# 10
# Glutamate and Depression

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## 1. INTRODUCTION

### *1.1. Prevalence and Symptoms of Depressive Disorders*

There are two principal types of mood disorder: major depression and bipolar disorder. Recurring episodes of major depression constitute unipolar depression whereas individuals who alternate periods of mania and depression are manic-depressive and suffer from bipolar disorder. Depression is a disease with a prevalence of 3–5% in developed countries and a lifetime morbidity of approx 15–18%. The disease is more prevalent in females than in males. Prevalence values are not, however, very precise because many depressed patients are still neither diagnosed nor treated. Clinical symptoms include depressed mood and loss of interest in almost everything, anhedonia and fatigue, as well as sleep disturbances, low self-esteem, guilty feelings, and suicidal tendencies. Other somatic symptoms such as gastrointestinal or cardiovascular disorders are also often present. The symptoms of mania are almost the exact opposite of those of depression. In major depressive disorders, there is a high risk not only for suicide but also for life-threatening effects on multiple organ systems, so it is considered that the mortality risk engendered by major depression is similar to that of the more severe cardiac and cerebrovascular diseases (reviewed in refs. *1* and *2*). It is supposed at present that depressive syndromes are the result of a combination of susceptibility genes and multiple environmental factors. The search for genetic substrates underlying depressive disorders has not as yet resulted, however, in any universally accepted finding.

### *1.2. Initial Theories on Depression: The Monoaminergic Hypothesis*

Early theories on the pathogenesis of depressive disorders have been entirely based on the mechanism of action of antidepressant drugs (*see also* Chapter 9). Because of the absence of animal models for a disease involving higher human emotions, it has been accepted that understanding the mechanisms underlying antidepressant treatment would provide substantial advance in the interpretation of pathological changes in depression. The initial biogenic amine hypothesis of depression was based on the effects on monoamine levels of reserpine, an antihypertensive drug, and antidepressants. Reserpine induces monoamine depletion as well as marked sedation and depressive symptoms, whereas clinically effective antidepressants increase monoamine levels and reverse reserpine-induced

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sedation. Most therapeutically useful antidepressants block the monoamine transporters providing increased extracellular levels of serotonin (5-HT) and/or noradrenaline (NA) or, alternatively, prevent monoamine degradation by monoamineoxidase (MAO) or act on presynaptic auto/heteroreceptors controlling monoamine release (Table 1). Lithium salts are of much value in the prophylaxis of bipolar disorder and electroconvulsive treatment (ECT) is still widely used in the treatment of depression. Another more recent physical therapy is rapid transcranial magnetic stimulation, which appears to improve mood in depression *(3)*. There is, however, a significant proportion of patients who do not respond to any antidepressant treatment and there is also a lag time of some weeks for the therapeutic effect of these agents, not correlated with the rapid increase in the availability of monoamines, suggesting that slower adaptive mechanisms, related or not to the monoaminergic systems, could be involved in the antidepressant effect (*see also* Chapter 9). In this regard, the so-called β-adrenoceptor downregulation hypothesis *(4,5)*, which assumed that suppression of signaling through β-adrenoceptors after chronic antidepressant treatment was indispensable for clinical efficacy, was the first widely accepted approach in the search for adaptive changes induced by chronic antidepressant treatment. However, this hypothesis was challenged since some more recently introduced antidepressants, such as the selective serotonin reuptake inhitors, did not downregulate β-adrenoceptors and some of them, such as citalopram, even produced the opposite effect *(6)*.

## 2. MORPHOLOGICAL CHANGES IN DEPRESSION

### *2.1. Neuroimaging and Neuropathological Studies in Depressed Patients*

A consistently observed neuroanatomical change in unipolar major depression has been the volume loss in the hippocampus. Reductions in hippocampal volume, evaluated using magnetic resonance imaging, were nearly 20% in some reports, and apparently dissociated from antidepressant medication or ECT; these reductions have been correlated with the total lifetime duration of depression *(7,8)*. Neuronal atrophy and cell death have been reported not only in the hippocampus but also in the prefrontal cortex of depressed patients. In brain-imaging studies, a decreased volume of the subgenual prefrontal cortex along with a reduced blood flow was found *(9)*. Decreased number and size of neurons, as well as decreased glial cells in the prefrontal and orbitofrontal cortex have been also reported *(10,11)*.

Other positron emission tomography studies have revealed increased cerebral blood flow and glucose metabolism, positively correlated with depression severity, in the amygdala *(12)*, one of the brain regions mediating emotional and stress responses. Antidepressant treatment producing symptom remission decreased amygdala metabolism, supporting the notion that chronic antidepressants have an inhibitory effect on amygdala function *(12)*. Conversely, areas that appear to inhibit emotional expression, such as the posterior orbital cortex, suffer histopathological abnormalities in depression. Excellent reviews on neuroimaging and postmortem studies in depression have been published in recent years *(11–13)*.

### *2.2. Stress-Induced Neuroanatomic Changes*

It is known that a significant percentage of major depression patients display some form of hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, such as hypercortisolemia and lack of feedback inhibition, and increased release of corticotropin-releasing factor (CRF). A high percentage of patients with Cushing disease also manifest

**Table 1**
**Representative Antidepressant Drugs Acting on the CNS 5-HT and NA Systems**

| |
|---|
| ***NA/5-HT reuptake inhibitors*** |
| Imipramine |
| Amitriptyline |
| Clomipramine |
| Venlafaxine (low anticholinergic side effects) |
| ***Selective 5-HT reuptake inhibitors*** |
| Fluoxetine |
| Paroxetine |
| Sertraline |
| Citalopram |
| ***Selective NA reuptake inhibitors*** |
| Desipramine |
| Reboxetine |
| ***Miscellaneous (non-MAO inhibitors)*** |
| Tianeptine (enhances 5-HT uptake) |
| Mirtazapine (antagonist at $\alpha_2$-adrenoceptors controlling monoamine release) |
| ***MAO inhibitors*** |
| Phenelzine |
| Tranylcypromine |

CNS, central nervous system; 5-HT, serotonin; NA, noradrenaline; MAO, monoamine oxidase.

depressive symptoms as well as hippocampal atrophy *(1,14,15)*. The reduced hippocampal volume may be a consequence of the increased release of glucocorticoids induced by stress, so depression may be ultimately considered as a stress-related disorder. Stress and glucocorticoids make certain neuronal populations more vulnerable to the neurotoxic effects of ischemia, hypoglycemia, and excitatory amino acids *(15)*. Chronic stress or repeated glucocorticoid administration induce in rodents dendritic atrophy in hippocampal neurons of the CA3 region, a suppression of the normal production of granule cells in the dentate gyrus, and even neurotoxic effects on pre-existing hippocampal neurons (reviewed in refs. *16* and *17*). It remains to be established with certainty whether stress is an epiphenomenon of depression or is rather critically involved in the pathophysiology of depression (cf. ref. *2*), although there is abundant evidence suggesting that there is a causal link between stressful experience and depression *(18)*.

Glucocorticoids, secreted during stress, contribute to neuronal atrophy in the hippocampus through two major mechanisms. One of them, shown not only in fat cells but also in cultured hippocampal neurons, is a decreased glucose uptake *(19)* that could result in increased sensitivity to other neurotoxic insults. The other major mechanism is an enhanced activation of glutamatergic transmission. It is known that an elevation of glucocorticoid levels from the low basal range to those typically excitotoxic increases glutamate levels by fourfold *(17)*. Excessive stimulation of glutamate receptors, in particular of the *N*-methyl-D-aspartate (NMDA) ionotropic receptor, may result in cell death through increased intracellular $Ca^{2+}$ levels (Fig. 1).

Antidepressant
PKA
pCREB
BDNF
trkB
AMPA
exocytosis
Enhanced synaptic connectivity
Ras
Raf
MEK
ERK
RSK-2
pCREB
Bcl-2
Cortisol
GR
glucose uptake
Stress Depression
Glutamate
NMDA
$Ca^{2+}$
ROS
Neuroplasticity
BDNF
trkB
PI3K
Akt
GSK-3
Bad
Bcl-x
Genetic vulnerability

Expression of the neurotrophin brain-derived neurotrophic factor (BDNF) is also downregulated by stress in different hippocampal subfields *(20)*. In the hippocampus and in other brain regions, BDNF influences neuronal survival, differentiation, and synaptic strength so reduced levels of this neurotrophic factor may also contribute to the atrophy and decreased function of different populations of hippocampal neurons. Like other neurotrophic factors, BDNF activates the mitogen-activated protein (MAP) kinase-signaling pathway, which inhibits cell death through different mechanisms, notably through an increased expression of the antiapoptotic protein Bcl-2 (ref. *21*; *see* Fig. 1). In the context of the present review, it is of interest that BDNF and NMDA receptor antagonists share protective effects on stress-induced neurotoxicity. Whereas NMDA antagonists prevent the reduction by corticosterone of cell proliferation in the adult dentate gyrus *(22)*, BDNF prevents neuronal cell death induced by corticosterone *(23)*.

### 2.3. *Antagonism of Stress-Induced Changes by Antidepressants*

Standard treatments for depression such as administration of antidepressant drugs or ECT have effects on the hippocampus that should counter those found in major depression, such as stress-induced retraction of dendritic processes in CA3 pyramidal neurons *(24)* or reduction of neurogenesis in the adult dentate gyrus *(25)*. Stress-induced changes in neural plasticity of the hippocampus can be prevented by representative antidepressants, such as imipramine and fluoxetine, and also by ECT *(26)*. In a model of psychosocial stress in primates, it was found that tianeptine, an antidepressant with an unconventional mechanism of action (*see* Table 1), prevented many of the morphological changes associated to stress, including the inhibition of cell proliferation in the hippocampus *(27)*. The ability of the NMDA antagonist MK-801 to prevent corticosterone-induced decrease of proliferating cells in the dentate gyrus *(22)*, suggests the possibility of common mechanisms for antidepressants and NMDA antagonists (*see* Section 5).

**Fig. 1.** Effect of stress and antidepressant treatment on the regulation of neuroplasticity and cell survival in affective disorders. Cellular plasticity and survival depend on genetic factors. Stress associated to depression increases cortisol and glutamate levels. Stimulation of glucocorticoid receptors reduces glucose uptake, increasing the sensitivity to neurotoxic insults. Excessive stimulation of *N*-methyl-D-aspartate receptors induces cell death through increased $Ca^{2+}$ levels and formation of reactive oxygen species. Stress also decreases brain-derived neurotrophic factor (BDNF) levels with the consequent attenuation in the PI-3K/Akt pathway, which promotes cell survival through inhibition of glucogen synthase kinase activity and reduced expression of the proapoprotic proteins Bad and Bcl-x. Different classes of antidepressants stimulate the cyclic adenosine monophosphate-protein kinase A signaling system and activate the transcription factor CREB (cAMP response element-binding protein). One of the target genes of CREB is BDNF, which inhibits cell death by activating the extracellular signal-regulated kinase mitogen-activated protein (MAP) kinase pathway and promoting the expression of the antiapoptotic protein Bcl-2. Antidepressants may also reduce NMDA receptor function and induce the membrane insertion of α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors, leading to enhanced synaptic connectivity and activation of the antiapoptotic mechanisms of the MAP kinase pathway. GR, glucocorticoid receptors; NMDA, *N*-methyl-D-aspartate; ROS, reactive oxygen species; BDNF, brain-derived neurotrophic factor; GSK-3, glucogen synthase kinase; PKA, protein kinase A; CREB, cAMP response element-binding protein; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein ERK, RSK, ribosomal subunit kinase-2.

## 3. GLUTAMATERGIC TRANSMISSION DYSFUNCTION IN DEPRESSION

### 3.1. Changes in Glutamate Levels

Glutamate in mood disorders has been studied using magnetic resonance spectroscopy. Although preliminary, some of these studies appear to reflect regionally specific alterations in glutamate turnover rates associated with mood disorders, such as reduced glutamate in the anterior cingulate cortex *(28,29)*.

The reported deficit of glial cells in mood disorders (*see* Subheading 2.1.) could cause complex changes in glutamate neurotransmission. Because glial glutamate uptake is critical for removing glutamate from the synapse, the reduced number of glial cells may produce toxic accumulation of extracellular glutamate *(30)*. In response to AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptor stimulation, glial cells release D-serine, which stimulates the glycine site of the NMDA receptor *(31)*. Glial deficits may consequently produce glutamatergic hyperactivity. Because glial cells also release trophic factors that participate in the development of synaptic networks, abnormalities in glial function could contribute to the pathophysiology of mood disorders *(32)*.

The effect of antidepressants on glutamate release has been analyzed in some studies. One of them *(33)* showed that desipramine enhanced the spontaneous vesicular release of glutamate in cultured hippocampal neurons. In contrast, it was found in an ex vivo study *(34)* that imipramine markedly blunted glutamate overflow in the prefrontal cortex but not in the striatum, although similar effects were found after acute or chronic antidepressant treatment. Because antidepressants are only efficacious on chronic treatment, the significance of these findings is unclear.

Stress increases extracellular levels of glutamate in the prefrontal cortex, nucleus accumbens and hippocampus *(35–37)*. In adrenalectomized rats, this effect is reduced in the prefrontal cortex or blocked in the hippocampus, indicating that corticosterone is involved in the stress-induced elevation of extracellular glutamate levels in brain regions *(36,37)*. Further studies on the effect of chronic antidepressant treatment on enhanced extracellular glutamate levels induced by acute or chronic stress would be no doubt of interest at the time of assessing the neuroprotective effect of antidepressants on stress-induced neurotoxicity. It is of note that in a single magnetic resonance spectroscopy study, a decreased caudate glutamate resonance was found following paroxetine treatment for obsessive-compulsive disorder *(38)*.

It has been suggested that inhibition of glutamate release could be a valid approach in the treatment of depression. Repeated administration of lithium, the prototype mood stabilizer, promotes glutamate uptake and reduces glutamate receptor function *(39)*. Lamotrigine, an anticonvulsant agent that among other effects, reduces glutamate release has antimanic and antidepressant efficacy *(40)*. Clinical trials for the efficacy in major depression of riluzole, another inhibitor of glutamate release that is used for the treatment of amyotrophic lateral sclerosis, are in progress.

### 3.2. Changes in Glutamate Receptor Function

NMDA receptor dysfunction has been studied in postmortem samples from suicide victims, many of whom could have been depressed patients. A reduced binding to the glycine site of the NMDA receptor complex was found in suicide victims as compared to sudden-death controls *(41)*. This study has been questioned, however, as diagnoses of the

suicide victims, as well as previous pharmacological treatments, were in general unknown. In another postmortem study on suicide victims with a firm diagnosis of depression, no change in the binding characteristics of the noncompetitive NMDA receptor antagonist, $^{3}$H-MK-801, was found *(42)*.

Postmortem studies in the striatum of patients with major depression or bipolar disorder revealed only minimal changes in mRNA expression of the different NMDA and AMPA receptor subunits, the only significant change being a reduced GluR1 mRNA expression in bipolar disorder *(43)*. The striatal expression of excitatory amino acid transporters was also analyzed in mood disorders. A decrease in neuronal EAAT3 and EAAT4 mRNA in bipolar disorder and a reduced EAAT4 mRNA in major depression was found *(44)*.

Exposure to stress has been shown to increase mRNA levels of NR1 and NR2 subunits of the NMDA receptor and the GluR1 subunit of the AMPA receptor in the rat hippocampus, as well as the expression of NR1 and GluR1 in the ventral tegmental area *(45,46)*.

## 4. ANTIDEPRESSANT TREATMENT AND SYNAPTIC PLASTICITY

Recent hypotheses on the pathophysiology of depressive disorders involve adaptive plasticity of neural systems. As proposed by Duman and colleagues *(47)*, depression could result from an inability to make the appropriate responses to stress as a consequence of a dysfunction of the normal mechanisms underlying neural plasticity. It has been supposed for some time that plastic changes should be involved in antidepressant actions since there is a lag time of several weeks for the therapeutic effect of antidepressants whereas acute effects on monoamine transporters or monoamine-inactivating enzymes are of rapid onset (*see also* Chapter 9). Indeed, abundant evidence indicates that antidepressants exert key effects on cell-signaling pathways regulating neuroplasticity and cell survival.

### *4.1. Effect of Antidepressant Treatment on the Cyclic Adenosine Monophosphate Signaling System*

Chronic treatment with different classes of antidepressants, including selective 5-HT and NA reuptake inhibitors, upregulates the cyclic adenosine monophosphate (cAMP) transduction cascade leading to the activation of cAMP-dependent protein kinase A (PKA), which phosphorylates proteins with a key role in cell signaling *(47–49)*. One of them is the transcription factor CREB (cAMP response element-binding protein), which mediates many of the actions of the cAMP system on cell signaling (Fig. 1). The time course for the induction of CREB is consistent with the lag time for therapeutic effectiveness of antidepressant treatment *(50)*. Furthermore, overexpression of CREB in the rat dentate gyrus produced an antidepressant-like effect in the forced swim and learned helplessness tests *(51)*. Consistent with these data, it was found in a postmortem study that CREB was decreased in the temporal cortex of depressed patients and this effect was reversed by antidepressant treatment *(52)*.

### *4.2. Effect of Antidepressant Treatment on BDNF Expression*

Among the target genes of CREB is the BDNF, which contributes to cellular processes underlying neuronal plasticity and cell survival. Chronic administration of antidepressants with different primary mechanisms of action increases BDNF mRNA and its receptor trkB in the rat hippocampus and blocks the downregulation of BDNF mRNA in the hippocampus in response to restraint stress *(53–55)*. It is of interest that BDNF induces

antidepressant-like effects in two widely used animal models of depression such as the learned helplessness and forced swim test *(56)*. The notion that BDNF may be regulated by antidepressant treatments is supported by postmortem studies in the hippocampus of depressed patients. An increased BDNF expression was found in dentate gyrus, hilus, and supragranular regions of subjects receiving antidepressant medication *(57)*. Consistent with this finding, decreased serum BDNF levels were found in major depressed patients *(58)*. The mechanisms that underlie BDNF inhibition of cell death include activation of the MAP kinase cascade, which leads to phosphorylation of CREB and to increased expression of the antiapoptotic protein Bcl-2 and inactivation of the apoptotic protein Bad *(59)*. Activation of trkB receptor by BDNF also enhances cell survival through the phosphatidylinositol-3 kinase (PI-3K)/Akt pathway (ref. *60; see* Fig. 1). Interestingly, it was recently reported that chronic antidepressant treatment is also able to increase the intensity of Bcl-2 immunostaining in rat hippocampus *(61)*.

### *4.3. Effect of Antidepressants on Neurogenesis in Adult Brain*

Neurogenesis has been demonstrated in the adult mammalian brain from different animal species, including humans *(62)*. Neurogenesis is restricted to two brain areas, the olfactory bulb and the dentate gyrus of the hippocampus. The new neurons are derived from the subventricular zone or from the subgranular zone of the hippocampus. Stress activates the HPA axis with the consequent release of glucocorticoids, which downregulate neurogenesis in the hippocampus through downstream actions on NMDA receptors *(63)*. Glutamate, by acting at NMDA receptors, suppresses neurogenesis whereas NMDA antagonists, such as MK-801, enhance it *(64)*. Exposure to stress, including learned helplessness, a paradigm of inescapable stress, decreases neurogenesis *(63)* and, conversely, exposure to an enriched environment *(65)* and chronic, but not acute, treatment with different classes of antidepressants, and also with the mood-stabilizing agent lithium, increases the neurogenesis of dentate gyrus granule cells *(25,66)*. Both the cAMP signaling system and BDNF, which are upregulated by chronic antidepressant treatment *(50,53,67)*, play a role in the regulation of neurogenesis. It has been suggested *(68)* that the lag time of several weeks for the therapeutic effectiveness of antidepressants is consistent with the time taken by newly born dentate gyrus neurons to migrate and to become integrated into the existing brain circuitry.

## 5. INVOLVEMENT OF NMDA RECEPTORS IN ANTIDEPRESSANT ACTIONS

### *5.1. Physiological and Pathophysiological Role of NMDA Receptors*

Excitatory synaptic transmission is mediated by three distinct classes of ionotropic receptors—NMDA, AMPA, and kainate—and by the three groups of metabotropic glutamate receptors (*see* Chapters 4 and 5). It is widely accepted that excitatory amino acid receptors are involved in numerous aspects of both normal and abnormal brain function. Activation of NMDA and AMPA receptors appears to underlie the vast majority of fast excitatory transmission in the central nervous system (CNS). Synaptically released glutamate results in a two-component EPSC on binding to NMDA and AMPA receptors. Activation of AMPA receptors mediates a component of rapid onset and decay, whereas the activation of NMDA receptors is more prolonged probably owing to the higher affinity of glutamate for NMDA than for AMPA receptors, at least one order of magnitude. NMDA

receptors are highly permeable to $Ca^{2+}$, whereas AMPA receptors are only permeable when they have no GluR2 subunits.

The elevation of cytosolic $Ca^{2+}$, leads to the activation of a variety of enzymes, including kinases with a critical role in paradigms of synaptic plasticity, such as long-term potentiation (LTP) in the hippocampus *(69)*, a long-lasting enhancement in the strength of synaptic connections between neurons that represents a widely accepted model for learning and memory. NMDA receptors are critical for the induction of LTP. In the expression and maintenance of LTP, there is an increase in AMPA receptor function, so a sequential activation of the two major classes of glutamate ionotropic receptors is necessary for this paradigm of synaptic plasticity. The effects of exposure to various types of uncontrollable stress on glutamate-mediated hippocampal synaptic plasticity has been studied. In general, LTP in rodent hippocampus is impaired by behavioral stress *(70)*, including manipulations such as the inescapable stress of learned helplessness, an animal model for depression. It is not clear however that antidepressant treatment can restore stress-induced impairment in hippocampal synaptic plasticity.

Excessive activation of ionotropic glutamate receptors can precipitate seizures and induce acute neuronal injury (excitotoxicity) and may also underlie some chronic neurodegenerative disorders. A significant proportion of the neuronal death associated with intense glutamate exposure is mediated by NMDA receptor activation, probably because lethal amounts of $Ca^{2+}$ influx are induced more rapidly than in the case of AMPA or kainate receptor activation. Sustained elevation in intracellular $Ca^{2+}$ initiate toxic cascades that ultimately result in neuronal cell death through free-radical production and lipid peroxidation, activation of nitric oxide (NO) synthase, and release of NO, which interacts with reactive oxygen species to generate peroxynitrite and uncoupling of mitochondrial electron transport enhancing production of free radicals *(71)*.

### *5.2. Modulation of NMDA Receptors by Antidepressants*

Acute and chronic treatment with antidepressants affects NMDA receptors. Chronic antidepresant treatment inhibits the binding of the uncompetitive NMDA antagonist $^{3}$H-MK-801 to mouse brain membranes *(72)* and reduces NMDA-induced behaviour *(73)*. Chronic, but not acute, administration of most clinically effective antidepressants down-regulates the strychnine-insensitive glycine site of the NMDA receptor in cortical membranes *(74)*. A transcriptional mechanism has been suggested for this down-regulation, as antidepressants such as citalopram and imipramine are able to produce after chronic administration to mice a region-specific altered expression of mRNA for NMDA receptor subunits *(75)*. In the latter study, a reduced NR1 subunit mRNA expression was found in different cortical areas, including the frontal cortex, and in subcortical regions, including striatum and amygdala. In the hippocampus, NR1 expression was not altered by antidepressant treatment but the expression of NR2 subunits was reduced to a varying extent in hippocampal fields by the two antidepressants tested. These studies suggest that chronic antidepressant treatment would be abating NMDA receptor function through a reduction in the proportion of active glycine sites *(76)*. To our knowledge, no electrophysiological study has been however performed as yet to confirm such assertion.

NMDA receptor antagonists exert a protective effect on multiple neuronal insults *(71)*. By reducing NMDA receptor expression, chronic antidepressants should exert also

neuroprotective actions. Furthermore, chronic antidepressants promote the expression of the neurotrophin BDNF with trophic and neuroprotective properties. A link between NMDA receptors and BDNF was found in a study on primary neuronal cultures where BDNF reduced NR2A and NR2C mRNA levels with a concomitant decrease in NMDA-induced $Ca^{2+}$ entry *(77)*. On this basis, it has been suggested *(78)* that, by promoting BDNF formation and by antagonizing NMDA receptors, antidepressants reach an identical functional end point, which results in a protection of vulnerable neurons.

### 5.3. Antidepressant-Like Actions of NMDA Receptor Antagonists

As previously indicated, NMDA receptor activation is required for LTP in the hippocampus and inescapable stress impairs the induction of LTP. On the basis of the ability of antidepressants to antagonize the syndrome of learned helplessness induced by inescapable stress, an animal paradigm that models aspects of depression, Skolnick and colleagues first suggested the possible utility of NMDA receptor antagonists as antidepressants. In their initial studies using the so-called "behavioral despair" models (forced swim and tail suspension tests), a dose-dependent reduction in immobility was found with a competitive NMDA antagonist (AP-7), a glycine partial agonist (ACPC), and MK-801, an uncompetitive channel blocker *(79)*. Studies from this and other groups have later reported similar effects in various animal models with different NMDA antagonists such as memantine, a low-affinity uncompetitive NMDA antagonist, CGP-39551 and CGP-37849, competitive NMDA antagonists, and eliprodil an NR2B-selective antagonist (refs. *80–82*; Table 2). Synergistic effects of weak uncompetitive NMDA receptor antagonists (memantine and amantadine) with different antidepressants (imipramine, fluoxetine, venlafaxine) also have been reported *(83)*, suggesting the potential utility of these combinations in treatment-resistant depressed patients. It is of interest that one of the principal events in the neurotoxic cascade following NMDA receptor activation is NO production. NO synthase inhibitors also significantly reduce the immobility time in the forced swim test *(84)*, suggesting that an antidepressant-like effect can be obtained by any interruption of the NMDA receptor signaling cascade (cf. ref. *78*). However, a 5-HT-dependent mechanism also appears to be involved in the antidepressant-like effects of NO synthase inhibitors *(85)*.

### 5.4. Effects of NMDA Antagonists in Depressed Patients

There have been few clinical studies with NMDA antagonists in depression (Table 2). In one of them *(86)*, ketamine, an intravenous dissociative anaesthetic that uncompetitively blocks the NMDA receptor channel, was given on a double-blind basis to a cohort of patients unresponsive to conventional antidepressants and a significant reduction in the scores of the Hamilton Depression Rating Scale was found. Positive results in clinical studies have been also obtained with other low-affinity NMDA receptor antagonists, such as metapramine, amantadine, memantine, and also D-cycloserine, a partial agonist/antagonist, at the glycine site of the NMDA receptor (*see* reviews in refs. *29* and *78*). Further clinical studies with memantine in major depression are in progress, as well as clinical trials in treatment-resistant bipolar depression with the anticonvulsant felbamate, an NMDA antagonist at the glycine site. Because highly potent NMDA receptor antagonists such as MK-801 or ketamine elicit a number of psychotomimetic side effects *(87)*, more subtle approaches aimed at dampening NMDA receptor function are probably necessary.

**Table 2**
**Preclinical and Clinical Data on the Antidepressant Efffect of Modulators of Glutamatergic Neurotransmission[a]**

| *Preclinical studies* | | | |
|---|---|---|---|
| Compound | Forced swim test (rats, mice) | Tail suspension test (mice) | Chronic mild stress (rats) |
| ***NMDA antagonists*** | | | |
| MK-801 | + | + | + |
| AP-7 | + | – | |
| ACPC | + | + | + |
| CGP 37849 | + | | + |
| CGP 40116 | | | + |
| CGP 39551 | + | | |
| Eliprodil | + | – | |
| Memantine | + | | |
| ***AMPA potentiators*** | | | |
| LY 392098 | + | + | |
| LY 404187 | + | + | |
| LY 451616 | + | + | |

| *Clinical trials* | | |
|---|---|---|
| Compound | Indication | Development status |
| ***NMDA antagonists*** | | |
| Ketamine | Major depression | Phase II |
| Memantine | Major depression | Phase III |
| Felbamate | Resistant bipolar disorder | Phase II |
| ***Glu release inhibitor*** | | |
| Riluzole | Major depression | Phase II |

[a](+) Significant effect; (–) nonsignificant effect.
NMDA, *N*-methyl-D-aspartate; AMDA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate.

## 6. INVOLVEMENT OF AMPA RECEPTORS IN ANTIDEPRESSANT ACTIONS

### *6.1. Physiological Role of AMPA Receptors*

AMPA receptors mediate most of the fast excitatory neurotransmission in mammalian brain and are an important target for mechanisms controlling synaptic strength. As already mentioned (Subheading 5.1.) an increase in AMPA receptor function is necessary for the expression and maintenance of LTP, a lasting enhancement in the strength of synaptic connections between neurons. Another main mediator of synaptic plasticity is the neurotrophin BDNF, which is induced by AMPA receptor activation, an effect found initially in vitro *(88)* and also in vivo *(89)*. Because chronic antidepressant treatment increases BDNF in the hippocampus, probably through activation of the cAMP transduction pathway and phosphorylation of the transcription factor CREB, there appears to be a close relationship between the effects of antidepressants and AMPA receptor activation, suggesting the interest of these ionotropic receptors in the search for new antidepressants.

### 6.2. Effect of Antidepressants on AMPA Receptors

Antidepressant treatment may potentiate AMPA-mediated transmission. It has been found that repeated electroconvulsive treatment increases GluR1 mRNA expression in different fields of the rat hippocampus *(90)*. Fluoxetine increases phosphorylation of the GluR1 subunit, preferentially at the Ser-845 PKA site *(91)*, a change that contributes to maintaining AMPA receptors at the synapses. We have found that chronic antidepressant treatment with either paroxetine, a selective 5-HT reuptake inhibitor, or desipramine, which is a more selective noradrenaline reuptake inhibitor, produced an increased expression of the AMPA receptor subunits GluR1 and GluR2/3 in rat hippocampus *(92)*. This effect was observed after chronic antidepressant treatment for 21 d but not after acute treatment and was restricted to membrane extracts and not to total protein extracts from rat hippocampus, suggesting a trafficking of these subunits from intracellular pools to synaptic sites. Changes in phosphorylation systems induced by chronic antidepressant treatment may account for the membrane insertion of AMPA receptors. Among the changes in phosphorylating enzymes, an increase of calcium/calmodulin-dependent protein kinase II (CaMKII) activity at postsynaptic sites *(93)* may result in the incorporation of GluR1-containing AMPA receptors into the synaptic membrane, where the upregulation of protein kinase A (PKA) by chronic antidepressants *(67)* contributes to prevent endocytosis of the membrane-inserted receptors. The increased number of AMPA receptors at the synapses may be a mechanism to enhance the strength of synaptic transmission. In subsequent immunoprecipitation studies (Frechilla et al., unpublished results), we found that desipramine increased the interaction of the GluR2/3 subunits of the AMPA receptor with the *N*-ethylmaleimide sensitive factor (NSF), which plays a critical role in protein trafficking, and also of the GluR1 subunit with the protein SAP 97, involved in the synaptic insertion of AMPA receptors, providing a mechanism for the enhanced expression of AMPA receptor subunits in hippocampal membranes. Because repeated antidepressant administration is required for increased expression of BDNF and its receptor trkB in the hippocampus and also for regulating AMPA receptor insertion into the synapses, the sequential correlation between both effects remains to be established *(55)*. AMPA receptor activation promotes BDNF expression but, reciprocally, BDNF increases the surface expression of AMPA receptor subunits *(94)*. The interplay between both molecular effectors probably represents a major contribution to the enhanced synaptic plasticity in the hippocampus induced by chronic antidepressant treatment.

### 6.3. AMPA Receptor Potentiators

AMPA receptor potentiators or Ampakines are compounds able to increase AMPA-mediated excitatory postsynaptic responses and to reduce the rate of desensitization/deactivation of the ligand-gated ionic channel *(95)*. Several classes of Ampakines have been identified, including benzothiazides, such as cyclothiazide, pyrrolidones with nootropic effect, such as piracetam and aniracetam, benzoylpiperidines (CX-516, CX-614) and, more recently, biarylpropylsulfonamide derivatives (LY392098 and LY404187). Different Ampakines (cyclothiazide, CX-614, LY392098, LY404187) are able to potentiate the AMPA-stimulated increase in the expression of BDNF or to potentiate BDNF expression by themselves. This effect has been found in vitro and also in vivo, after daily administration for 5–7 d, in hippocampal subfields, notably in the dentate gyrus *(88,96,97)*. Increased BDNF mRNA is blocked by selective antagonists, of

AMPA receptors, such as NBQX, but not by NMDA receptor antagonists, such as MK-801 *(96)*. Chronic administration of LY451646 also increased, like clinically effective antidepressants, progenitor cell proliferation in the rat dentate gyrus in a dose-dependent manner *(98)*. Ampakines are not able to affect AMPA channel opening in the absence of glutamate or other AMPA receptor agonist, so it is to be supposed that these compounds also augment glutamate levels in neuronal cultures. AMPA receptor-mediated increase in BDNF expression has been linked to activation of voltage-sensitive L-type calcium channels because this increase can be blocked by nimodipine, a typical calcium channel blocker *(77)*. Increase in $[Ca^{2+}]_i$ can then activate BDNF expression through multiple mechanisms including the activation of calcium response elements located in the promoter region of the BDNF gene *(99)*. Activation of a MAP kinase pathway that may be activated by Lyn kinase (a member of the Src family of protein tyrosine kinases) may be also involved in the increased BDNF expression induced by AMPA receptor potentiators *(77,100)*.

Ampakines not only promote, like chronic antidepressants, BDNF expression but the biarylpropylsulfonamides LY392098 and LY404187 are also effective in animal models of depression with high predictive validity such as the forced swim test and the tail suspension test (refs. *77* and *101*; *see* Table 2). The antidepressant-like effect of this class of compounds can be blocked by the noncompetitive AMPA receptor antagonist LY300168. The rapid antidepressant-like effect of Ampakines in these behavioral despair tests do not appear in principle to be linked to a significant increase in BDNF levels. Some studies have shown however that BDNF can be induced as an immediate early gene, in only 30 min, in response to behavioral manipulation *(102)*. Potentiation of AMPA-mediated glutamatergic transmission may consequently exert an antidepressant-like effect, probably mediated through an increased expression of the neurotrophin BDNF.

## 7. METABOTROPIC GLUTAMATE RECEPTORS AND ANTIDEPRESSANT TREATMENT

Metabotropic glutamate receptors form a family divided into three subgroups. Group I includes the subtypes mGlu1/5 coupled to PI hydrolysis, group II includes mGlu2/3 coupled to $G_i$ proteins, and group III includes mGlu4/6/7/8, also coupled to $G_i$ proteins in heterologous expression systems (*see* Chapter 5). It has been shown that repeated imipramine treatment attenuates the neuronal responsiveness to the selective group 1 mGlu receptor agonist dihydroxyphenylglycol (DHPG) in the CA1 field of rat hippocampus with a time course correlated with the delayed therapeutic effect of antidepressants in humans *(103)*. It is of note that in this study the attenuation of the response to DHPG was still detectable 1 wk after imipramine withdrawal. Chronic imipramine, and also chronic ECT, enhanced the expression of group I mGlu receptors, located postsynaptically and generally connected with the enhancement of glutamatergic transmission, in different rat hippocampal fields. The most pronounced effects were the increased expression of the splice variants mGluR1a in CA3 and mGluR5a in CA1 *(104)*. An antidepressant-like activity has been found with a mGlu5 receptor antagonist, MPEP, although it is possible that this effect is rather related to the additional interaction of this compound with the serotonergic system *(105)*.

An upregulation of mGlu2/3 receptor protein in the hippocampus, cerebral cortex, striatum, and nucleus accumbens was found in rats chronically, but not acutely, treated

with imipramine *(106)*. Some functional effects associated with mGlu receptors such as the amplified PI response to combined activation of group 1 and group 2 mGlu was also enhanced by the chronic antidepressant treatment. It has been proposed that endogenous activation of group II mGlu receptors negatively modulate the activity of the HPA axis *(107)*. Accordingly, these findings suggest that agonists at these receptors would oppose the effects of stress.

## 8. CONCLUSIONS AND FUTURE DIRECTIONS

The monoaminergic hypothesis of depression has provided the basis for extensive research into the pathophysiology of mood disorders and has been of great significance for the development of effective antidepressants. Current antidepressant treatments not only increase serotonin and/or noradrenaline bioavailability but also originate adaptive changes increasing synaptic plasticity. Novel approaches to depression and to antidepressant therapy are now focused on intracellular targets that regulate neuroplasticity and cell survival. Accumulating evidence indicates that there is an anatomical substrate for such a devastating neuropsychiatric disease as major depression. Loss of synaptic plasticity and hippocampal atrophy appear to be prominent features of this highly prevalent disorder. A combination of genetic susceptibility and environmental factors make hippocampal neurons more vulnerable to stress.

Abundant experimental evidence indicates that stress causes neuronal damage in brain regions, notably in hippocampal subfields. Stress-induced activation of glutamatergic transmission may induce neuronal cell death through excessive stimulation of NMDA receptors. Both standard antidepressants and NMDA receptor antagonists are able to prevent stress-induced neuronal damage. NMDA antagonists are effective in widely used animal models of depression and some of them appear to be effective also in the few clinical trials performed to date.

Chronic antidepressant treatment increases the expression in hippocampal subfields of the neurotrophin BDNF, which promotes processes underlying neuronal plasticity and cell survival. Antidepressants also increase AMPA receptor insertion into synapses of the hippocampus, a mechanism contributing to enhanced synaptic strength and to increased BDNF expression. The interplay between AMPA receptors and BDNF appears to be a key factor in the enhanced synaptic plasticity induced in the hippocampus by chronic antidepressants. In this context, the development of AMPA receptor potentiators, which promote BDNF expression and show antidepressant-like effects in animal models, may represent a novel approach to the treatment of mood disorders.

We are still far from understanding the complex cellular and molecular events involved in mood disorders. Yet, there appears to be an emerging role for glutamate neurotransmission in the search for the pathogenesis of major depression. Mechanisms for potentiation of AMPA-mediated neurotransmission, for attenuation of NMDA receptor function, and for increased neurotrophic factor signaling appear to be promising targets in the search for a more effective antidepressant therapy.

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