging studies have revealed a change in blood flow in several brain regions including prefrontal and cingulate cortex, hippocampus, striatum,
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Amygdala and thalamus during depression (Liotti and Mayberg, 2000) has been revealed that specific deactivation of the prefrontal cortex more prominent deactivation of the posterior parietal cortex leads to sadness in depression. This suggests the involvement of cortical systems in the pathophysiology of depression (Liotti et al., 2000). It has been found that these brain areas operate as a series of highly interacting circuits (Fig.2) and there have been various neurotransmitters viz. γ-butyric acid (GABAergic), glutamatergic, dopaminergic, peptidergic, adrenergic or serotonergic innervations identified in these areas (Nestler et al., 2002). It is believed that disturbances in any of the circuits or neurotransmitters may lead to various symptoms of depression.

Table 3: Examples of proposed subtypes of depression

<table>
<thead>
<tr>
<th>Depression subtype</th>
<th>Main features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melancholic depression or endogenous depression</td>
<td>Innate depression; Severe symptoms; prominent neurovegetative abnormalities</td>
</tr>
<tr>
<td>Reactive depression or exogenous depression</td>
<td>Moderate symptoms apparently in response to external factors</td>
</tr>
<tr>
<td>Psychotic depression</td>
<td>Severe symptoms, associated with psychiatric symptoms</td>
</tr>
<tr>
<td>Atypical depression</td>
<td>Associated with labile mood, hypomania, increased appetite, and weight gain</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>Milder symptoms, but with a more extended course</td>
</tr>
<tr>
<td>Seasonal Affective disorders</td>
<td>Depression due to seasonal variation, for example, winter depression</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>Alternate phase of depression and mania</td>
</tr>
</tbody>
</table>

Adapted from Nestler et al., 2002

The hippocampus is a limbic structure located along the medial surface of each temporal lobe. It is known that hippocampal dysfunction in the frontal cortical regions is involved in mediating various co-aspects of depression such as memory impairment, feeling...
worthlessness, excessive guilt, and suicide thoughts. Many studies using 3-dimensional magnetic resonance imaging (MRI) volumetric measurements have reported decrease in hippocampal volume among depressed subjects compared with control (Sheline et al., 2002). Both the noradrenergic (from the locus ceruleus located within the dorsal wall of the rostral pons in the lateral floor of the fourth ventricle) and cholinergic system (innervations from the medial septum) innervates the hippocampal region and are required for learning and memory processes (Scheiderer et al., 2008).

Fig. 2. Various neural circuits in the brain involved in the pathophysiology of depression. PFC: Prefrontal Cortex; NAc: Nucleus Accumbens; VT: Ventral Tegmental area; DR: Dorsal Raphe Nuclei; LC: Locus Ceruleus (Reproduced from Nestler et al., 2002)
Therefore, any disturbance in the cholinergic system or noradrenergic may be one of the key factors that leads to memory impairment in the depressed patients. This is further evidenced from the fact that Flinder Sensitive Line (FSL) of rats, a genetic animal model of depression have an abnormal cholinergic system and have been demonstrated to possess cognitive difficulties and other depression-like symptoms (Overstreet, 1993; Overstreet and Djuric, 2001).

The amygdala that is best studied for its role in establishing associations between aversive or rewarding stimuli has also been strongly implicated in the pathophysiology of depression (Keedwell et al., 2005). Anhedonia (relative lack of pleasure in response to formerly rewarding stimuli) in depressed patients is associated with decreased activity of amygdala region of the brain (Keedwell et al., 2005). However, contrary to this, both the hyperactivity and hypoactivity of amygdala is known in the depressed patients, depending on the causating factor of depression. It has been proposed that amygdala hyperactivity is present in subjects suffering from depression because of some environmental factors and amygdala hypoactivity in those at risk mainly through genetic factors (Wolfensberger et al., 2008).

The Locus ceruleus (LC) is a nucleus in the brain stem involved with physiological responses to stress and panic. The region has noradrenergic innervations (Berridge and Waterhouse, 2003) and failure of its function could explain the basic impairments in the processing of novel information, intensive processing of irrational beliefs, and anxiety (Harro and Oreland, 2001). In fact, locus ceruleus (LC) is the sole source of norepinephrine projections to the forebrain (Devilbiss and Waterhouse, 2004). In one of the studies, it was found that tyrosine hydroxylase activity, a rate limiting enzyme for the formation of norepinephrine or dopamine was decreased in locus ceruleus region following forced running stress in rats (Komori et al., 1990). However, contrary to this, overactivity of locus ceruleus region has also been demonstrated in the postmortem studies of patients suffering from major depressive disorders. It is being discussed that the activity of locus ceruleus is under the control of both excitatory and
inhibitory inputs (Zhu et al., 2006). The major inhibitory input being GABAergic innervations, in further sections of this review, it has been discussed that there is decrease in GABA levels in the cerebrospinal fluid of depressed patients. Therefore, the low levels of GABA in the cerebrospinal fluid (CSF) and plasma of subjects with major depressive disorder raises the possibility that locus ceruleus overactivity in depression may be secondary to reduced GABAergic input to the this region (Zhu et al., 2006).

Hypothalamic region of the brain is known to play an important role in producing various neurovegetative symptoms in depression including too much or too little sleep, appetite, and energy, as well as a loss of interest in sex and other pleasurable activities (Nestler et al., 2002). Excessive activation of the hypothalamic-pituitary-adrenal (HPA) axis has been demonstrated in individuals suffering from depression (Holsboer, 2001). Activation of this axis may lead to increased release of glucocorticoids such as cortisol which may damage hippocampal neurons and produce cognitive impairment in depressed patients (Fig. 3).

**Fig. 3.** Hypothalamic-pituitary-adrenal (HPA) axis and participation in depression symptomology
Another important area that is involved in depression is Nucleus accumbens (NAc). Nucleus accumbens is a collection of neurons within the forebrain and has been found to play a critical role in domains which are prominently affected in most depressed patients. Many studies point to the NAc as the major region involved in, and mediating, activities relating to motivation and hedonia in which the dopaminergic and serotonergic systems are involved (Serova et al., 1998; Malesman et al., 2007).

The Ventral tegmental area (VTA)-Nucleus Accumbens (NAc) tract is important in mediating various symptoms of depression. The Ventral tegmental area (VTA) (part of the striatum) provides dopaminergic inputs to the Nucleus Accumbens (NAc), amygdala, prefrontal cortex (PFC) and other limbic structures (Fig. 2) (Nestler et al., 2002). These dopaminergic inputs are basically involved in the reward phenomenon and have been projected for the presence of anhedonia and decreased motivation and energy levels in individuals suffering from depression. The VTA-NAc tract is also known to innervate hippocampus region of the brain and plays an important role in memory formation (Nestler et al., 2002).

Recently, the role of Dorsal Raphe (DR) nucleus has come into picture. It has been debated that serotonin neurons of the dorsal raphe nucleus receive dense noradrenergic innervations (O’Leary et al., 2007) from other regions of the brain. In a recent study, it has been demonstrated that hypoactivity of DR neurons is a distinct phenomenon in depression, specific only for suicidal subgroup of depressed patients (Gos et al., 2008).

Some of the important brain areas and their role in depression are summarized in Fig. 4.

**Theories of mental depression**

**Monoaminergic theory of depression**

The discoveries of chlorpromazine and imipramine in the early 1950s and 1960s have caused a paradigm shift in the treatments of psychiatric disorders (Hayashi and Su, 2008). The first generation of these
psychotherapeutic agents and their analogs significantly improve prognosis and quality of life for patients (Hayashi and Su, 2008) suffering from psychiatric disorders.

**Major brain areas and symptoms of depression**

- **Frontal cortex and hippocampus**
  - Mediate cognitive aspects of depression such as
    - Memory impairment
    - Feeling of worthlessness
    - Hopelessness
    - Guilt
    - Suicidability

- **Striatum (Ventral striatum or Nucleus Accumbens) and amygdala**
  - Responsible for
    - Anhedonia
    - Anxiety
    - Reduced motivation

- **Hypothalamus**
  - Involved in mediating neurovegetative symptoms of depression such as
    - Too much or too little of sleep
    - Appetite and eating
    - Loss of interest in sexual and other pleasurable activities

**Fig. 4. Brain areas and their role in various symptoms of depression**

Virtually all antipsychotics and antidepressants presently used in clinical practice target monoaminergic system(s) in the central nervous system, leading to the formation of monoamine theory as therapeutic basis of the treatment (Delgado, 2000).

The catecholamines, norepinephrine and dopamine, and indoleamine, serotonin are the brain monoamines that have received greatest research attention in the pathophysiology of depression (Schildkraut et al., 1965; Ressler and Nemeroff, 2000; Delgado, 2004). These monoamines are widely distributed in mammalian central nervous system. This regulates a considerable arra
behaviors including mood, appetite, cognition, libido, anxiety, aggression, and others (Nemeroff, 2002). A number of roles can be ascribed to neurotransmitters. For example, stress responsiveness, energy, and behavior have primarily been associated with norepinephrine (Morilak and Frazer, 2004), impulsivity with serotonin systems (Nemeroff, 2002), and motivation and reward with dopamine projections (Alcaro, 2007). However, as drawn in figure (Fig. 5.), there is considerable overlap between functions of monoamines. Both norepinephrine and serotonin are important in mediating anxiety response, impulsivity and irritability; norepinephrine and dopamine in motivation and attention and dopaminergic functions in sexual behavior, appetite and aggression (Nemeroff, 2002). Therefore, all three monoamines are important in regulating mood, emotion and cognitive functions (Nemeroff, 2002) which form the basis of monoaminergic theory of depression.

The monoamine theory of depression proposes that depression is due to a deficiency in one or another of three monoamines, norepinephrine (Coppen, 1967), noradrenaline (Schatzberg and Schild, 2007), and serotonin (Nemeroff, 2002).
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1995; Bunney and Davis, 1965) and/or dopamine (Kapur and Mann, 1992). Monoamine oxidase is an enzyme that degrades all the three neurotransmitters and terminates their action and monoamine oxidase inhibitors (MAO) and monoamine reuptake blockers by enhancing the availability of monoamines possess antidepressant properties (To et al., 2005).

Monoamine theory of depression is supported by various facts such as

- MHPG (3-methoxy-4-hydroxy-phenylglycol), a major metabolite of norepinephrine is known to be decreased in the blood, urine and cerebrospinal fluid samples of the patients suffering from depression (Muscettola et al., 1984).
- Reserpine, an alkaloid obtained from Rauwolfia serpentina is known to deplete neurotransmitters in the brain and produces severe mental depression in humans; particularly in hypertensive patients who are treated with relatively larger doses (Musselman et al., 1998; Nutt, 2006b). Similarly, animals when injected with reserpine displayed increased immobility period in both forced swim (Kulkarni and Mehta, 1985; Aley and Kulkarni, 1989) and tail-suspension (O’Leary et al., 2007a) tests. Reserpine depletes biogenic amines by degranulating the catecholamines (norepinephrine and dopamine) and serotonin storage vesicles within the neurons (Baumeister et al., 2003).
- Drugs, which increase the brain levels of norepinephrine, serotonin and/or dopamine, are known to have antidepressant action (Demitrack, 2002). Most of the currently available antidepressants like imipramine (tricyclic antidepressant), fluoxetine (selective serotonin reuptake inhibitor), venlafaxine (dual reuptake inhibitor of serotonin and norepinephrine) follow monoaminergic theory of depression (Arroll et al., 2005).
- Treatment with AMPT (α-methyl-para-tyrosine), a tyrosine hydroxylase inhibitor or PCPA (para-chlorphenylalanine), a tryptophan hydroxylase inhibitor is known to produce depression like symptoms (Fig. 6)
(Kulkarni et al., 1973; O'Leary et al., 2007; Machado et al., 2008). Both tyrosine hydroxylase and tryptophan hydroxylase are the rate limiting enzymes for the biosynthesis of norepinephrine and serotonin, respectively.

- A recent study has demonstrated that there are increased levels of monoamine oxidase-A (MAO-A) isoenzyme in depressed patients. Increased MAO-A activity may lead to increased degradation of norepinephrine and serotonin, thus, producing depression in patients (Meyer et al., 2006).

**Norepinephrine in depression**

The noradrenergic system is intimately involved in the intervention of depression response. The main noradrenergic cell body region in the brain is Locus Ceruleus (LC), which gives rise to diverse projections to a variety of brain structures (Berridge and Waterhouse, 2003). This region receives inputs from numerous other neurotransmitter systems including serotonin, opioidergic, GABA, corticotrophin releasing factor, dopamine and glutamate (Anand and Charney, 2000) and is sensitive to both external environmental stimuli and internal changes in homeostasis.

The norepinephrine released following activation of its neurons mediates the effects through interaction with both α- and β-adrenoceptors present presynaptically as well as postsynaptically (Ressler and Nemeroff, 2000). Further, norepinephrine released at central and peripheral synapses are inactivated through active transport into terminals by the presynaptically localized norepinephrine transporter (NET) (Iversen, 1961). The human NET gene is a single-copy gene (SLC6A2) located on chromosome 16 containing 16 exons (Hahn and Blakely, 2002a) and is a member of the SLC6A family of Na⁺/Cl⁻-dependent transporters with a predicted protein topology of 12 transmembrane domains with intracellularly localized NH₂ and COOH termini (Pacholczyk et al., 1991; Hahn and Blakely, 2002b).
Fig. 6. Metabolic pathways of dopamine, norepinephrine and serotonin. AMPT: alpha-methyl-p-tyrosine; PCPA: p-chloro-phenylalanine

MHPG (3-methoxy-4-hydroxy-phenylglycol), a major metabolite of norepinephrine, is known to be decreased in the blood, urine, cerebrospinal fluid samples of the patients suffering from unipolar depression (Traskman et al., 1981) and also bipolar disorders (Mus et al., 1984). Further investigations have indicated that this is not a general finding for a subgroup of bipolar patients and not typical for unipolar depression (Harro and Oreland, 2001; Sher et al., 2006).

It is known that norepinephrine transporter binding site is decreased in the brains of patients with major depression (Klimek, 1997). It is well documented that selective norepinephrine transport inhibitors such as maprotiline, desipramine and raboxetine are effective antidepressants (Peng et al., 2007; Rygula et al., 2008). Related to the relative norepinephrine deficiency and the symptoms of depression was suggested on the basis that the antidepressant efficacy...
above-mentioned agents was related to their ability to increase norepinephrine levels (Schildkraut et al., 1965).

However, contrary to this monoamine theory of depression, it has been debated that depressed patients have hyper-responsive noradrenergic system and there is higher rather than lower noradrenergic tone in depressed patients (Wong et al., 2000). Wong et al., have demonstrated a pronounced increase in the levels of noradrenaline in cerebrospinal fluid of patients suffering from major depression with melancholic syndrome, which was present around the clock for the entire 30-hr sampling period in patients (Wong et al., 2000). It was a notable observation that the patients were free of any medication for at least 2 weeks. Some of these contrary statements had forced researchers to think the alternative mechanism of action of various noradrenergic reuptake inhibitors in depression.

To this alternative, it has been documented that there is an increase in \(\alpha_2\) adrenoceptor agonist binding in depression and an increased agonist-induced platelet aggregation (Gurguis et al., 1999). \(\alpha_2\)-adrenergic receptors are mainly located presynaptically and check the release of norepinephrine into the synapse. This fact is further supported by the studies carried out in our laboratory (Parale and Kulkarni, 1986). It was shown that clonidine, BHT 920 and guanfacine, all \(\alpha_2\) adrenergic receptor agonists by blocking the release of norepinephrine were able to significantly prolong the total immobility duration in mouse forced swim test, thus producing depression like state, while yohimbine an \(\alpha_2\) receptor antagonist was able to reverse the symptoms of depression (Parale and Kulkarni, 1986). This is further evident from some of the clinical studies. It has been demonstrated that higher densities of \(\alpha_2\) adrenergic receptors have been found in the brains of depressed patients who committed suicides (De Paermentier et al., 1997; Garcia-Sevilla et al., 1999). There is up-regulation of \(\alpha_2\) adrenergic receptors by social stress in tree shrews (Flugge et al., 1997), and by isolation stress in rats (Fulford et al., 1994). Increase in the number of presynaptic \(\alpha_2\) adrenergic receptors density
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could lead to reduced release of noradrenaline or serotonin. Further, clonidine, a $\alpha_2$ adrenergic receptor agonist and one of the centrally acting anti-hypertensive agents is known to reduce blood pressure by decreasing the release of noradrenaline. Some interpretations of radioligand binding studies have demonstrated that $\alpha_2$ adrenergic receptor numbers are not primarily increased but the receptors are in a higher affinity state in the depressed suicide victims (Kaneko et al., 1992). Long-term antidepressants treatment can decrease radioligand binding at $\alpha_2$ adrenergic receptors (Kaneko et al., 1992).

It is generally acknowledged that $\beta$-adrenoceptors are up-regulated in the brains of suicide victims, strongly indicative of an altered noradrenergic transmission in depression (Biegon and Israeli, 1988). There is a large body of literature suggesting that $\beta$-adrenoceptor antagonists may produce depression (Waal, 1967). Postsynaptic $\beta$-adrenoceptor down-regulation is a well-documented phenomenon that occurs during long-term administration of some antidepressants, particularly those affecting norepinephrine (Harro and Oreland, 2001). Binding studies have revealed that chronic but not acute treatment with antidepressant down-regulates $\beta$-adrenoceptors in the rat forebrain (Harro and Oreland, 2001). This change in $\beta$-adrenoceptor density appears to be a homeostatic response to the action of antidepressants (Harro and Oreland, 2001). Thus, it is likely that some antidepressants increase level of endogenous norepinephrine leading to prolonged stimulation of the $\beta$-adrenoceptors. This may induce adaptation of intracellular signal transduction pathways and changes in the expression, phosphorylation, and/or subcellular distribution of $\beta$-receptors, which manifest as the observed reduction in receptor number.

These careful studies emphasize the need to re-evaluate some of the dogmas in the neurobiology of depression. Summary of the role of norepinephrine in depression has been depicted in Fig. 7.
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Up-regulation of \( \beta \) adrenergic receptors in depression and down-regulation during chronic antidepressant treatment

\( \beta \) adrenergic receptor antagonists produce depression-like effect. Chronic antidepressant therapy down regulates \( \beta \)-adrenoceptors and that may be responsible for 2-3 weeks lag period observed for the clinical response of these drugs.

Norepinephrine levels decreases/increases or no change in the brain in depression

Norepinephrine reuptake inhibitors are known to possess antidepressant properties. Most of the first generation antidepressants acted by this mechanism.

Decreased excretion of MHPG, a noradrenaline metabolite in urine

This observation is a supportive evidence for monoaminergic theory of depression.

Increase adrenergic \( \beta_2 \) binding in depression

\( \beta_2 \) adrenergic antagonists have a dual action and the depression-like effect of antidepressants started by this mechanism.

Serotonin in depression

Serotonin is an imperative neurotransmitter that mediates inhibitory and excitatory neurotransmission throughout the central nervous system (Ressler and Nemeroff, 2000). As discussed, serotonergic cell bodies are usually concentrated in the dorsal raphe nuclei and extend to a variety of brain areas, including the hypothalamus, amygdala, cortex, hippocampus, basal ganglia, and the brain stem, the areas believed to be associated with the symptoms of depression. To be more particular, the hypothalamus is known to be involved in appetite disturbance, the cerebral cortex and hippocampus are associated with cognitive dysfunction, and the brain stem with sleep disturbance respectively (Nemeroff, 2002).

Similar to norepinephrine, serotonin is also taken from the synapse into the neurons with the help of respective transporters. The serotonin transporter is a 630-amino acid long receptor with 12 transmembrane domains (Lesch et al., 1993a; 1993b).
The serotonin transporter gene is localized on chromosome 17. In the human brain, the density of serotonin transporter varies by region: superior and inferior raphe nuclei > hypothalamus > thalamus = amygdala > putamen > caudate = hippocampus insular cortex > prefrontal cortex > white matter > cerebellar cortex (Laruelle et al., 1988; Cortes et al., 1988; Kish et al., 2005).

Dysfunction in the serotonergic system is a well-established theory explaining the pathophysiology of depression. There are evidences that indicate a relative deficiency of serotonin in most or all forms of the depression (Ressler and Nemeroff, 2000). A study of 68 patients with depression found that the cerebrospinal fluid concentration of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) varied among different people (Asberg et al., 1976). One group had a concentration of 5-HIAA in the cerebrospinal fluid similar to that measured in healthy patients, but approximately 40% had cerebrospinal fluid levels of 5-HIAA that were greatly reduced in comparison with healthy volunteers (Asberg et al., 1976). This reduction in the levels of 5-HIAA was related to the degree of severity of depression. This finding has been confirmed in later studies and the studies suggested that reduced availability of serotonin, indicated by decreased 5-HIAA levels in the CSF, was related to symptoms of depression (Risch and Nemeroff, 1992). Even, there were significantly lower 5-HIAA levels in the cerebrospinal fluid of depressed patients having committed suicides as compared to control population (Traskman et al., 1981).

Following its release from nerve endings, serotonin is rapidly transported by a reuptake mechanism into the presynaptic nerve terminals with the help of serotonin transporters. Inhibitors of the serotonin reuptake system, which includes imipramine and the selective serotonin transporter inhibitors like fluoxetine, fluvoxamine, citalopram results in antidepressant action. It has been reported that the numbers of serotonin transporter sites are known to be significantly reduced in occipital cortex and hippocampus of the depressed patients as evidenced by [3H]-imipramine binding (Perry et al., 1981).
et al., 1983). This may be the homeostatic mechanism of the body against decreased monoamine synthesis. In another study, binding of \(^{3}\text{H}\)-imipramine was significantly reduced by 44\% in frontal cortex obtained from suicide victims as compared with the control group (Stanley et al., 1982).

The platelets provided a useful source of tissue for the study of the serotonergic system. Platelets share a common embryological origin with serotonergic neurons and although, not capable of synthesizing serotonin, but have many characteristics identical to serotonergic neurons, including serotonin transport, 5-HT\(_2\) serotonin receptors and \(^{3}\text{H}\)-imipramine binding sites (Da Prada et al., 1988; Lesch et al., 1993a; 1993b). It has been observed that the serotonin transporters are reduced by 54\% in the platelets of depressed patients (Briley et al., 1980).

The effect of serotonin are mediated through serotonin receptors, of which at least 13 molecular subtypes have been identified at present, including three major receptor families such as 5HT\(_{1A}\), 5HT\(_{2AC}\), and 5HT\(_3\), respectively (Hoyer and Martin, 1997). These receptors are present both at pre- and postsynaptic sites, in addition to their location on serotonergic nerve-cell bodies.

The major evidence supporting a role for serotonergic circuit dysfunction in the development of major depression is the observation that all of the selective serotonin reuptake inhibitors like fluoxetine, fluvoxamine, citalopram are effective antidepressants (Owens and Nemeroff, 1994; Blier and De Montigny, 1994). However, like all antidepressants, SSRIs therapeutic activity is not apparent until 2-3 weeks after commencing treatment (Stahl, 1998). This has led to the hypothesis that reuptake inhibition is a necessary first step that results in long term adaptive changes and eventually leads to persistent enhancement of serotonergic neurotransmission (Blier and De Montigny, 1994). The adaptive changes that occur in antidepressant therapy have been suggested to involve progressive desensitization of somatodendritic 5HT\(_{1A}\) and 5HT\(_{1D}\) serotonin autoreceptors. Following the administration of selective serotonin reuptake inhibitors, there is an initial rise in
extracellular serotonin concentration which further results in an attenuation of neuronal firing through activation of somatodendritic 5HT1A serotonin autoreceptors (Nemeroff, 2002). Therapeutic efficacy is observed when these autoreceptors get desensitized following chronic antidepressant treatment (Nemeroff, 2002). These events result in a gradual enhancement of serotonergic neurotransmission following initiation of SSRI therapy. It has been demonstrated that, pindolol, a 5-HT1A autoreceptor antagonist when administered in combination with selective serotonin reuptake inhibitors, may enhance and/or accelerate the therapeutic efficacy of latter (Geretsegger et al., 2008). The study was carried out in fifty patients, meeting ICD-10 criteria for major depressive disorder or bipolar depression.

Many effects of serotonin such as regulation of mood, anxiety, and body temperature and control of sexual functions are thought to be mediated by an interaction with 5-HT2 serotonin receptors (Stahl, 1998). An important property of 5-HT2 serotonin receptors is that 5-HT2 receptor density has an inverse relation to extracellular serotonin levels, such that the density of 5-HT2 receptors in the cortex increases after chronic serotonin depletion and decreases after chronically raising extracellular serotonin. In patients with depression, an increased density of post-synaptic 5-HT2 serotonin receptors has been reported in both frontal cortex and platelets. It is therefore possible that these changes reflect an up-regulation of 5-HT2 serotonin receptors as an adaptative response to reduction in synaptic serotonin concentration. This up-regulation of 5HT2 serotonin receptors can be reversed by the treatment with chronic antidepressants (Owens and Nemeroff, 1994).

Recently, the involvement of 5-HT3 serotonin receptors has come into picture and ondansetron, a 5-HT3 serotonin receptor antagonist is known to have antidepressant action in various behavioral models of despair (Ramamoorthy et al., 2008). Chronic treatment with ondansetron is known to augment the antidepressant effects of fluoxetine or venlafaxine (Ramamoorthy et al., 2008). Summary of the role of serotonin in depression has been depicted in Fig. 8.
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5-HT transporter site is significantly reduced and 5-HT2 serotonin receptor density is increased in depression. This indicates a reduction in the number of serotonergic terminals and/or number of terminals expressing the reuptake site. Up-regulation of 5-HT2 serotonin receptors is the adaptation response of the neurons to reduction in synaptic serotonin concentration.

Serotonin levels decreases in brain as well as platelets of depressed patients. Selective serotonin reuptake inhibitors are effective antidepressants.

Decreased excretion of 5-HIAA, a serotonin metabolite. 5-HIAA concentrations in the cerebrospinal fluid are related to the degree of severity of depression.

Role of 5-HT1A or 5-HT2 serotonin autoreceptors antidepressant action. 5-HT1A and 5-HT2 serotonin autoreceptors are located presynaptically and the main action is to check the release of serotonin. Therefore, a latency of 2-3 weeks is generally observed after SSRI therapy. Desensitization of these receptors is required before the antidepressant response comes.

Fig. 8. Summary of role of serotonin in depression. 5-HIAA: 5-hydroxyindole acetic acid.

Dopamine in depression

Central dopaminergic system is crucial for a broad spectrum of behaviors including reward-seeking, motivation, emotions and environment responsivity (Swerdlow and Koob, 1987). Disruption of these behaviors may lead to anhedonia, social isolation, and psychomotor retardation and other behavioral symptoms that are the core psychopathology of depression (Klimek et al., 2002). The dopamine hypothesis of depression is relatively new and has emerged third among the monoamine hypotheses. The role of dopamine was overlooked even though dopamine depletors like reserpine and α-methyldopa altered human as well as animal behavior. Bupropion, a dopamine reuptake inhibitor has been now clinically used as an antidepressant (Cooper et al., 1980).

The dopaminergic system in the brain arises from groups of the midbrain and the hypothalamus. Dopaminergic neurons are majorly organized in three main pathways: mesolimbic-mesocortical pathway linked midbrain dopamine cell...
with limbic and cortical regions; the nigrostriatal pathway, which connects substantia nigra to striatum (caudate and putamen); and the tubero-infundibular pathway, which comprises an intrinsic hypothalamic pathway that modulates the anterior pituitary gland (Dailly et al., 2004) (Fig. 9). Recently, it has been demonstrated that lesions of dopaminergic neurons in the substantia nigra pars compacta and in the ventral tegmental area enhance depression-like behavior in rats (Winter et al., 2007). Evidence from the studies of neuroanatomical substrates of animal behavior suggests that mesolimbic dopaminergic system may be involved in the pathophysiology of affective disorders such as mental depression (Willner, 1991). In addition, there is high degree of co-morbidity between depression and Parkinson’s disease, a disorder well documented to be attributable to the loss of dopaminergic neurons in the nigrostriatal pathway (Brown and Gershon, 1993). Dopaminergic neurons, therefore, innervate brain areas associated with behavioral and physiological functions that are altered in depression (e.g. the cortex, limbic structures, and the pituitary gland). These brain areas are involved in cognition and modulation of behaviors linked with motivation and reward (Creese, 1985).

Data obtained from brain-imaging studies, postmortem tissue studies, and analyses of dopamine and its metabolite, homovanillic acid in biological fluids, have indicated that the alteration of dopaminergic systems may be involved in the pathophysiology of depression. Levels of homovanillic acid are reduced in patients with depression, suggesting that a decrease in dopamine turnover is associated with the pathophysiology of depression (Lambert et al., 2000). Recently, it has been found that the homovanillic acid/5-hydroxyindoleacetic acid (HVA/5-HIAA) ratios are reduced in cerebrospinal fluid of depressed suicides (Jokinen et al., 2007). It is being suggested that that the HVA/5-HIAA ratio may be a biomarker of suicide intent (Jokinen et al., 2007).
**Fig. 9.** Dopaminergic pathways in brain

L-deprenyl (selegiline) is propargylamine derivative that forms irreversible covalent bond with the monoamine oxidase isoenzyme (MAO-B). MAO-B is the predominant form of the enzyme in the human brain and dopamine is the preferred substrate for it whereas norepinephrine and serotonin are the preferred substrates for monoamine oxidase A (MAO-A). The selectivity of the drug for the MAO-B subtype is dose dependent and at higher dose, it becomes non-selective and inhibits MAO-A. The overall effect of the drug is to cause a slight significant increase in the level of dopamine content in the nigrostriatal system and an enhanced sensitivity of the dopaminergic neuronal physiological and pharmacological influences (Knoll, 1983). L-deprenyl is known to have antidepressant property at a dose of 15 mg/day or (dose selective for MAO-B inhibition). Others have observed antidepressant action only at non-selective doses (Knoll, 1983). How on the contrary, Mann et al (1989) found no antidepressant effect with L-deprenyl after 3 weeks of its treatment (Mann et al., 1989).
Tyrosine, an amino acid, is converted into dihydroxyphenylalanine (DOPA), which in turn is converted to dopamine by L-aromatic acid decarboxylase (Fig. 6.). In noradrenergic neurons, the enzyme dopamine-β-hydroxylase converts dopamine to norepinephrine (Fig. 6). Under basal conditions, the exogenous administration of tyrosine leads to a rather specific enhancement of norepinephrine without much effect on the dopaminergic transmission (Gelenberg et al., 1982). However, under conditions of dopamine deficiency, tyrosine causes an enhancement of dopaminergic transmission and has been tried as an antidepressant in clinical studies (Gelenberg et al., 1982). These workers have reported the useful effects of oral administration of L-tyrosine in mood disorders (Gelenberg et al., 1982). Similarly, several studies have reported the effect of administration of L-DOPA in conjunction with carbidopa (a peripheral dopa decarboxylase inhibitor) in mood disorders (Henry et al., 1976). Although it is evident that L-DOPA has definite effects on mood, however, its antidepressant efficacy was found to be unimpressive (Henry et al., 1976).

Effects of dopamine in the central nervous system were initially considered to mediate via two receptor subtypes D₁ and D₂. The D₁ dopamine receptors activated the adenylate cyclase system and the D₂ dopamine receptors inhibited it (Missale et al., 1998). Now, the other subtypes D₃, D₄ and D₅ dopamine receptors have also been cloned. Initially, only D₂ dopamine receptors had identifiable effects in the central nervous system, based on the action of D₂ receptor antagonists, which are effective as antipsychotics and caused extrapyramidal side effects. However, recent behavioral studies using selective agents have shown that D₁, D₃, D₄ and D₅ dopamine receptors also play an important role in the expression of effects of dopaminergic system. Various dopamine agonists show therapeutic efficacy in depression. Electroconvulsive therapy, an effective antidepressant therapy used mostly in case of resistant depression is also known to enhance dopamine function (Verma and Kulkarni, 1991; Andrade et al., 2002).
Dopamine transporter (DAT) belongs to a large family of Na\(^+\) and Cl\(^-\) dependent transporters similar to norepinephrine and serotonin transporters (NET and SERT, respectively), but located on short arm of chromosome 5 (Storch et al., 2004). In mammalian brain, DAT mRNA is localized in cell bodies and is restricted to dopamine neurons (Augood et al., 1992; Amara and Kuhar, 1993; Lorang et al., 1994; Nirenberg et al., 1996). DAT mRNA has been detected in the substantia nigra (SN), the midbrain region and the brain stem with the highest expression levels in the substantia nigra pars compacta and pars lateralis and ventral tegmental area (VTA) (Usdin et al., 1991). The major physiological role of dopamine transporter is the termination of neurotransmission by rapid reuptake of dopamine from the synaptic cleft into presynaptic terminals, and it is believed to control the intensity and duration of dopaminergic neurotransmission by setting the concentration of dopamine in the extracellular space.

Various studies have shown the involvement of dopamine in depression. Dopamine reuptake is inhibited by some antidepressants, such as bupropion and nomifensine (Yamada et al., 2004), dopamine metabolism is altered during administration of MAO-B inhibitors (Kapur and Mann, 1992), and dopamine agonists such as pramipexole and ropinirole (Lemke, 2007; Rogoz and Skuza, 2006) have antidepressant properties. In one of the studies, oxocarbazepine, a keto-analogue of carbamazepine (an anti-epileptic drug) is known to possess antidepressant-like effect in animal models of despair possibly by modulating the dopaminergic neurotransmission (Joca et al., 2000). Amphetamine, a dopamine agonist is known to promote the release of norepinephrine and dopamine from the nerve terminals and inhibits their reuptake. Because the euphoriant effect of amphetamine is blocked by pimozide, a dopamine antagonist, and not by alpha-adrenergic or beta-adrenergic blocking agents, the behavioral effect of amphetamine appears to be mediated by the dopaminergic system (Kulkarni and Dandiya, 1972;
Dandiya and Kulkarni, 1974; Randrup and Munkwad, 1975). It has been demonstrated that amphetamine-induced stereotypic behavior was potentiated by concomitant administration of imipramine (Kulkarni and Dandiya, 1972). Bromocriptine, an ergot alkaloid derivative, has significant dopamine agonistic properties and found to have antidepressant activity similar to tricyclic antidepressants (Muscat et al., 1992). Interestingly, bromocriptine produced an earlier response as compared with standard tricyclics and was associated with higher incidence of psychomotor activation and precipitation of mania (Theohar et al., 1982). Similarly, pirebedil, another dopamine receptor agonist with little effect on noradrenergic and serotonergic system stimulates postsynaptic dopamine receptors at higher doses and exert an antidepressant action (Brocco et al., 2006). Bupropion has been reported to be a weak inhibitor of norepinephrine reuptake (Cooper et al., 1980). However, bupropion differs from tricyclic antidepressants drugs in that it is approximately 2-fold more potent as an inhibitor of dopamine reuptake (Cooper et al., 1980).

Interestingly, in one of the studies carried out using postmortem brain tissues from psychiatrically depressed subjects, it was found that a lower density of radioligand binding to the dopamine transporter was observed in the basal and central nuclei of the amygdala from subjects diagnosed with major depression compared with normal control subjects (Klimek et al., 2002). These findings are highly suggestive of neurochemical abnormalities in the limbic dopamine system in major depression (Klimek et al., 2002).

Another important area to be discussed is that of dopamine-serotonin interaction. Numerous studies investigated the role of different serotonin receptors in the control of brain dopamine transmission. Serotonergic stimulation of the prefrontal cortex (Chen et al., 1992), the striatum (Parsons et al., 1996), or the nucleus accumbens (Parsons and Justice, 1993) potently releases dopamine. The increase of dopamine release in the prefrontal and frontal cortex by 5-HT₁A serotonin agonists
has also been demonstrated (Lejune and Millan, 1998). Lejeune demonstrated that the selective activation of 5HT₁A postsynaptic also elicits an increase in ventral tegmental area dopamine (Lejune and Millan, 1998). The role of dopamine in depression illustrated in Fig 10.

However, still there is less evidence to support a preemin dopaminergic system in depression. Recently, it has been ropinirole when combined with other standard antidepressants i suffering treatment-resistant depression produce symptoms c hallucination (Pae et al., 2008), therefore, this area needs investigations. It is therefore reasonable to assume that an ant monoamine reuptake inhibitor, may offer additional benefit in the of depression over a single-site agent. Nevertheless, if monoaminergic systems contribute to the neurobiology of deeph the primary defect underlying the symptoms is unclear or multif simultaneous targeting of all the three systems may be advanta

![Diagram of Dopamine in Depression]

**Fig. 10.** Summary of role of dopamine in depression
Limitations of monoaminergic theory of depression

- The lag period with antidepressant drug therapy (a delay of few weeks) before the onset of a clinically measurable effect
- Monoaminergic hypothesis does not account for several issues such as why antidepressants are effective in anxiety disorders such as phobia?
- Some of the studies have not found any reduction in the brain concentrations of noradrenaline or its metabolites in depression.
- Tianeptine, which acts by increasing serotonin reuptake, calls for further exploration in the exact mechanism of action of antidepressants.

Dysregulation of Hypothalamic-pituitary-adrenal (HPA) axis

The concept of stress and the role of hypothalamic-pituitary-adrenal axis were previously given by Hans Selye in 1950s (Selye, 1956). A prominent mechanism by which the brain reacts to acute and chronic stress is activation of hypothalamic-pituitary-adrenal (HPA) axis (Hindmarch, 2002; Nestler et al., 2002). Although stress and depression are relatively different terminologies, it is debated that both are related to each other (Krackow and Rudolph, 2008). Neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotrophin releasing factor (CRF), which stimulates the synthesis and release of adrenocorticotropin (ACTH) from the anterior pituitary gland. ACTH then stimulates the synthesis and release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal cortex (Nestler et al., 2002) (Fig. 3.). Abnormal, excessive activation of the HPA axis is observed in approximately half of individuals with depression, and these abnormalities are corrected by antidepressant treatment (Sachar and Baron, 1979; De Kloet et al., 1988; Holsboer, 2001).

Glucocorticoids released can profoundly affect on the general metabolism and also dramatically affect the behavior via direct actions on numerous brain regions (Dranovsky and Hen, 2006). Glucocorticoids, by regulating hippocampal and paraventricular neurons, exert powerful
feedback effects on the HPA axis. Sustained elevations of glucocorticoids seen under conditions of prolonged and severe stress may damage hippocampal neurons particularly CA3 pyramidal neurons (Sapolsky, 2000). Current hypothesis focuses on the increased levels of cortisol as a culprit in damaging hippocampal neurons (Fuchs and Gould, 2000) but the exact mechanism is yet to be explored.

Similar to this, hypothalamic-pituitary-thyroid axis is also disturbed in depressed patients. Hypothyroidism and depression are known to share many overlapping clinical symptoms such as fatigue, apathy, depressed mood and cognitive impairment (Hindmarch, 2002). Indeed, some authors recommend the treatment of all depressed patients with thyroxine therapy (Jackson, 1998).

**Neurotrophic theory of mental depression**
Neurotrophic factor is the recently proposed theory for explaining the pathophysiology of mental depression (Duman et al., 1997; Grassi-Oliveira et al., 2008; Aso et al., 2008). Neurotrophic factors regulate neural growth and neuronal differentiation during the development (Kang et al., 2002). This theory states that a deficiency of neurotrophic support may contribute to hippocampal pathology during the development of depression (Smith et al., 1995) and the reversal of this deficiency by antidepressants may contribute to the resolution of depressive symptoms (Koponen et al., 2005; Rantamaki et al., 2007). Brain derived neurotrophic factor (BDNF) is one of the most prevalent neurotrophic factors in the brain (Aso et al., 2008). BDNF is known to play an important role in mediating long-term neural and behavioral plasticity in response to aversive social experiences (Berton et al., 2006). Acute and chronic stress decreases levels of BDNF expression in the dentate gyrus and pyramidal cell layer of hippocampus in rodents (Fig. 11). This reduction appears to be mediated partly via stress-induced glucocorticoids and partly via other mechanisms (Fig. 11). All the antidepressants including tranylcypromine, sertraline, desipramine, or mianserin, when administered chronically are known to increase the BDNF
expression in the brain (Nibuya et al., 1995). Furthermore, active TrkB (the receptors for BDNF) may contribute to the antidepressant (Rantamaki et al., 2007).

![Diagram](image)

Fig. 11. Neurotrophic mechanisms in depression (Adapted from Nibuya et al., 2002)

(A) Normal hippocampal pyramidal neuron and its innervating glutamatergic, monoaminergic, and other neurons. Its regulation by BDNF (derived from hippocampus or other brain areas) is also shown. (B) Glucocorticoids stress causes several changes in these neurons, including a reduction in their dendritic arborizations, and a reduction in BDNF expression. This reduction in BDNF is mediated partly by excessive glucocorticoids, which could interfere with the normal transcriptional mechanism. Antidepressants produce the opposite effects: they increase dendritic arborizations and BDNF expression of these hippocampal neurons.

In contrast, chronic administration of other psychotropic agents including morphine, cocaine, or haloperidol, did not increase levels of BDNF mRNA (Nibuya et al., 1995). Together these supportive evidence, it can be argued that antidepressant-induced up-regulation of BDNF could help repair some stress-induced damage to hippocampal neurons and protect other vulnerable neurons from further damage. These
could also explain why an antidepressant response has a lag period of 2-3 weeks? It can be hypothesized that there requires a sufficient time for levels of BDNF to gradually rise and exert their neurotrophic effects.

**GABAergic theory of depression**

γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter (Roberts, 1986) and approximately 10-40 % of the nerve terminals in cerebral cortex, substantia nigra and hippocampus are GABAergic in nature (Hendry *et al.*, 1987). GABA is known to modulate an array of behavioral and physiological mechanisms including sleep, feeding behavior, aggression, sexual behavior, pain, cardiovascular regulations, thermoregulation, locomotor activity and mood (Paredes and Agmo, 1992). Because of its ubiquitous nature in the central nervous system, its function is intimately linked to other neurotransmitter systems. In neurochemical terms, GABA is considered to be an inhibitory neurotransmitter, based on decrease neuronal activity observed when GABA is applied to biogenic amine cell bodies in the brain stem. However, in prefrontal cortex and hippocampus, GABA actually facilitates noradrenergic release through a presynaptic GABA<sub>A</sub> mechanism (Sujdak and Gianutsos, 1985). Owing to its close ties with other neurotransmitters and its role as the major inhibitory neurotransmitter, GABA is well positioned to be associated with mood disorders (Sujdak and Gianutsos, 1985).

Action of GABA is mediated through three receptors *viz.* GABA<sub>A</sub>, GABA<sub>B</sub> and GABA<sub>C</sub> located on various neurons (Macdonald and Olsen, 1994; Johnston, 1996; Czuczwar, 1999; Schechter *et al.*, 2005). Activation of GABA<sub>A</sub> or GABA<sub>C</sub> receptors leads to an increase in Cl<sup>-</sup> conductance (Rabow *et al.*, 1995) whereas GABA<sub>B</sub> receptor agonists produce a G-protein coupled activation of inward K<sup>+</sup> currents (Kaupmann *et al.*, 1997). GABAergic drugs are mainly useful in the treatment of epilepsy, anxiety and sleep disorders (Nutt, 2006a). These disorders are often associated
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with mood disturbances and drug therapy with GABAergic drugs may be useful in treating depression secondary to these disorders.

It has been demonstrated that patients suffering from depression often have decreased levels of GABA in their cerebrospinal fluid (Gold et al., 1980; Petty, 1995). Comparing with low GABA levels in depression, GABA_A receptors are reported to be up-regulated in the brains of depressed suicide cases (Cheetham et al., 1988). One of the studies from our laboratory has demonstrated the depressogenic effect of FG-7142, a beta-carboline GABA-benzodiazepine receptors inverse agonist in various behavioral paradigms of despair, the action being reversed by the treatment with desipramine (Chopra et al., 1988). Further, it has been claimed that activities of glutamate decarboxylase and GABA aminotransferase (enzymes that synthesize and metabolically degrade GABA) is lower in the blood of certain depressed patients (Kaiya et al., 1982; Berrettini et al., 1982; Sanacora and Saricicek, 2007). These observations indicate that low plasma levels of GABA are a potential marker for clinical depression. Progabide and fengabide, GABA-mimetic agents have demonstrated to be effective in alleviating the symptoms of depression in certain patients (Lloyd et al., 1989). Similarly, acute treatment with high doses of desipramine (50 mg/kg) or pargyline (100 mg/kg) and other monoamine oxidase inhibitors have been reported to alleviate decreased brain GABA concentrations (Patel et al., 1975). However, lower doses of these compounds have no effect on GABA levels (Patel et al., 1975). On meta-analysis, it has been found that alprazolam, a benzodiazepine is as effective antidepressant as tricyclic antidepressants in the acute phase treatment of outpatients suffering from major depressive syndrome (Weissman et al., 1992). Simultaneous treatment of desipramine and alprazolam lead to a greater treatment response as compared to the effect per se in the patients suffering from major depressive disorders (Kravitz et al., 1990). Alprazolam seems to decrease core symptoms of depression such as anhedonia and hopelessness, as
well as improving psychic anxiety and sleep related disturbances. Advantage of alprazolam as an antidepressant is that it had a more rapid onset of therapeutic action than tricyclics in several studies, with clinically significant response usually apparent during the first two weeks of treatment (Weismann et al., 1992). Further, the side effects of alprazolam were favorable compared to tricyclics in many studies. In one of the studies, oral fluoxetine administration is reported to increase the concentration of GABA in cerebrospinal fluid by 2-folds in the rat brain (Goren et al., 2007).

Animal studies have also established a role of GABAergic neurotransmission in the pathophysiology of depression. Administration of GABA directly to the hippocampal area protected the rats from developing learned helplessness behavioral paradigms of despair (Tunnicliff and Malatynska, 2003), the effect being reversed by bicuculline or picrotoxin (Sherman and Petty, 1982; Poncelet et al., 1987). Muscimol, a GABA receptor agonist is known to decrease immobility period in mouse forced swim test, action being reversed by prior administration of bicuculline (Tunnicliff and Malatynska, 2003).

Neurosteroids are steroid structure hormones that are synthesized in the central nervous system either de novo from cholesterol or from steroid hormone precursors (Fig. 12) and are involved in a wide variety of psychopathological processes (Reddy and Kulkarni, 2000). Several neurosteroids have been shown to be formed de novo in mammalian brain via classical steroid metabolic pathways (Fig. 12) (Corpechot et al., 1983; Baulieu and Robel, 1990). Cytochrome P450 catalyzed side-chain cleavage of cholesterol to pregnenolone has been demonstrated in mitochondria of glial cultures of oligodendrocyte-rich embryonic rat brain (Hu et al., 1989). Incubation of primary cultures of rat forebrain glial cultures with a precursor to cholesterol led to the formation of cholesterol itself, pregnenolone, progesterone and 20-hydroxypregnenolone, respectively (Hu et al., 1989; Jung-Testas et al., 1989).
Biochemical, immunohistochemical and molecular biologies have also revealed the presence of CYP450A enzyme in mammalian brain (Baulieu, 1997). It is mainly found in cerebrum, amygdala, hippocampus and mid brain and is expressed in central and peripheral nervous system of the developing rat and foetus, as well as throughout the adult brain (Warner and Gu, 1995). This suggests that neurosteroids are biosynthesized embryogenesis and development of nervous system.

![Biosynthetic pathway of neurosteroids. DOC; Deoxycorticosterone](Adapted from Reddy and Kulkarni, 2000)

Some of the neurosteroid structures are depicted in Fig. 1. Neurosteroids are known to modulate mainly GABA\(_A\) receptors, calcium channels, NMDA, sigma, and glycine receptors, respectively (Table 4) (Reddy and Kulkarni, 2000).
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Progestrone

Deoxycorticosterone

Alphaxolone

Allopregnanalone

Allotetrahydro-DOC

Pregnenolone sulphate

DHEA - sulphate

Pregnanolone
Non-conjugated metabolites of progesterone such as allopregnenolone are potent positive modulators of GABA\textsubscript{A} receptors. They open ion channels for Cl\textsuperscript{−} and known to possess analgesic, hypnotic,
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anxiolytic and anticonvulsant effects. On the contrary, neurosteroids such as dehydroepiandrosterone and its sulphate are negative modulators of GABA_A receptors (Table 4) acting as excitants and proconvulsants. They are able to modulate positively N-methyl-D-aspartate (NMDA) receptors and open Ca^{2+} ion channels.

### Table 4: Potential neurotransmitter receptor sites for neuro-active steroid modulation of brain functions (Adapted from Reddy and Kulkarni, 2000)

<table>
<thead>
<tr>
<th></th>
<th>GABA-A</th>
<th>NMDA</th>
<th>Glycine</th>
<th>VGCC</th>
<th>Sigma</th>
<th>Nicotinic</th>
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<tr>
<td>Progesterone</td>
<td>PAM</td>
<td>NCA</td>
<td>NCA</td>
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<tr>
<td>Allopregnenolone</td>
<td>PAM</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
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<tr>
<td>Deoxytocosterone</td>
<td>PAM</td>
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<tr>
<td>THDOC</td>
<td>PAM</td>
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<tr>
<td>Pregnenolone</td>
<td>NCA</td>
<td>PAM</td>
<td>NCA</td>
<td>NCA</td>
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<tr>
<td>Pregnenolone sulfate</td>
<td>NCA</td>
<td>PAM</td>
<td>NCA</td>
<td>NCA</td>
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<td>DHEAS</td>
<td>NCA</td>
<td>PAM</td>
<td>NCA</td>
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<tr>
<td>Epipregnanolone</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
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<tr>
<td>Ganaxolone</td>
<td>PAM</td>
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<td>Minaxolone</td>
<td>PAM</td>
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<td>Alphaxolone</td>
<td>PAM</td>
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<tr>
<td>Alphadolone</td>
<td>PAM</td>
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</tbody>
</table>

THDOC: Tetrahydrodeoxytocosterone, DHEAS: Dehydroepiandrosterone sulfate, VGCC: Voltage gated Ca^{2+} channels, PAM: Positive allosteric modulator; NCA: Non-competitive antagonist

Neurosteroids are shown to have antidepressant actions. In one of the studies, allopregnenolone (3α-hydroxy-5α-pregnan-20-one), a neurosteroid was observed to have antidepressant-like activity in mouse forced swim test (Khisti et al., 2000). This action is potentiated by the additional administration of muscimol and blocked by bicuculline suggesting the involvement of GABAergic neurotransmission in its antidepressant-like action (Khisti et al., 2000).

Analysis of the cerebrospinal fluid levels of neuroactive steroids in healthy volunteers suffering from depression revealed that pregnenolone is decreased in subjects with affective illness, particularly during episodes
of severe depression (George et al., 1994). Administration of dehydroepiandrosterone to the patients suffering from Alzheimer’s disease resulted in improvement in mood, energy, confidence, interest and activity levels (Roberts and Fitten, 1990). However, interestingly both pregnenolone and dehydroepiandrosterone are negative modulators of GABA receptors (Reddy and Kulkarni, 2000); but they possessed antidepressant activity. In one of the recent studies, it has been debated that reduced dehydroepiandrosterone is associated with an early onset of depressive-behavior (Malkesman et al., 2007).

The cross-talk between antidepressant-like effects of neurosteroids and sigma-1 receptor modulation is the topic of debate, suggesting a role for central sigma receptors in the antidepressant-like effects of neurosteroids (discussed subsequently). Our previous studies, using moderate doses of dehydroepiandrosterone sulfate or pregnenolone sulfate displayed an antidepressant-like effect in the Porsolt’s forced swim test, which is sensitive to NE-100, a putative sigma-1 receptor antagonist, or progesterone, a neurosteroid sigma receptor antagonist (Reddy et al., 1998). This area needs further attention.

Contrary to the above-mentioned studies, some researchers have found no alterations in GABA levels in patients suffering from major depressive disorders. Even there were no changes in the GABA concentrations in several different regions of forebrain tissues from suicide victims who presumably had suffered from depression (Korpi et al., 1988). Therefore, the area of GABA and depression is open for further exploration and scientific scrutiny.

**Glutamatergic theory of depression**

Glutamate, a major excitatory neurotransmitter in the brain, might be a promising target for a novel antidepressant therapy (Palucha and Pilc, 2005). Glutamate acts by stimulation of two distinct groups of receptors: ionotropic glutamate receptors [including N-methyl-d-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor...
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(AMPA) and kainate], which are coupled to ion channels, and metabotropic glutamate receptors (mGlRs), a family of G-protein coupled receptors (Nakanishi, 1992). The mGlRs are further divided into three groups according to their sequence homology, effector coupling and agonist selectivity. Group I mGlRs (mGl 1 and mGl 5) are coupled to the phosphatidylinositol hydrolysis/Ca²⁺ signal transduction pathway, while Group II (mGlR2 and mGlR3) and group III mGlRs (mGlR4, mGlR6, mGlR7, mGlR8) are both coupled in an inhibitory manner to adenylyl cyclase signal transduction pathway (Conn and Pin, 1997).

In 1990, Trullas and Skolnick provided the first evidence for the involvement of NMDA subtype of glutamate receptors in the pathophysiology of depression. They found that NMDA receptor antagonists viz. 2-aminophosphoheptanoic acid (competitive NMDA receptor antagonist) or MK-801 (non-competitive NMDA receptor antagonist) reduce the immobility period of mice in the forced swim test (Trullas and Skolnick, 1990). Similarly, competitive NMDA receptor antagonists CGP-37849 and CGP-39551 were effective in the forced swim test in rats (Maj et al., 1993). MK-801 has been thoroughly investigated in various animal models of depression and was effective in forced swim test in rats (Maj et al., 1992), tail-suspension test in mice (Panconi et al., 1993), foot-shock-induced fighting behavior in chronically stressed rats (Ossowska et al., 1997), chronic mild stress model of depression (Papp and Moryl, 1996) and the olfactory bulbectomy model of depression (Redmond et al., 1997), respectively.

These findings indicate that substances capable of reducing neurotransmission at NMDA receptors complex might represent a new class of drugs. However, the greater hopes have been hampered by the adverse side effect profile of these drugs. These drugs are known to produce psychomimetic effects and neurodegeneration that further leads to disturbed motor performances. Further, their higher doses may lead to learning and memory impairment, ataxia and muscle relaxation (Danysz et al., 1996). However, the newly discovered NMDA receptor antagonist viz.
memantine has been shown to have promising antidepressant-like effects in preclinical studies and is free of adverse effects typical of high affinity NMDA receptor blockers (Parson et al., 1999). One clinical study has demonstrated the antidepressant effect of ketamine, a NMDA receptor antagonist. Ketamine produced a significant reduction in scores of Hamilton depression rating scale after 40 minutes of its infusion (0.5 mg/kg) to patients not responding to the conventional antidepressant therapy (Berman et al., 2000; Liebrenz et al., 2007). However, on the contrary, it has been shown that ketamine neither produces antidepressant-like effects in rodents nor does it display antidepressant-like behavioral or neurochemical effects after chronic treatment (Popik et al., 2008).

A reduction in NMDA receptor reactivity has also been found after chronic treatment with both classical and atypical antidepressants. In 1999, Skolnick proposed that brain-derived neurotrophic factor (BDNF) may be the mediator linking the action of conventional antidepressants and attenuation of NMDA receptor function (Skolnick, 1999). As discussed previously, it is known that chronic treatment with antidepressants causes an increase in the expression of BDNF mRNA (Nibuya et al., 1995). Further, BDNF was shown to decrease NMDA receptor function, thus producing an effect similar to that produced by NMDA receptor antagonists (Brandoli et al., 1998).

Contrary to this, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor potentiators are known to have antidepressant-like action. It has been found that mice with deletion of the main AMPA receptor subunit GluR-A represent a depression model, and display various behavioral and neurochemical features of human depression (Chourbazi et al., 2008). Also, chronic treatment with LY-451646, AMPA receptor potentiator increases cell proliferation in adult rat hippocampus (Bai et al., 2003). Further, AMPA receptor activation has shown to increase the expression of BDNF (Skolnick, 1999).
Several data indicates that antagonists of group I mGluRs (mGluR1 and mGluR5) produced antidepressant-like effects in behavioral tests in rodents. mGluR5 antagonists MPEP [2-methyl-6- (phenylethynyl)-pyridine] reduced immobility period in tail-suspension test in mice without affecting the locomotor activity (Pile et al., 2002). Moreover, repeated MPEP administration reversed the olfactory bulbectomy-induced behavioral deficits, similarly to the classic antidepressant desipramine (Pile et al., 2002). The mechanism behind the antidepressant-like effect of group I mGluR antagonists may be connected with their ability to reduce NMDA receptor activity, which was observed in several brain areas (Attucci et al., 2001).

Similarly, Group-II mGluR antagonists MGS-0039 and LY-341495 dose-dependently reduced immobility time of mice in TST (Chaki et al., 2004). It has been demonstrated that repeated administration of MGS-0039 increases cell proliferation in the adult mouse hippocampus (Yoshimizu and Chaki, 2004).

Little is known about the potentials of antidepressant activity of group III mGluRs ligands, as they are not systemically active drugs. ACPT-1, a group III mGluR agonists as well as RS-PPG, a mGluR8 agonist produced a dose-dependent decrease in the immobility time of rats in FST (Palucha et al., 2004). However, the exact mechanisms of glutamatergic neurotransmission have to be explored in depression.

**Purinergic theory of depression**

Adenosine, a purine ribonucleoside is a ubiquitous and essential component of every living cell. Adenosine is formed from 5'-adenosine monophosphate by the cytosolic 5'-nucleotidase and is converted back to 5'-AMP by adenosine kinase (which requires ATP as a phosphate donor). Adenosine may also be formed by the action of S-adenosylhomocysteine (SAH) hydrolase (Fig. 14) (Dunwiddie and Masino, 2001).
Adenosine and related nucleosides are reported to have neuromodulatory or neurotransmitter role (Kulkarni and Mehta, 1985). Adenosine, and 2-chloroadenosine treatment is known to prolong the immobilization period in mice, the action being potentiated by treatment with dipyridamole, an adenosine uptake inhibitor, antagonized by caffeine and theophylline, purinoceptor antagonists (Kulkarni and Mehta, 1985). It has been discussed that adenosine and 2-chloroadenosine probably by reducing the norepinephrine outflow their action on presynaptic purinoceptors on noradrenergic neurons causes prolongation of immobility in animals. The results are supported by the fact that adenosine and 5'-adenosine monophosphate when applied to cerebral cortex by microiontophoresis technique, depressed the neuronal firing rates in cerebral cortex (Stone and Fink, 1979). Contrary to this, it has been found that adenosine has an antidepressant-like action in mouse forced swim test and...
that are prerequisite for physiological functions of neurons, for exam-
neurotransmitter release. Indeed, it has been demonstrated that sigm
receptor ligands, including neurosteroids that have affinities for sigm
receptors, modulate NMDA-induced dopamine release via calc
sensitive protein kinase C (Nuwayhid and Werling, 2003).

Fig. 17. Molecular function of the sigma-1 receptor: At the resting s
most sigma-1 receptors resides at globules of endoplasmic reticulum (I
Sigma-1 receptors at the endoplasmic reticulum thus regulate calc
efflux from the endoplasmic reticulum by associating with IP3 recepl
After stimulation by ligands, sigma-1 receptors translocate to pla:
membrane, thus regulating ion channels located at the membrane (K
NMDA channels). Therefore, sigma-1 receptors can modulate intracell
signaling involving IP3 receptors at the endoplasmic reticulum and
channels. GPCR: G-protein coupled receptors; ER: Endoplasmic reticulum; IP3: Inositol triphosphate

Sigma receptor agonists are found to be useful in the treatm
neurodegenerative disorders, stroke and depression. In 1998, two stu
have proposed the potent neuroprotective action of sigma-1 rece
agonists in the NMDA-treated primary neurons, the action b
agonized by NE-100 (Nakazawa et al., 1998; Bhardwaj et al., 1996
is also being debated that sigma-1 receptors potentiate the nerve gr
factor-induced neurite overgrowth (Takebayashi et al., 2002).

Diverse kind of drugs, including antipsychotics, antidepressants
neurosteroids bind to sigma-1 receptors (Table 5) (Hayshi and Su, 20
Among antipsychotics, haloperidol has the highest affinity for sigm
receptors (Su, 1982), and atypical antipsychotics like sulpiride and clozapine have no affinity. Selective serotonin reuptake inhibitors like fluvoxamine have been report to possess higher affinity for sigma-1 receptors (Narita et al., 1996) while tricyclic antidepressants to have moderate affinity (Su, 1982). A recent positron emission tomography (PET) study demonstrated that a single oral administration of fluvoxamine occupies sigma-1 receptors in the human brain (Ishikawa et al., 2007). Based on the data generated from highly selective sigma-1 ligands, it is certain that some of the useful psychotropic drugs like antipsychotics or antidepressants might be acting through sigma-1 receptors (Skuja, 2003; Hayashi and Su, 2008). However, an interesting feature of sigma receptors is that in contrast to the classical observations of a linear dose-response curve followed by a plateau phase, many drugs show a biphasic bell-shaped dose response curve for sigma receptors in various behavioral, biochemical and electrophysiological paradigms (Maurice et al., 1994; Bergeron et al., 1995; Monnet et al., 1996; Hayashi et al., 2000).

It has been proposed that at low doses the sigma ligands activate high affinity sites and these high affinity sites in turn may activate another subtype of sigma receptor(s) for which they have low affinity (Bergeron et al., 1995).

More direct evidence of potential antidepressant properties of sigma ligands were obtained from the fact that SA 4503, (+)-pentazocine, DTG, JO-1784 and SKF-10047, all sigma receptor agonists dose-dependently decrease the immobility period in mouse forced swim test (Matsuno et al., 1996a; Urani et al., 2004). In addition, SA 4503 and (+)-pentazocine also decreased immobility period in tail-suspension test (Ukai et al., 1998). These effects were blocked by the sigma receptor antagonist NE-100 (Ukai et al., 1998). OPC-14523, a high affinity sigma-1 receptor agonist with 5-HT1A serotonin receptor agonistic property produced a marked antidepressant-like effect in forced swim test (Oshiro et al., 2000).
Table 5: Sigma-1 receptor ligands (Adapted from Hayashi and Su, 2008)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Agonists/antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neuroleptic</strong></td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td>Antagonist</td>
</tr>
<tr>
<td><strong>Antidepressants</strong></td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>Agonist</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Agonist</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>Agonist</td>
</tr>
<tr>
<td><strong>Benzomorphans</strong></td>
<td></td>
</tr>
<tr>
<td>(+) SKF-10047</td>
<td>Agonist</td>
</tr>
<tr>
<td>(+) Pentazocine</td>
<td>Agonist</td>
</tr>
<tr>
<td><strong>Neurosteroids</strong></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>Antagonist</td>
</tr>
<tr>
<td>Pregnenolone sulfate</td>
<td></td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>Agonist</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
</tr>
<tr>
<td>DTC</td>
<td>Agonist</td>
</tr>
<tr>
<td>BD-1008</td>
<td>Antagonist</td>
</tr>
<tr>
<td>PRE-084</td>
<td>Antagonist</td>
</tr>
</tbody>
</table>

**Synthetic compounds introduced/considered for clinical trial**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Agonists/antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE-100</td>
<td>Antagonist</td>
</tr>
<tr>
<td>BD-1047</td>
<td>Antagonist</td>
</tr>
<tr>
<td>Dup-734</td>
<td>Agonist</td>
</tr>
<tr>
<td>SA-4503</td>
<td>Agonist</td>
</tr>
<tr>
<td>OPC-14523</td>
<td>Agonist</td>
</tr>
<tr>
<td>Rimcazole</td>
<td>Antagonist</td>
</tr>
<tr>
<td>BMY-14802</td>
<td>Antagonist</td>
</tr>
<tr>
<td>Panamesin</td>
<td>Antagonist</td>
</tr>
</tbody>
</table>

Neurosteroids are shown to possess antidepressant activity in forced swim test possibly by modulating the sigma-1 receptors (Reddy and Kulkarni, 1996). It has been debated that sigma-1 receptor agonists induced a significant effect on the firing rates of serotonergic neurons in the dorsal raphe nucleus area of the brain (Bermack and Debonnel, 2005).
Sigma receptors may rapidly modulate the NMDA receptor-mediated transmission in the hippocampus, and potentially other forebrain regions, which in turn would lead to modulation of serotonergic neurotransmission in the dorsal raphe nucleus (Peyron et al., 1998).

Therefore, it is proposed that sigma-1 receptors by modulating the glutamatergic, dopaminergic and serotonergic neurotransmission may display antidepressant activity in animal models of despair. However, further studies are required to understand the concept fully.

**Development of antidepressants**

Discovery and development of antidepressants have been divided into various era’s

**Era of 1950s:** The first of the antidepressants were discovered initially by serendipity. For example, some of the agents such as iproniazid, classified as monoamine oxidase inhibitor (MAOI) or imipramine, a tricyclic antidepressant (TCA) were being developed as antitubercular and antihistamine/antipsychotic drugs, respectively (Nutt, 2002; Slattery et al., 2004). These drugs were further shown to possess antidepressant activity.

**Era of 1960s-70s:** The mechanism of actions of monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs) were begun to understand during this period. Researchers explored out that MAOIs block the metabolism of norepinephrine and serotonin and increase levels of these monoamines in brain samples (Iversen, 1965; Cotzias et al., 1974). The action of tricyclic antidepressants was however, more difficult to understand. To this end, Axelrod et al demonstrated the process of norepinephrine reuptake phenomenon (Axelrod et al., 1961). While attempting to determine the mechanism of action of imipramine, it was found that the brain deals with most neurotransmitters by taking them back into terminals or glial cells (Axelrod et al., 1961). Soon thereafter, it was realized that imipramine blocked not only reuptake of norepinephrine (Axelrod et al., 1961; Iversen, 1965) but also that of serotonin (Ross et al.,
This discovery leads to the development of selective serotonin reuptake inhibitors (SSRIs) as antidepressants in the late 1970s.

**Era of 1970s:** During the 1970s, two isoenzymes of monoamine oxidase were identified and hence two subtypes of MAOIs namely, monoamine oxidase-A selective inhibitor (pargylne) and monoamine oxidase-B selective inhibitor (selegilene) were discovered. Selegilene developed initially as a dopamine sparing agent and a possible neuroprotective agent in Parkinson's disease (Mendelwicz and Youdim, 1978; Rinne, 1978) has recently been developed as a possible alternative antidepressant treatment. More recently, Food and Drug Administration (FDA) has approved a patch preparation of selegilene for the treatment of mental depression (Wecker *et al.*, 2003).

**Era of 1980s:** Once the mechanisms of the tricyclic antidepressants were unraveled, a range of tricyclics and selective serotonin reuptake inhibitors were discovered. The first generation antidepressants desipramine, protriptyline, nortriptyline, lofepramine had mainly norepinephrine reuptake properties (Nutt, 2002). The second generation antidepressants with selective serotonin reuptake (SSRIs) properties emerged as front runners during this period. Fluoxetine became a block-buster drug.

**Era of 1990s and beyond:** The concept of dual reuptake inhibitors was a major development during this period. The SNRIs (dual reuptake inhibitor of serotonin and norepinephrine) namely venlafaxine became main agent amongst these days.

The evolution of antidepressants is summarised in Fig. 18

Currently available antidepressant treatments and their mode of action are depicted in table below (Table 6) (Holtzheimer and Nemeroff, 2006b; Berton and Nestler, 2006).
Enzyme inhibitor | Reuptake blockers | Reuptake blocker
---|---|---
1950's | MAOIs | TCAs
1960's | Subtype selective MAOIs | NE selective 5-HT selective Mianserin
1970's | MAOI's | Trazodone
1980's | RIMAs | SSRI's Nefazodone
1990's | NDRIs | SNRI's Mirta
2000's | Triple reuptake inhibitors and new mechanisms of action of exantidepressants to fully elucidate the pathophysiology of depression

Fig. 18. Evolutionary discovery of antidepressants

5-HT=serotonin, MAOI=monoamine oxidase inhibitor, NDRI=norepinephrine dopamine reuptake inhibitor, NE=norepinephrine, RIMA=reversible inhibitor monoamine oxidase type-A, SNRI=serotonin-norepinephrine reuptake inhibitor SSRI=selective serotonin reuptake inhibitor, TCA=tricyclic antidepressant

(Adapted from Nutt, 2002).

**MAO-inhibitors (MAOIs)**

Monoamine oxidase (MAO), a mitochondrial outer membrane enzyme catalyzes the degradation of neurotransmitters in the central nervous system and is the target for antidepressant drugs (Ma et al., 2004). This enzyme is found in both neurons and liver cells. There is a high probability of criminality, psychopathy, childhood conduct disorders, as well as with sensation seeking, impulsivity, and drug abuse (especially alcoholism) associated with low monoamine oxidase activity.
### Table 6: Currently available antidepressants

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Mode of action</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Medication</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricyclics and tetracyclic antidepressants (TCAs)</td>
<td>Inhibition of noradrenaline and/or serotonin reuptake into the presynaptic terminal</td>
<td>Imipramine, Desipramine, Amitriptyline, Clomipramine, Doxepine, Nortriptyline, Protriptyline, Trimipramine</td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitors (SSRIs)</td>
<td>Inhibition of selective serotonin reuptake into the presynaptic terminal</td>
<td>Fluoxetine, Citalopram, Escitalopram, Paroxetine, Sertraline</td>
</tr>
<tr>
<td>Noradrenaline reuptake inhibitor (NRIs)</td>
<td>Inhibition of noradrenaline-selective reuptake</td>
<td>Atomoxetine, Reboxetine</td>
</tr>
<tr>
<td>Serotonin and noradrenaline reuptake inhibitors (SNRIs)</td>
<td>Inhibition of mixed noradrenaline and serotonin reuptake into the presynaptic terminal</td>
<td>Venlafaxine, Duloxetine</td>
</tr>
<tr>
<td>Monoamine oxidase(s) inhibitors (MAOIs)</td>
<td>Inhibition of monoamine oxidase A (MAO-A) or monoamine oxidase B (MAO-B) within the presynaptic terminal</td>
<td>Isocarboxizid, Tranylcypromine, Phenelzine, Moclobemide, Selegiline</td>
</tr>
<tr>
<td>Lithium</td>
<td>Lithium has many molecular actions but the exact mechanism of antidepressant action is not clear.</td>
<td>-</td>
</tr>
<tr>
<td>Atypical antidepressants</td>
<td>Unknown. Although these drugs have purported monoamine-based mechanisms (for example, bupropion inhibits dopamine reuptake, mirtazapine is an $\alpha_2$-adrenergic receptor antagonist and tianeptine is an activator of monoamine reuptake), these actions are not necessarily the mechanisms that underlie their drug therapeutic actions</td>
<td>Bupropion, Mirtazapine, Tianeptine, Nefazodone, Trazodone, Mianserin, Raboxetine</td>
</tr>
<tr>
<td>Type of treatment</td>
<td>Mode of action</td>
<td>Examples</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Electroconvulsive therapy</td>
<td>General brain stimulation</td>
<td>-</td>
</tr>
<tr>
<td>Magnetic stimulation</td>
<td>A magnetic field is thought to affect the brain by inducing electrical currents and neuronal depolarization</td>
<td>-</td>
</tr>
<tr>
<td>Vagal nerve stimulation</td>
<td>Unknown</td>
<td>Cognitive-behavioral therapy (CBT), interpersonal therapy (IPT)</td>
</tr>
<tr>
<td>Psychotherapies</td>
<td>Exact mechanism is uncertain, but is thought to involve learning new ways of coping with problem</td>
<td>-</td>
</tr>
<tr>
<td>Deep brain stimulation</td>
<td>In severely ill patients, stimulation of a region of the cingulated cortex found to function abnormally in brain imaging scans reportedly has antidepressant effects</td>
<td>-</td>
</tr>
</tbody>
</table>

*Many patients respond to several types of treatment, although it is not yet possible to predict which group of patients will respond optimally to a particular treatment. Although they alleviate mood in patients with depression, antidepressants do not elevate mood in healthy individuals and are non-addictive (Berton and Nestler, 2006).

There are two subtypes of MAO viz. MAO-A and MAO-B (Ma et al., 2004). These are distinct proteins with high amino acid homology, coded by separate genes both located on the short arm of the human chromosome X (Shih et al., 1998). The enzyme subforms show different substrate specificities in vitro and different distributions within the central nervous system and in peripheral organs. In the central nervous system, MAO-A seems to be mainly involved in the metabolism of serotonin and noradrenaline, whereas 2-phenylethylamine and dopamine are predominantly deaminated by MAO-B (Benedetti, 2001). As depression is associated with decreased levels of all the monoamines viz. norepinephrine, serotonin and/or dopamine, therefore, various MAO-inhibitors (both MAO-A as well as MAO-B inhibitors) could be a useful drug therapy in the treatment of mental depression.
This class of drugs were introduced in the mid 1950s (Keller, 2003; Pacher and Kecskemeti, 2004) and it was soon discovered that MAOIs block the metabolism of norepinephrine, serotonin and/or dopamine.

Currently available MAOIs include phenelzine (non-selective MAO-inhibitor), clorgyline (selective MAO-A inhibitor), L-deprenyl (selective MAO-B inhibitor) and tranylcypromine (non-selective MAO-inhibitor) (Wooters and Bardo, 2007) (Fig. 19).

MAOIs are associated with considerable side effects and toxicity such as a dangerous rise in blood pressure when high-tyramine foods (soy sauce, beans and cheeses) are ingested (cheese reaction) by the patients (Gumnick and Nemeroff, 2000). Other side effects include dizziness,
orthostatic hypotension, weight gain or sexual dysfunction (Gumnick and Nemeroff, 2000).

Most of these side effects are due to irreversible blockade of monoamine oxidase enzyme. The novel antidepressant moclobemide (Fig. 19) is a reversible inhibitor of monoamine oxidase (MAO), preferentially of type A and free of above-mentioned side effects (Burkard et al., 1989). In contrast to imipramine-like antidepressants, it lacks anticholinergic activity and it differs from classic MAO inhibitors by potentiating only weakly the pressor effect of tyramine.

MAOIs are also contraindicated in patients who are on the medications which increase serotonin concentrations in the synapse (Martinez and Marangell, 2004). Most antidepressants increase serotonin availability through various mechanisms, thus the use of such agents with MAOIs is contraindicated because excessive serotonin could be induced in the synapse, a condition known as serotonin syndrome. Signs and symptoms of serotonin syndrome include altered mental alertness, agitation, diaphoresis, hyperthermia and hypertonicity (Martinez and Marangell, 2004).

Because of the above mentioned serious drug interaction profile, MAOIs generally are not prescribed as a first-line treatment for depression and are considered by many healthcare professionals to be appropriate only for treatment-resistant depression (Keller, 2003).

**Tricyclic antidepressants (TCAs)**

Another way to increase the neurotransmitter availability involves blocking the process of reuptake in the presynaptic neuron, which subsequently increases the concentration of neurotransmitters in the synaptic cleft. The first generation namely tricyclic antidepressants, introduced in mid 1950’s had reuptake inhibiting properties (Pacher and Kecskemeti, 2004).

TCAs are considered to be superior to MAOIs because of their reduced toxicity. In contrast to MAOIs, TCAs inhibit the reuptake of norepinephrine and serotonin neurotransmitters. Unfortunately, TCAs are
not very selective and interact with several types of receptors inside and outside the brain such as cholinergic, histaminergic, and adrenergic receptors causing uncomfortable to miserable side-effects in many patients (Peretti et al., 2000). The examples of TCAs include imipramine, amitryptiline, desipramine, nortriptyline, protriptyline, clomipramine and lofepramine (Holtzheimer and Nemeroff, 2006a) (Fig. 20). Out of these, desipramine, protriptyline, nortriptyline and lofepramine are selective norepinephrine reuptake inhibitors whereas imipramine, amitryptiline and clomipramine are reuptake inhibitors of both norepinephrine as well as serotonin.

![Imipramine and Desipramine](image)

**Fig. 20. Structures of tricyclic antidepressants**

**Selective serotonin reuptake inhibitors (SSRIs)**

The selective serotonin reuptake inhibitors (SSRIs) have emerged as a major therapeutic advance in the treatment of mental depression. SSRIs are rationally designed class of psychotropic medications, and represent a new era of second generation antidepressant drugs. These drugs which were developed in the 1970s and introduced in the mid 1980s (Holtzheimer and Nemeroff, 2006a), act like TCAs by inhibiting the reuptake of neurotransmitters by presynaptic cell. However, their activity is specific to the serotonin reuptake transporter protein, resulting in more available serotonin in the synapse (Lieberman, 2003). They are currently among the most prescribed therapeutic agents in the market. Some of the SSRIs include fluoxetine, citalopram (Fig. 21), escitalopram (S-enantiomer of citalopram) (Robert et al., 2006), sertraline, fluvoxamine, paroxetine etc. Under steady-state concentrations, their half-lives range between 1 and 4
days for fluoxetine (7 and 15 days for norfluoxetine) and between 21 and 36 hr for paroxetine and citalopram, respectively. Sertraline and citalopram show linear and fluoxetine, fluvoxamine, and paroxetine display nonlinear pharmacokinetics (Hiemke and Hartter, 2000). Their serotonin specificity is believed to result in a decreased side effect profile (Peretti et al., 2000). Common side effects with SSRI s include nausea, nervousness, insomnia, sexual dysfunction and headache (Kelsey, 2001).

![Fluoxetine and Citalopram](image)

**Fig. 21.** Structures of selective serotonin reuptake inhibitors

### Atypical antidepressants

Antidepressant therapies that are not categorized as SSRIs, TCAs or MAOIs are referred to as atypical antidepressants. The drug includes bupropion, mirtazapine, nefazodone and trazodone (Fig. 22). Nefazodone was removed from United States market in the year 2004 due to its propensity to cause liver damage.

Bupropion is considered to be a dopaminergic antidepressant based on its ability to inhibit the reuptake of dopamine somewhat more selectively than it inhibits the reuptake of norepinephrine or serotonin (Cooper et al., 1980, 1994). However, the exact mechanism of action of bupropion remains unclear after many years of studies (Ascher et al., 1995). Its efficacy is similar to TCAs. When administered at a dose of 37.5, 75 and 150 mg/kg per day for 21 days, the drug had no effect on β-adrenergic, α₂ adrenergic, or serotonin (5HT₂) receptors in the brain of rats as determined by scatchard analysis of the binding data (Ferris and
The most serious effect of bupropion is increased seizure risk (Kuate et al., 2004), especially in patients with a history of seizure or head trauma.

Mirtazapine is a tetracyclic antidepressant that increases the amount of norepinephrine and serotonin in the brain through unknown mechanism (Hartmann, 1999) by blocking $\alpha_2$ adrenoceptors and selectively antagonizing $5\text{HT}_2$ and $5\text{HT}_3$ serotonin receptors (De Boer et al., 1988; Ruigt et al., 1990; Frazer, 1997). Its efficacy has been shown to be equal to that of the amitryptiline, a tricyclic antidepressant. Mirtazapine has a high affinity for the histamine postsynaptic receptors (Gumnick and Nemeroff, 2000). Inhibition of postsynaptic histamine receptors appears to be responsible for its most common side effects such as sedation, fatigue, increased appetite and weight gain (Gumnick and Nemeroff, 2000).

Fig. 22. Structures of atypical antidepressants

Bupropion

Mirtazapine

Nefazodone

Trazodone
Nefazodone and trazodone are chemically related to each other. Both antidepressants weakly inhibit the reuptake of norepinephrine and serotonin and are potent inhibitors of postsynaptic serotonin type 2 receptors (Golden et al., 1998). Studies have demonstrated that trazodone is equally efficacious as compared to TCAs in mild to moderate depression (To et al., 2005). However, it may produce excessive sedation on administration which may limit its use (To et al., 2005). Common side effects of nefazodone include nausea, somnolence, dry mouth, dizziness, and constipation. It is also an inhibitor of CYP3A4 isoenzyme in the liver, therefore, producing drug-drug interactions (Gumnick and Nemeroff, 2000).

**Dual reuptake inhibitors**

Antidepressants that inhibit reuptake of more than one neurotransmitter may have an efficacy advantage compared with single-active agents in specific subpopulation of major depression patients. Specifically, results of traditional meta-analysis, pooled analysis, and some individual studies have suggested a greater benefit with dual-acting agents, such as the tricyclic antidepressants, amitryptiline and cloimipramine compared with single-acting agents, including the selective serotonin reuptake inhibitors. It has been demonstrated that a combination of SSRIs with a noradrenergic TCA desipramine is more efficacious in the treatment of major depression (Nelson et al., 2004). The results suggest that simultaneous increase in the serotonergic as well as noradrenergic synaptic concentrations may have a synergistic effect that is beneficial for the remission of major depressive disorders (Nelson et al., 2004). On the similar grounds, dual acting TCAs may provide better efficacy than SSRIs in depressed patients. In one of the studies, dual acting cloimipramine was found to be more efficacious as compared to the citalopram (Danish University Antidepressant Group, 1986) or paroxetine (Danish University Antidepressant Group, 1990).
Venlafaxine and duloxetine (Fig. 23), referred to as dual-action antidepressants, uniquely block the reuptake of serotonin and norepinephrine. Whereas TCAs block 5-HT and NE reuptake in a relatively fixed ratio, the SNRIs block 5-HT at lower doses and, 5-HT and NE at medium to high doses and 5-HT, NE and DA at the highest doses (Lee and Keltner, 2006).

In double-blind randomized clinical trials, venlafaxine, a dual reuptake inhibitor of serotonin and norepinephrine was significantly more efficacious than fluoxetine in patients hospitalized with major depressive syndrome and melancholia (Nemeroff et al., 2007; 2008).

![Venlafaxine](image1.png)  ![Duloxetine](image2.png)

Fig. 23. Structures of dual reuptake inhibitors

**Triple reuptake inhibitors**

An important development in the treatment of depression has been the emergence of triple reuptake inhibitors (serotonin, norepinephrine, dopamine reuptake inhibitors; SNDRIs), which inhibit besides serotonin and norepinephrine, the reuptake of dopamine (Chen et al., 2007). These triple reuptake inhibitors have also been mentioned as “broad spectrum antidepressants” (Skolnick et al., 2003).

Advantages of triple reuptake inhibitors in mental depression are (Skolnick et al., 2006):

- A drug inhibiting reuptake of all three of these neurotransmitters could produce more rapid onset of action and greater efficacy than traditional antidepressants (Chen and Skolnick, 2007).
A triple reuptake inhibitor may have fewer side effects than the classical antidepressants. For example, antidepressants like paroxetine, fluoxetine and sertraline (all selective serotonin reuptake inhibitors), already carry a “black box” warning highlighting an increased risk of suicidal behaviour amongst children and adolescents which may be less with triple reuptake inhibitors [http://www.drugresearcher.com/news/ng.aspid=61172-antidepressant glaxosmithkline-merck].

The classical antidepressants display a two to six week lag period (the therapeutic lag) before they show clinical effects, a low rate of response and a low rate of remission which can be overcome by using a triple reuptake inhibitor (Chen and Skolnick, 2007).

There are presently three molecules in clinical trials that belong to the category of triple reuptake inhibitors. These molecules include GlaxoSmithKline's NS2359 and Merck's DOV216303 and DOV21947. Both candidates are in phase 2 clinical trial. Recently, Sepracor Inc. (SEPR) has initiated a phase I, single-blind, randomized, placebo-controlled safety, tolerability and pharmacokinetic study for SEP-225289, another molecule belonging to the category of triple reuptake inhibitors for the treatment of major depressive disorder (MDD). DOV 216303 is equipotent as an inhibitor of [3H] norepinephrine, [3H] serotonin uptake in HEK 293 cells expressing the corresponding recombinant human transporter proteins (Skolnick et al., 2003). It is approximately 4 folds potent in inhibiting [3H] dopamine uptake, albeit with a potency of <100nM (Skolnick et al., 2003). Nonetheless, in phase-I studies, plasma concentration of DOV 216303 are sufficient to inhibit the reuptake of three amines at a dose that is safe and well tolerated (Skolnick et al., 2003). NS2359 has the advantage that besides triple reuptake inhibiting mechanism, it also increases release of the neurotransmitter acetylcholine [www.neurosearch.com]. This mode of action is expected to produce a better and faster reduction of the symptoms associated with depression.

Recently, DOV 21947, another triple reuptake inhibitor is effective in causing a sustained and selective reduction in fat content and triglyceride levels in animal models of obesity without significantly altering vital organ function (Tizzano et al., 2008).

While all the dopamine, serotonin and norepinephrine transporters are the members of twelve transmembrane domain gene superfamily (Povlock and Amara, 1997), the design and synthesis of orally available, safe and well tolerated compounds active at all three amine transporters remain a synthetic challenge.

**Animal models of depression**

Research in non-humans, primarily rodents, has been always central to understand the neural systems involved in mediating emotions. Further, these animal models can suggest the system dysfunction under pathological conditions and how they can be therapeutically modulated (Cryan and Holmes, 2005). For decades, the rat was the species of choice in preclinical research-in part, because rats perform well in many of the cognitive and operant tasks that are the pillars of behavioral pharmacology. This may be due to various factors such as convenient size and robustness of rats as compared to smaller animals (Cryan and Holmes, 2005). This may aid in the application of invasive procedures such as catherization and cannula implantation. However, in the past decade, there has been an explosion in the use of mice in neuropsychiatric disorders. Advantages of using mice in research include the relatively ease to breed and house them in large numbers and further, genetic manipulation in mice is easy to perform.

So far, about 80 different mutant lines have been reported to have phenotypes interpreted as abnormal “depression-related” or anxiety-related behaviors” (Cryan and Mombereau, 2004). For example, the
phenotype found in noradrenaline transporter (NET) knock-out mice may fit the profile of antidepressant efficacy of drugs that blocks norepinephrine transporter such as desipramine (Xu et al., 2000). Similarly, serotonin transporter (SERT) knock-out mice are being employed for screening antidepressant drugs (Olivier et al., 2008). However, the problem with genetic models in depression is that, not a single gene is involved in its pathophysiology. Unlike Huntington's disease or sickle-cell anemia, which is unifactorial in nature, depression is multifactorial in nature (Romm, 2003) which limits the use of such models in the screening of antidepressants. Therefore, now-a-days various models of behavioral despair are being tried which are easier to perform and also are less time-consuming.

An ideal animal model should have identical causative factors, symptomology and responsiveness to treatment modalities. A number of symptoms of depression, however, are clearly not measurable in preclinical paradigms, such as recurrent thoughts of death or suicide, or excessive thought of guilt. It is clear that evolutionary progression has provided humans with a much more elaborated cerebral cortex that facilitates integration of complex psychological concepts also relevant to human depression, such as self-esteem and the ability to perceive the future, which are absent in rodents. Some of the depression associated phenotypes that can be modeled in mice or rats are tabulated below (Table 7).

Animal models of depression, in the proper meaning, are expected to present with sufficient face validity and to shape the underlying disease etiology. Many different animal models of depression have been proposed and evaluated on the basis of different criteria, of which predictive validity appears to be the most salient (Harro, 2004). As antidepressant action can be elicited by different molecular mechanisms, in vivo animal models remain of utmost value. As discussed above, depression is clearly related to functional alterations in monoaminergic systems and is precipitated by severe life events in predisposed individuals.
Thus, by introducing persistent disturbances in the function of monoaminergic neurotransmission using genetic, environmental, or neurochemical means, a number of different models can be created. Caution should be exercised in interpreting behavioral changes in neurobiologically altered animals, since simple behavioral characteristics can be affected by different parameters. Therefore, neurobiological including in vivo neurochemical estimations of various neurotransmitters may provide viable alternatives as end points in these behavioral models. It has been shown that fluoxetine, fluvoxamine and citalopram all increased extracellular levels of cortical serotonin while desipramine and imipramine increased extracellular norepinephrine and dopamine levels (Kobayashi et al., 2008). Some of the behavioral models used in the screening of antidepressants have been tabulated in table 8.

### Forced swim test

Out of all the procedures, the forced swim test (FST) and the tail-suspension tests (TST) are mainly employed for the screening of antidepressant drugs. The FST described originally by Porsolt et al. (1977a, 1978a), is the most widely used model for assessing the predictive antidepressant activity of a compound in animals. The test is based on the observations that when rodents are forced to swim in a jar of water from where they cannot escape, the animals surrender to the situation and adopt immobile posture (float). The immobility is described as helplessness or a state of depression (despair) (Porsolt et al., 1977a,b; 1978 a,b; Kulkarni and Mehta, 1985). The immobility period is measured during a total of 6-min test duration. The treatment with antidepressant drugs decrease the period of immobility, and the animals show increased or prolonged active escape behaviors during the test (Kulkarni and Mehta, 1985). In rats, drugs are generally administered in two to three doses between the initial drug-free swimming session and the second swim that follows twenty four hours later, while in mice, even acute single administration may show antidepressant activity.
Table 7: Depression associated phenotypes that can be modeled in mice and rat

<table>
<thead>
<tr>
<th>Symptoms of depression</th>
<th>Mimicking animal models</th>
</tr>
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<tbody>
<tr>
<td>Anhedonia</td>
<td>The loss of interest in pleasurable and rewarding actions is a core symptom of depression. Anhedonia in rodents can be assessed by the preference for a palatable reward such as sucrose solution or by intracranial self stimulation.</td>
</tr>
<tr>
<td>Anxiety-related behavior</td>
<td>Anxiety is a co-morbid symptom with depression. There is a high prevalence of anxiety symptoms in person suffering from depression. Therefore, animal models that are used to elucidate mechanisms underlying depression often display anxiety-related behaviors.</td>
</tr>
<tr>
<td>Behavioral despair</td>
<td>Behavioral despair may be assessed by using tests such as forced swim test and tail-suspension test.</td>
</tr>
<tr>
<td>Neuroendocrine disturbances</td>
<td>Disturbances of the hypothalamic pituitary adrenocortical (HPA) axis are one of the most consistent symptoms of depression. The functionality of the HPA axis can readily be assessed by challenges test such as dexamethasone suppression test.</td>
</tr>
<tr>
<td>Change in appetite or weight gain</td>
<td>Depression in humans is often associated with large changes in appetite or weight gain, which can be measured in rodents</td>
</tr>
<tr>
<td>Alteration in sleep architecture</td>
<td>Disturbances in the circadian rhythms and especially in the sleep architecture are often observed in depressed patients. Various EEG recordings can be carried out.</td>
</tr>
<tr>
<td>Neuroanatomy</td>
<td>Depressed subjects display decreased hippocampal volume as demonstrated by magnetic resonance imaging. Rodents exposed to chronic stress or excess glucocorticoids exhibits similar signs of hippocampal loss of neurons and dendritic atrophy</td>
</tr>
</tbody>
</table>
### Table 8: Animal models of depression

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tail suspension test</strong></td>
<td>Rodents, mainly mice, when hung from tail will adopt an immobile posture. Antidepressant treatment increases the time animal spend in active behaviors</td>
<td>Steru <em>et al.</em>, 1985; Porsolt <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><strong>Reserpine-induced depression</strong></td>
<td>Reserpine produced depression in animals by depleting monoamines in the brain. Various antidepressant treatments are known to reverse reserpine-induced immobility period in both forced swim test and tail-suspension test</td>
<td>Sharma and Kulkarni, 1994</td>
</tr>
<tr>
<td><strong>Learned helplessness (rats and mice)</strong></td>
<td>Animals exposed to inescapable shocks subsequently fail to escape. Antidepressant treatment increases the number of escapes</td>
<td>Anisman and Merali, 2001</td>
</tr>
<tr>
<td><strong>Olfactory bulbectomy</strong></td>
<td>Removal of the olfactory bulbs causes a constellation of behavioral and neurochemical alterations, which are only reversed by chronic antidepressant treatment</td>
<td>Pistovcakova <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>Animal model</td>
<td>Description</td>
<td>References</td>
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<tr>
<td>Maternal deprivation/separation</td>
<td>When animals are separated from the mother during early postnatal life they can develop a number of depression-like behavioral characteristics. These behaviors are not present in all animals subjected to this treatment.</td>
<td>Ruedi-Bettischen et al., 2006</td>
</tr>
<tr>
<td>Social stress in shrews</td>
<td>Tree shrews form stable dominant/subordinate relationship with subordinate showing behavioral and neurochemical alterations similar to depressed patients. A number of these alterations can be reversed with antidepressant treatment but the model requires further validation.</td>
<td>Fuchs, 2005</td>
</tr>
<tr>
<td>Chronic mild stress</td>
<td>Animals are subjected to a variety of unpredictable stressors, which leads to a constellation of symptoms, that are reversed by antidepressant treatment.</td>
<td>Grippo et al., 2008</td>
</tr>
<tr>
<td>Pro-inflammatory cytokine-induced sickness behavior</td>
<td>Exposure to endotoxins such as lipopolysaccharide or cytokines induces a sickness behavior syndrome that is characterized by anhedonia, increased sleep, and decrease in food intake, body weight, locomotor activity, social interaction, sexual behavior and grooming.</td>
<td>La Garza II, 2005</td>
</tr>
</tbody>
</table>
The FST is credited for having good predictive validity for detecting antidepressant activity (Kulkarni and Parale, 1986; Borsini and Meli, 1988). The test also investigates the mechanism of action of antidepressant drugs and has been effectively employed in the preclinical identification and development of tricyclics (TCA) and selective serotonin reuptake inhibitors (SSRI) (Borsini 1995; Redrobe et al., 1996; 1998a; 1998b; Redrobe and Bourin, 1997). Forced swim test relatively has high selectivity for antidepressants as compared to other classes of central nervous system drugs (Cryan et al., 2005b). For example, the benzodiazepines are not active in FST (Cryan et al., 2005b) with the exception of alprazolam, the only benzodiazepine which exhibit antidepressant-like effects (Flugy et al., 1992). However, the psychomotor stimulants like amphetamine which reduces immobility period in FST and are probably not effective as antidepressants. Because of this pattern, most studies continue to employ tests of locomotor activity with FST. Although drugs that decrease immobility period in the FST and also increases the locomotor activity may still have antidepressant activity, viz. dopamine reuptake inhibitors such as bupropion and GBR 12909 (Hemby et al., 1997).

Genetic factors, such as strain, may also contribute to the behavioral performance of mice in this model of depression. Mouse strain differences have been reported in response to drugs in the FST (Porsolt et al. 1978a,b; Tadano et al. 1997; Bai et al. 2001, Lucki et al. 2001). It has been found that Swiss mice are the most sensitive strain to detect the drugs that affect serotonin and/or noradrenaline neurotransmission (David et al., 2003). The DBA/2 inbred mice did not show immobility period in the FST (David et al., 2003). The lack of sensitivity to antidepressant treatment in DBA/2 strains could be due to high dopamine, noradrenaline and serotonin contents in their brain (David et al., 2003). Similar studies have also been carried out in our laboratory using laca strain of mice (Kulkarni and Mehta, 1985).
Tail-suspension test
Another prominent test, which relies on similar assumptions and interpretations as FST, is the tail-suspension test (TST) (Steru et al., 1985). In the TST, mice are suspended by their tails for a defined period of time and their immobility is assessed. The model is valid for a broad range of antidepressants, which significantly decrease immobility period (Steru et al., 1985). Despite the apparent conceptual similarity with the forced swim test, behavior in tail-suspension test may have a different neurochemical basis (Bai et al., 2001; Renard et al., 2003). A major advantage of TST is that it is simple, inexpensive and allows for automation. A major drawback of TST is that its application is limited to strains that do not climb their tail (Deussing, 2006). Moreover, neither the FST nor the TST reflect the slow onset of antidepressant action as it is observed in clinics.

Olfactory bulbectomy
Another reliable screening technique for novel antidepressants has been the bilateral removal of the olfactory bulbs (Dennis et al., 1993). This procedure has been described to elicit a variety of behavioral, neurochemical, neuroendocrine, and neuroimmune alterations, many of which parallel symptoms of depression in humans (Kelly et al., 1997). It is a very robust model with predictive power. An additional interesting characteristic of this model has been that the bulbectomy is found to elicit increases in intake of amphetamine and alcohol, both attenuated by chronic antidepressant treatment (Katkov et al., 1994; Holmes et al., 2002). This may be useful for studies on association of drug abuse and mood disorders. The model is particularly suitable for studying comorbidity.

Learned helplessness
Another animal model useful for the screening of antidepressant drugs is learned helplessness. After uncontrollable electric shocks have been administered repeatedly, animals display escape deficits sensitive to
various antidepressants. The model is referred to as learned helplessness (Overmier and Seligman, 1967) even though it can be argued that it is simply the extreme stressful situation of uncontrollable shock that is producing the various depression-like behaviors. One of the limitations with this model is that the changes persist for a couple of days and there would be a need to repeatedly administer shocks which may be an objection to the ethical committees. Recently, modifications of the technique that should increase its reliability have been described (Gambarana et al., 2001; Vollmayr and Henn, 2001). These include introduction of chronic mild stress as a background to shock administration (Harro, 2004).