Regulation of cellular plasticity and resilience by mood stabilizers: the role of AMPA receptor trafficking

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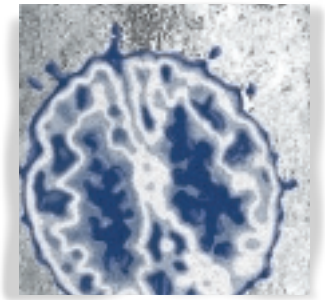

Despite the devastating impact that mood disorders have on the lives of millions worldwide, there is still a dearth of knowledge concerning their underlying etiology and pathophysiology. The brain systems that have heretofore received the greatest attention in neurobiological studies of mood disorders have been the monoaminergic neurotransmitter systems, which are extensively distributed throughout the network of limbic, striatal, and prefrontal cortical neuronal circuits thought to support the behavioral and visceral manifestations of mood disorders.[1-3] Thus, clinical studies over the past 40 years have attempted to uncover the specific defects in these neurotransmitter systems in mood disorders by utilizing a variety of biochemical and neuroendocrine strategies.

There is increasing evidence from a variety of sources that severe mood disorders are associated with regional reductions in brain volume, as well as reductions in the number, size, and density of glia and neurons in discrete brain areas. Although the precise pathophysiology underlying these morphometric changes remains to be fully elucidated, the data suggest that severe mood disorders are associated with impairments of structural plasticity and cellular resilience. In this context, it is noteworthy that a growing body of data suggests that the glutamatergic system (which is known to play a major role in neuronal plasticity and cellular resilience) may be involved in the pathophysiology and treatment of mood disorders. Glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) GluR1 receptor trafficking plays a critical role in regulating various forms of neural plasticity. It is thus noteworthy that recent studies have shown that structurally dissimilar mood stabilizers lithium and valproate regulate GluR1 receptor subunit trafficking and localization at synapses. These studies suggest that regulation of glutamatergically mediated synaptic plasticity may play a role in the treatment of mood disorders, and raises the possibility that agents more directly affecting synaptic GluR1 represent novel therapies for these devastating illnesses.

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Selected abbreviations and acronyms

AMPA	*α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid*
CAMKII	*calcium/calmodulin-dependent protein kinase II*
EAAT	*excitatory amino acid transporter*
LTD	*long-term depression*
LTP	*long-term potentiation*
NMDA	N-*methyl-D-aspartate*
MAPK	*mitogen-activated protein kinase*
PCP	*phencyclidine*
PKA	*protein kinase A*
PP1	*protein phosphatase 1*
WMH	*white matter hyperintensities*

While such investigations have been heuristic over the years, they have been of limited value in elucidating the unique biology of mood disorders, which must include an understanding of the underlying basis for the predilection to episodic and often-profound mood disturbance, which can become progressive over time. These observations have led to the appreciation that, while dysfunction within the monoaminergic neurotransmitter systems is likely to play important roles in mediating some components of the pathophysiology of mood disorders, they do not fully explain all the facets of these complex neuropsychiatric disorders.[4,5]

In addition to the acknowledgement that investigations into the pathophysiology of complex mood disorders have been excessively focused on monoaminergic systems, there has been a growing appreciation that progress in developing truly novel and improved medications has consequently also been limited. A recognition of the clear need for better treatments and the lack of significant advances in our ability to develop novel, improved therapeutics for these devastating illnesses has led to the investigation of the putative roles of intracellular signaling cascades and nonaminergic systems in the pathophysiology and treatment of mood disorders. Consequently, recent evidence demonstrating that impairments of neuroplasticity may underlie the pathophysiology of mood disorders, and that antidepressants and mood stabilizers exert major effects on the signaling pathways that regulate cellular plasticity and resilience, have generated considerable excitement among the clinical neuroscience community, and are reshaping views about the neurobiological underpinnings of these disorders.[1,2,6-8]

Somewhat surprisingly, the potential role of the glutamatergic system in the pathophysiology and treatment of bipolar disorder has only recently begun to be investigated in earnest. Glutamate is the major excitatory synaptic neurotransmitter regulating numerous physiological functions in the mammalian central nervous system (CNS), such as synaptic plasticity, learning, and memory, and represents a major neurotransmitter system in the circuitry thought to subserve many of the symptoms of severe, recurrent mood disorders.[3] In this perspectives paper, we review the growing body of data that suggests that severe mood disorders are associated with impairments of cellular plasticity and resilience, effects that may arise from perturbations of neurotrophic signaling cascades and the glutamatergic system. We follow with a discussion of the emerging data that suggests regulating the balance of glutamatergic throughput via *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors may play an important role in the actions of our most effective thymoleptic agents, and represent very attractive targets for the development of novel therapeutics for these devastating disorders.

What is the evidence for impairments of cellular plasticity and resilience in severe mood disorders?

Structural imaging studies have demonstrated reduced gray matter volumes in areas of the orbital and medial prefrontal cortex (PFC), ventral striatum, and hippocampus, and enlargement of third ventricle in mood-disordered patients relative to healthy control samples.[3,9,10] Postmortem neuropathological studies have shown abnormal reductions in glial cell counts/density, neuron size/density, and cortical volume/thickness in the subgenual PFC, orbital cortex, dorsal anterolateral PFC, amygdala, and in basal ganglia and dorsal raphe nuclei and hippocampus.[11-16] Morphometric studies also have reported layer-specific reductions in interneurons in the anterior cingulate cortex (ACC), and reductions in non-pyramidal neurons (~40% lower) in CA2 of the hippocampal formation in bipolar disorder subjects compared with controls.[17] Overall, the layer-specific cellular changes observed in several distinct brain regions, including the PFC, ACC, and hippocampus suggest that multiple neuronal circuits underlie the neuropathology of mood disorders. This is not altogether surprising since the behavioral and physiological manifestations of the illnesses are complex and include cognitive, affective,

motoric, and neurovegetative symptomatology, as well as alterations of circadian rhythms and neuroendocrine systems, and are thus undoubtedly mediated by networks of interconnected neurotransmitter systems and neural circuits.[13] In addition to the accumulating neuroimaging evidence, several postmortem brain studies are now providing direct evidence for reductions in regional CNS volume, cell number, and cell body size. Baumann and associates[18] reported reduced volumes of the left nucleus accumbens, the right putamen, and bilateral pallidum externum in postmortem brain samples obtained from patients with unipolar depression or bipolar depression. The abnormal presence of white matter hyperintensities (WMH) has been reported in multiple magnetic resonance imaging (MRI) studies of geriatric patients with affective disorder, particularly those with late-onset depression (ie, elderly depressed patients who experience their first depression after age 60). Elderly adults (>60 years old) with severe WMH are 3 to 5 times more likely to have depressive symptoms as compared with persons with only mild or no white matter lesions.[19] Tupler and colleagues[20] reported that late-onset depressed patients had more severe hyperintensity ratings in deep white matter than early-onset patients and controls, and that late- and early-onset patients had more severe subcortical gray matter hyperintensities (particularly in the putamen) compared with controls. Recently, Silverstone and colleagues[21] reported that bipolar patients showed more severe deep WMH on brain MRI than age-matched unipolar and control subjects. WMH severity has been suggested to predict poorer response to antidepressant therapy.[22] In fact, these lesions have been also found to be increased in children with psychiatric disorders, but are highest among bipolar patients, when compared with controls, particularly in the frontal lobes,[23] and also early in the course of bipolar illness in adolescent subjects.[24] Although the cause of WMH in mood disorders is unknown, their presence—particularly in the brains of young bipolar patients—suggests importance in the pathophysiology of the disorder.[25,26] Together, these results support the contention that WMH indicate damage to the structure of brain tissue, and likely disruption of the neuronal connectivity necessary for normal affective functioning.

It is not known whether these structural brain changes seen in patients with severe mood disorders constitute developmental abnormalities that may confer vulnerability to abnormal mood episodes, compensatory changes to other pathogenic processes, or the sequelae of recurrent affective episodes per se. Understanding these issues will partly depend upon experiments that delineate the onset of such abnormalities within the illness course and determine whether they antedate depressive episodes in individuals at high familial risk for mood disorders. Nevertheless, these prominent atrophic changes and impairments of plasticity have drawn much attention to the glutamatergic system, since—as we discuss in detail below—the glutamatergic system is known to play critical roles in regulating various forms of plasticity. Furthermore, as is discussed extensively in this issue and elsewhere,[27] alterations in glutamatergic signaling, mediated by both NMDA and non-NMDA receptors, are known to play important roles in stress-induced morphometric brain changes.[14,28,29] Since some clinicians may be less familiar with the intricacies of the regulation of glutamate receptor subtypes, we now present a brief overview of the functioning and regulation of NMDA and AMPA glutamatergic receptors. We follow with a discussion of the exciting emerging data suggesting that glutamatergic signaling represents a very attractive target for the development of novel therapeutics for severe mood disorders.

A primer on glutamatergic signaling: critical roles in cellular plasticity and resilience

As the principal mediator of excitatory synaptic transmission in the mammalian brain, glutamate participates in wide-ranging aspects of both normal and abnormal CNS function. Unlike the monoamines, which require transport of amino acids through the blood–brain barrier, glutamate and aspartate cannot adequately penetrate into the brain from the periphery and are produced locally by specialized brain machinery.[30] The metabolic and synthetic enzymes responsible for the formation of these nonessential amino acids are located in glial cells as well as neurons.[30,31] The major metabolic pathway in the production of glutamate is derived from glucose and the transamination of α-ketoglutarate; however, a small proportion of glutamate is formed directly from glutamine. The latter is actually synthesized in glia, via an active process (requiring adenosine triphosphate [ATP]), and is then transported to neurons where glutaminase is able to convert this precursor to glutamate *(Figure 1)*. Following release, the concentration of glutamate in the extracellular space is highly

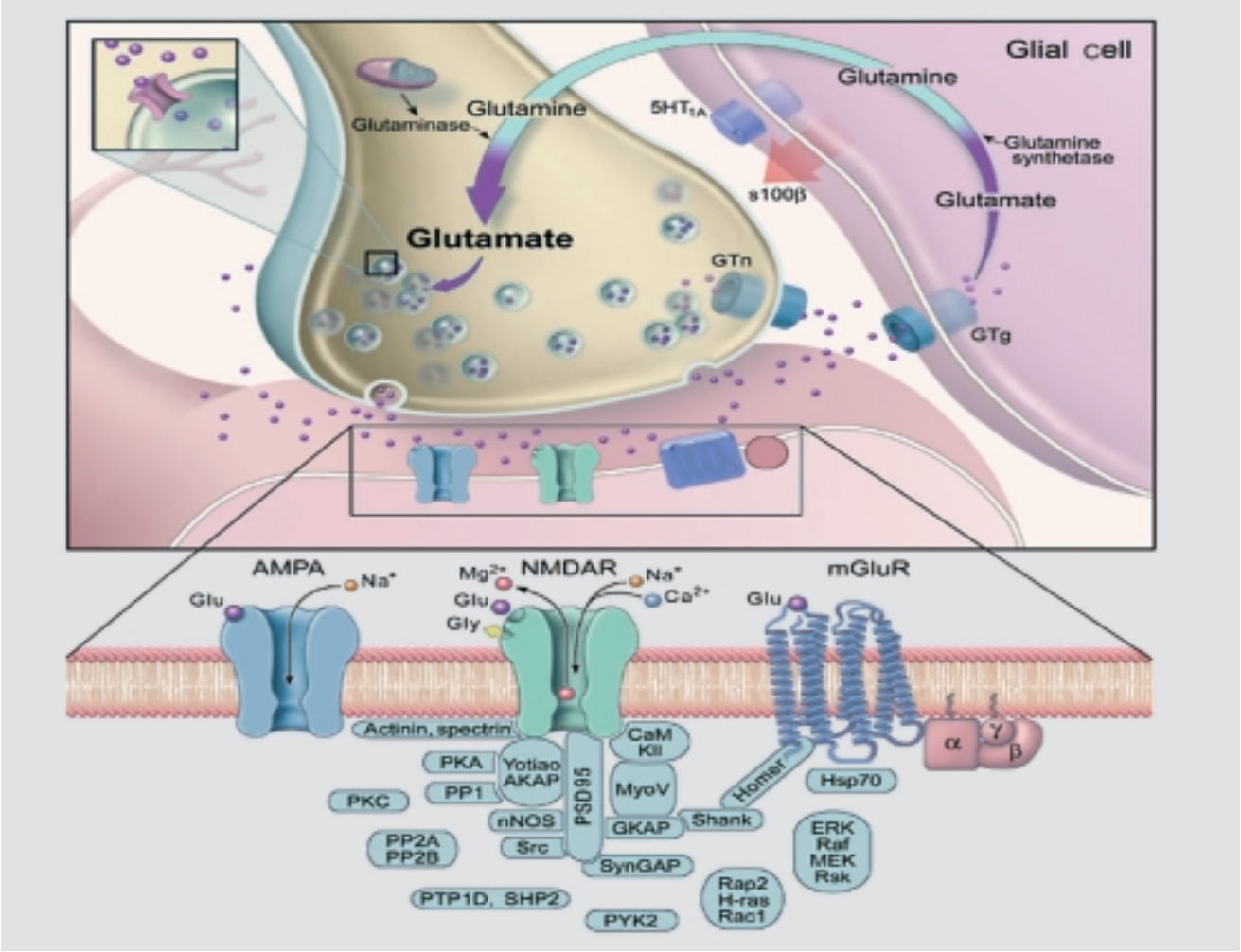

Figure 1. Glutamatergic system. This figure depicts the various regulatory processes involved in glutamatergic neurotransmission, as described in the text. In astrocytes, glutamine can undergo oxidation to yield α-ketoglutarate, which can also be transported to neurons and participate in glutamate synthesis. Glutamate is either metabolized or sequestered and stored into secretory vesicles by vesicular glutamate transporters (VGluT). Glutamate can then be released by a calcium-dependent excitotoxic process. Glutamate has its action terminated in the synapse by reuptake mechanisms utilizing distinct GLU transporters (GLUTs), which exist not only on presynaptic nerve terminals, but also on astrocytes; indeed, current data suggests that astrocytic glutamate uptake may be more important for clearing excess glutamate, raising the possibility that astrocytic loss (as has been documented in mood disorders) may contribute to deleterious GLU signaling, but more so by astrocytes. It is now known that there are a number of important intracellular proteins, which are able to alter the function of glutamate receptors. Gly, glycine; GTn, glutamate transporter; GTg, glutamate transporter (glial); 5-HT_{1A}, 5-hydroxytryptamine (serotonin) receptor 1A; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NMDAR, NMDA receptor; PKA and PKC, protein kinase A and C; PP1, PP2A, and PP2B, protein phosphatase 1, 2A, and 2B; Yotiao, NMDA receptor accessory protein; AKAP, protein A kinase anchoring protein; nNOS, nitric oxide synthetase; Src, a family of protein tyrosine kinases; PTP1D, protein-tyrosine phosphatase 1D; SHP2, src homology 2 domain–containing tyrosine phosphatase; PSD95, postsynaptic density protein 95; CAMKII, calcium/calmodulin-dependent protein kinase II; MyoV, myosin V; SynGAP, Ras guanosine triphosphatase (GTPase)–activating protein; GKAP, guanylate kinase–associated protein; PYK2, proline-rich tyrosine kinase-2; Shank; Shank family of multidomain proteins; Homer, a family of dendritic multidomain proteins; Rap2, a small GTPase; H-ras, Harvey rat sarcoma viral oncogene homologue; Rac1, a Rho family GTPase; ERK, extracellular signal–regulated kinase; Raf, MEK, and Rsk, ribosomal S6 kinases; Hsp70, heat-shock protein 70.

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regulated and controlled, primarily by a sodium-dependent reuptake mechanism involving several transporter proteins. The major glutamate transporter proteins found in the CNS include excitatory amino acid transporters (EAATs): EAAT1 (or GLAST-1), EAAT2 (or GLT-1), and EAAT3 (or EAAC1), with EAAT2 being the most predominantly expressed form in the forebrain. Additionally, these transporters are differentially expressed in specific cell types, with EAAT1 and EAAT2 being found primarily in glial cells, EAAT3 localized in neurons, and EAAT4 mainly localized in cerebellum. The physiological events regulating the activity of the glutamate transporters are not well understood, though there is evidence that phosphorylation of the transporters by protein kinases may differentially regulate glutamate transporters and therefore glutamate reuptake (discussed in reference 30). Glutamate concentrations have been shown to rise to excitotoxic levels within minutes following traumatic or ischemic injury, and there is evidence that the function of the glutamate transporters becomes impaired under these excitotoxic conditions.[32] Moreover, microdialysis studies have shown that severe stress increases extracellular levels of glutamate in hippocampus, and NMDA glutamate receptor antagonists attenuate stress-induced atrophy of CA3 pyramidal neurons.

Glutamate receptor subtypes: a focus on NMDA and AMPA receptors

The many subtypes of glutamatergic receptors in the CNS can be classified into two major subtypes—the ionotropic and metabotropic receptors *(Table I)*. The ionotropic glutamate receptor ion channels are assemblies of homo-oligomeric or hetero-oligomeric subunits integrated into the neuron's membrane. Every channel is assembled of (most likely) four subunits associated into a dimer of dimers, as has been observed in crystallographic studies.[33,34] Every subunit consists of an extracellular amino terminal and ligand-binding domain, three transmembrane domains and a re-entrant pore loop (located between the first and second transmembrane domains), and an intracellular carboxyl terminal domain.[35] The subunits associate through interactions between their amino terminal domains forming a dimer that undergoes a second dimerization mediated by interactions between the ligand-binding domains and/or between transmembrane domains.[33,34] Three different subgroups of glutamatergic ion channels have been identified utilizing their pharmacological ability to bind different synthetic ligands, each of which is composed of a different set of subunits. These are the NMDA receptor (NMDAR), the AMPA receptor (AMPAR), and the kainate receptor (KAR). The latter two groups are often referred to together as the "non-NMDA" receptors, but undoubtedly subserve unique functions *(Table I)*. In the adult mammalian brain, NMDA and AMPA glutamatergic receptors are colocalized in approximately 70% of the synapses.[36] By contrast, at early stages of development, synapses are more likely to contain only NMDA receptors. Radioligand binding studies have shown that NMDA and AMPA receptors are found in high density in the cerebral cortex, hippocampus, striatum, septum, and amygdala.

Ionotropic receptors	
NMDA	• NR1
	• NR2A, NR2B, NR2C, NR2D
	• NR3A, NR3B
AMPA	• GluR1, GluR2, GluR3, GluR4
Kainate	• GluR5, GluR6, GluR7
	• KA1, KA2
Metabotropic receptors	
Group I	• mGlu1a, mGlu1b, mGlu1c, mGlu1d
Group II	• mGlu2, mGlu3
Group III	• mGlu4a, mGlu4b, mGlu4c, mGlu4d
	• mGlu6
	• mGlu7a, mGlu7b, mGlu7c, mGlu7d
	• mGlu8a, mGlu8b, mGlu8c, mGlu8d

Table I. Receptor subtype units. Once released from the presynaptic terminal, glutamate is able to bind to numerous excitatory amino acid (EAA) receptors, including both ionotropic (eg, *N*-methyl-D-aspartate [NMDA]) and metabotropic receptors. Presynaptic regulation of glutamate release occurs through metabotropic glutamate receptors (mGluR2 or mGluR3), which subserve the function of autoreceptors. However, these receptors are also located on the postsynaptic element. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid.

NMDA receptors

The NMDA receptor is activated by glutamate and requires the presence of a coagonist, namely glycine or D-serine, to be activated. However, the binding of both glutamate and glycine is still not sufficient for the NMDA receptor channel to open, since, at resting membrane potential, the NMDA ion channel is blocked by Mg^{2+} ions. Only when the membrane is depolarized (eg, by the activation of AMPA or kainate receptors on the same postsynaptic neuron) is the Mg^{2+} blockade relieved. Under these conditions, the NMDA receptor channel will open and permit the entry of both Na^{+} and Ca^{2+} *(Figure 1)*.

The NMDA receptor channel is composed of combination of NR1, NR2A, NR2B, NR2C, NR2D, NR3A, and NR3B subunits *(Table I)*. The binding site for glutamate has been localized to the NR2 subunit and the site for the coagonist glycine has been localized to the NR1 subunit, which is required for receptor function. Two molecules of glutamate and two of glycine are thought to be necessary to activate the ion channel. Within the ion channel, two other sites have been identified called the sigma (σ) site and the phencyclidine (PCP) site. The hallucinogenic drug PCP, ketamine, and the experimental drug dizocilpine (MK-801), all bind at the latter site and are considered noncompetitive receptor antagonists that inhibit NMDA receptor channel function. In preclinical studies, drugs of this type have been shown to have neuroprotective properties against anoxia and hypoglycemia; these studies await clinical confirmation. In clinical psychiatric studies, ketamine has been shown to transiently induce psychotic symptoms in schizophrenic patients, and to produce rapid antidepressant effects in depressed patients.[37] These latter observations have led to the investigation of NMDA antagonists as putative novel antidepressants.[29,37]

NMDA receptors play a critical role in regulating synaptic plasticity.[38] The best-studied forms of synaptic plasticity in the CNS are long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission. The molecular mechanisms of LTP and LTD have been extensively characterized and have been proposed to represent cellular models of learning and memory.[38] Induction of LTP and LTD in the CA1 region of the hippocampus and in many regions of the brain has now clearly been demonstrated to be dependent on NMDA receptor activation. During NMDA-receptor–dependent synaptic plasticity, Ca^{2+} influx through NMDA receptors can activate a wide variety of kinases and/or phosphatases that, in turn, modulate synaptic strength. An important recent development is the finding that two of the primary molecules involved—Ca^{2+}/ calmodulin-dependent protein kinase II (CAMKII) and the NMDA subtype of glutamate receptor—form a tight complex with each other at the synapse.[39] Interestingly, this binding appears to enhance both the autophosphorylation of the kinase and the ability of the entire holoenzyme, which has 12 subunits, to become hyperphosphorylated.[39] This hyperphosphorylated state has been postulated to represent a "memory switch," which can lead to long-term strengthening of the synapse by multiple mechanisms. One important mechanism involves direct phosphorylation of the glutamate-activated AMPA receptors, which increases their conductance. Furthermore, once CAMKII is bound to the NMDA receptor, it may organize additional anchoring sites for AMPA receptors at the synapse.

It is intriguing that activation of synaptic NMDA receptor versus nonsynaptic receptor has an opposite effect on cell survival via differential regulation of CREB (cyclic adenosine monophosphate [cAMP]–response element binding protein) function. Calcium entry through synaptic NMDA receptors induced CREB activity and brain-derived neurotrophic factor (BDNF) gene expression as strongly as did stimulation of L-type calcium channels. In contrast, calcium entry through nonsynaptic NMDA receptors, triggered by glutamate exposure or hypoxic/ischemic conditions, activated a general and dominant CREB shut-off pathway that blocked induction of BDNF expression. Synaptic NMDA receptors have antiapoptotic activity, whereas stimulation of extrasynaptic NMDA receptors caused loss of mitochondrial membrane potential (an early marker for glutamate-induced neuronal damage) and cell death.[40]

AMPA receptor trafficking plays critical roles in the regulation of various forms of neural plasticity

The AMPA receptor is stimulated by the presence of glutamate and characteristically produces a fast excitatory synaptic signal that is responsible for the initial reaction to glutamate in the synapse. In fact, as discussed above, it is generally believed that it is the activation of the AMPA receptor that results in neuronal depolarization sufficient to liberate the Mg^{2+} cation from the NMDA receptor, thereby permitting its activation. The AMPA receptor channel is composed of the combination of GluR1, GluR2, GluR3, and GluR4 subunits, and requires two molecules of glutamate to be activated *(Table I)*. AMPA receptors have a lower affinity for glutamate than the NMDA receptor, thereby allowing for more rapid dissociation of glutamate and therefore a rapid deactivation of the AMPA receptor (reviewed in reference 41).

Emerging data suggest that AMPA receptor trafficking, including receptor insertion and internalization, and delivery to synaptic sites, provides an elegant mechanism for activity-dependent regulation of synaptic strength. AMPA receptor subunits undergo constitutive endocytosis and exocytosis; however, the process is highly regulated with a

variety of signal transduction cascades being capable of producing short- or long-term changes in synaptic surface expression of AMPA receptor subunits. Indeed, although the mechanisms of LTP and LTD have not been completely elucidated, it is widely accepted that AMPA receptor trafficking is the key player in these phenomena.
Most importantly for the present discussion, AMPA receptor trafficking is highly regulated by the protein kinase A (PKA), protein kinase C (PKC), CAMKII, and mitogen-activated protein kinase (MAPK) signaling cascades; these are the very same signaling cascades that mood stabilizers and antidepressants exert major effects on.[42-45] These observations have led to an extensive series of studies, which have clearly demonstrated that AMPA receptor trafficking is highly regulated by antidepressants and mood stabilizers[46,47] (see below).

Regulation of AMPA receptor trafficking by signaling cascades

Most vesicle trafficking requires the ordered coating of a donor membrane, budding and fusion to form transport vesicles, transport by passive or active delivery along microtubule, and final fusion with the target membrane.[48] AMPA receptors adopted this mechanism to be delivered to the neuronal membrane surface. AMPA receptors are multimeric assemblies of the subunits GluR1 to GluR4. Each subunit is composed of N-terminal extracellular domain, membrane-spanning domain, and C-terminal intracellular domain.[49,50] AMPA receptor trafficking is subunit-specific and regulated by phosphorylation of its C-terminal domain, and subsequent alteration of protein-protein interactions.

PKA pathway

The GluR1 subunit appears to govern the trafficking behavior of heteromeric GluR1/GluR2 receptors, preventing constitutive exchange and conferring inducible delivery of the heteromer.[51] Phosphorylation of GluR1 at the PKA site p845 facilitates the insertion of GluR1 onto the membrane and synapses, and is often associated with LTP.[52] Dephosphorylation of the GluR1 by protein phosphatases (eg, calcineurin and protein phosphatase 1 [PP1]) target GluR1 to recycling endosomes, where rephosphorylation by PKA may occur and the receptors will be reinserted onto the membrane.[53] Phosphorylation of GluR1 at PKA site can be enhanced by synapse-associated protein 97 (SAP97)/protein A kinase anchoring protein (AKAP79) complex that direct PKA to GluR1 via a PDZ (PSD95, disk large, ZO1) domain interaction.[54]

CAMKII pathway

Numerous studies have demonstrated that CAMKII is required for the proper formation of LTP in slice preparations, and in regulating learning and memory in rodents.[55] In response to stimulation, CAMKII translocates to the postsynaptic site, where it has two major effects on AMPA receptor activity at the postsynaptic site during the formation of LTP.[55] First, the AMPA single conductance is directly increased by CAMKII at Ser831 of GluR1 subunit.[56] Second, CAMKII is required for the delivery of AMPA receptor to the synapse, which is lacking AMPA receptors.[51,57,58] This enhancement of synaptic GluR1 level by activation of CAMKII requires an intact C-terminal domain of GluR1, and is possibly involved in interaction with SAP97.[59] PP1, which is also known to be a important modulator for learning and memory, can dephosphorylate the phosphorylation of GluR1 at p831 site by CAMKII.[60]

Extracellular signal–regulated kinase (ERK) MAPK pathway

A recent study reported that the small guananine triphosphatases (GTPases) Ras and Rap are involved in AMPA receptor trafficking through a postsynaptic signaling mechanism. Ras mediates activity-evoked increase in GluR1/GluR4–containing AMPA receptor surface expression at synapses via a pathway that requires p42/44 MAPK activation. In contrast, Rap mediates NMDA-dependent removal of synaptic GluR2/GluR3–containing vesicles via a pathway that involves p38 MAPK. The regulation through Ras and Rap, which work as molecular switches, may in turn control the AMPA receptor level at synapses.[61]

PKC pathways

AMPA GluR2 receptors respond to secondary signals by constitutive receptor recycling. Phosphorylation of Ser880 on GluR2 provides a switch from receptor retention at the membrane by binding to ABP (AMPA receptor–binding protein)/GRIP (glutamate receptor–interacting protein), to receptor internalization by binding to PICK 1 (protein interacting with C kinase-1). Therefore,

phosphorylation of GluR2 at Ser880 by PKC may release the AMPA receptor from the anchoring proteins and initiate the internalization of receptors.[62-65]

The mechanism for AMPA receptor trafficking is specific for brain region and neuronal type. For example, the endocytosis of AMPA receptors mediating LTD is triggered by very different signaling cascades in different cell types despite the fact that a conserved cell biological mechanism (ie, clathrin/dynamine-dependent endocytosis) always seems to be involved. Specifically, in CA1 pyramidal cells, protein phosphatases seem to be involved in triggering LTD through dephosphorylation of GluR1 and phosphorylation of PKA site on GluR1 is associated with LTP.[53] However, in midbrain dopamine cells, activation of PKA appears to trigger LTD and endocytosis of AMPA receptors.[66]

AMPA receptor trafficking and mood disorders: implication for development of new medications

In view of the critical role of AMPA receptor trafficking in regulating various forms of plasticity, our laboratory has sought to determine if two structurally highly dissimilar antimanic agents, lithium and valproate, exert effects on AMPA receptor trafficking. Lithium, a monovalent cation, and valproic acid (VPA), an 8-carbon fatty acid, are the two most commonly used agents in the treatment of mania. Because lithium and valproate both require several weeks to exert their therapeutic effects, it is widely believed that adaptive changes in intracellular signaling and/or cellular physiology underlie the beneficial effects; interestingly, these two agents have been shown to exert robust effects on the very same signaling pathways known to regulate AMPA receptor trafficking (vide supra). Thus, we investigated whether lithium and valproate regulate synaptic plasticity and AMPA receptor trafficking in the hippocampus, a brain region presumed to be involved in the circuitry of mood disorders.[3] We have found that the structurally highly dissimilar antimanic agents lithium and valproate have a common effect on downregulating AMPA GluR1 synaptic expression in the hippocampus after prolonged treatment with therapeutically relevant concentrations as assessed both in vitro and in vivo. In cultured hippocampal neurons, lithium and valproate attenuated surface GluR1 expression after long-term treatment. Further supporting the therapeutic relevance of the finding, we found that an agent that provokes mania, namely the antidepressant imipramine, has an opposite effect as it upregulates AMPA synaptic strength in the hippocampus.[47,67]

Since chronic administration of mood stabilizers bring about numerous biochemical effects, our laboratory[8,68] and others[69] have established several criteria that findings should meet in order to maximize the likelihood of their therapeutic importance:

- This effect of mood stabilizers on GluR1 is a common effect of the structurally dissimilar antimanic agents lithium (a monovalent cation) and valproate (which is an 8-carbon branched fatty acid).
- This attenuation of synaptic GluR1 by lithium and valproate occurs in the hippocampus, a brain region known to be involved in critical affective neuronal circuits.
- This effect of lithium and valproate on synaptic GluR1 occurs at therapeutic concentrations both in vivo and in vitro.
- Similar to the clinical therapeutic effects, the changes in GluR1 were observed only after chronic (and not acute) administration.
- The effects were specific for antimanic agents, as a promanic antidepressant produced opposite effects. While it is impossible to determine whether synaptic GluR1 attenuation occurs in patients being treated with lithium or valproate, our experimental conditions attempt to mimic this situation as closely as possible.

Further supporting our data are recent studies that show that AMPA receptor antagonists attenuate several "manic-like" behaviors produced by amphetamine administration. Thus, AMPA antagonists have been demonstrated to attenuate psychostimulant-induced development or expression of sensitization and hedonic behavior without affecting spontaneous locomotion; additionally, some studies have demonstrated that AMPA receptor antagonists reduce amphetamine- or cocaine-induced hyperactivity.[70-75] The need to use caution in the appropriate application of animal models to complex neuropsychiatric disorders has been well articulated, and in fact it is unlikely we will ever develop rodent models that display the full range of symptomatology clinically expressed in man.[76,77] However, one current model of mania, which has been extensively used and has reasonable heuristic value in the study of mood disorders, involves the use of psychostimulants in appropriate paradigms. Thus, psychostimulants like amphetamine and cocaine are known to induce manic-like symp-

toms in healthy volunteers, and trigger frank manic episodes in individuals with bipolar disorder.[78] Thus, the best-established animal models mania utilize the administration of amphetamine or cocaine to produce hyperactivity, risk-taking behavior, and increased hedonic drive—all very important facets of the human clinical condition of mania. Moreover, these psychostimulant-induced behavioral changes are attenuated by the administration of chronic lithium in a therapeutically relevant time frame. Thus, the fact that AMPA receptor antagonists are capable of attenuating psychostimulant-induced sensitization, hyperactivity, and hedonic behav-

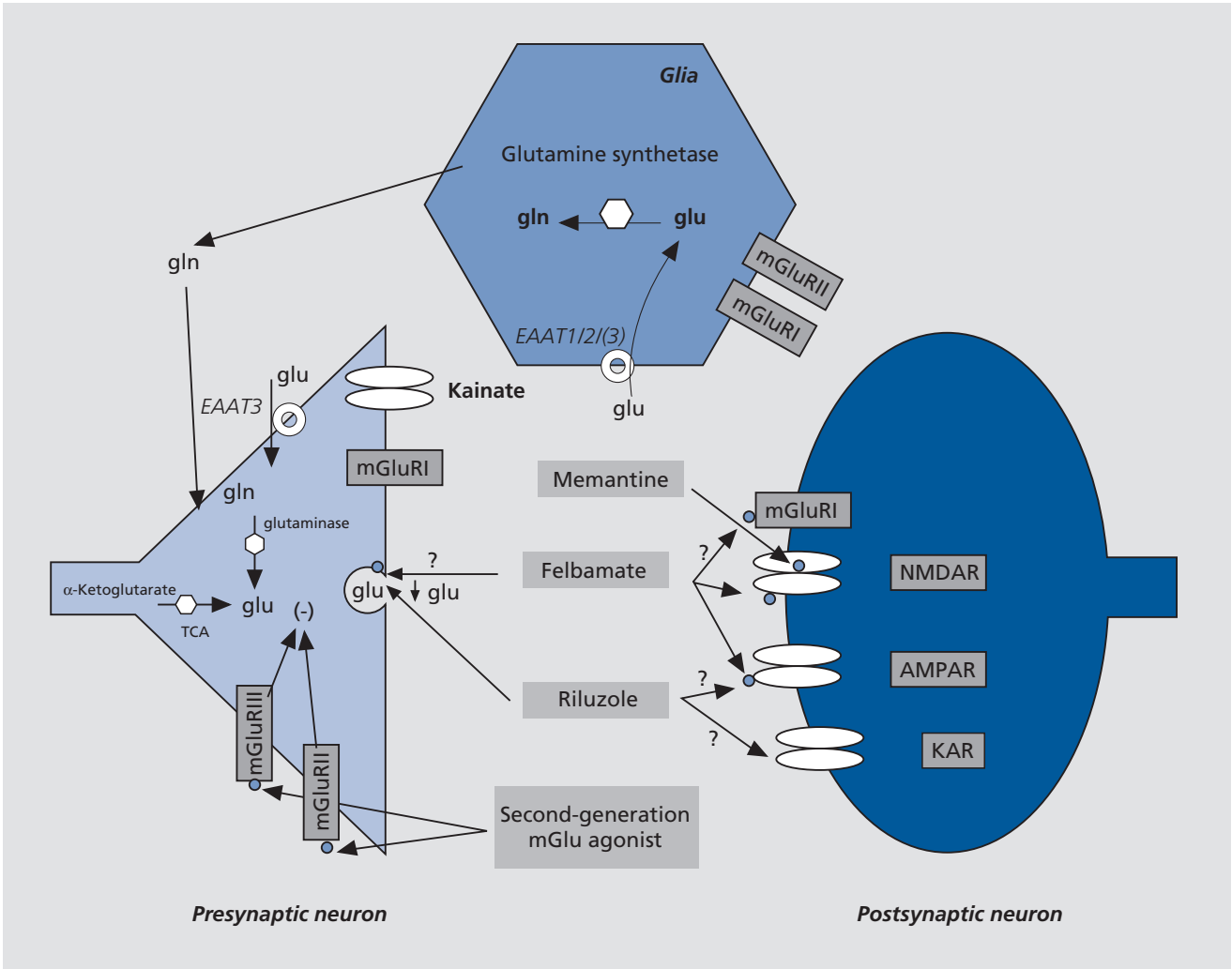

Figure 2. Thymoleptic agents, which exert major effects on the glutamatergic system. The various glutamate receptors and the presumed antiglutamatergic drug sites of action are presented. Memantine is a noncompetitive antagonist at the *N*-methyl-D-aspartate (NMDA) receptor. Felbamate is a noncompetitive NMDA receptor antagonist (glycine NR1 and glutamate NR2B), an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor antagonist, an mGlu group I receptor (mGluRI) antagonist, and a glutamate release inhibitor. Riluzole is a glutamate release inhibitor (acting through blockade of Na^+ voltage dependent channels), a γ-aminobutyric acid $GABA_A$ agonist, and probably an AMPA and kainate (KA) antagonist. The sites for second-generation mGlu group II and III receptor agonists are also depicted. NMDAR, NMDA receptor; AMPAR, AMPA receptor; KAR, KA receptor; glu, glutamate; gln, glutamine; mGluRII (or mGluRIII), mGlu group II (or III) receptor; EAAT, excitatory amino acid transporter; TCA, tricarboxylic acid cycle.

ior[70-75] provides compelling behavioral support for our contention that AMPA receptors play important roles in regulating affective behavior.

As mentioned already, in striking contrast to the effects seen with the antimanic agents lithium and valproate, we found that the chronic administration of the antidepressant imipramine—which is capable of triggering manic episodes in susceptible individuals[78]—*increased* hippocampal synaptic expression of GluR1. Very recent studies from other laboratories have also demonstrated that chronic administration of antidepressants enhances membrane expression of GluR1 as well as phosphorylation of GluR1 at the PKA site (p845) and the CAMKII/PKC site (p831).[79,80] Furthermore, it is noteworthy that AMPA potentiating agents reportedly have efficacy in preclinical models of depression.[81] Additionally, chronic exposure to the psychostimulants amphetamine and cocaine caused an increase in GluR1 level in the ventral tegmental area (VTA), and these effects have been postulated to represent a trigger for sensitization to drug abuse.[82] An elegant series of studies has recently provided insights into how dopamine receptors, which are activated during psychostimulant administration, might influence glutamate-dependent forms of synaptic plasticity, which are being increasingly recognized as important to drug addiction.[83] They showed that surface GluR1 labeling on processes of medium spiny neurons and interneurons was increased by brief incubation with a dopamine D_1 agonist.[83] Although these studies were designed to investigate the role of GluR1 in mediating the effects of drugs of abuse, it is noteworthy that many of the symptoms of mania resemble the effects of psychostimulants (eg, locomotor hyperactivity, racing thoughts, reduced sleep, and psychosis). Taken together, the biochemical and behavioral studies investigating the effects of antimanic (lithium and valproate) and promanic (antidepressants, cocaine, and amphetamine) agents on GluR1 strongly suggest that AMPA receptor trafficking is an important target in the pathogenesis and treatment of certain facets of bipolar disorder. The mechanisms by which glutamate receptors are actively recruited to synapses have long intrigued the neuroscience community; the information reviewed here suggests that they may also play important roles in the pathophysiology and treatment of complex neuropsychiatric disorders.

Concluding remarks

Regionally selective impairments of structural plasticity and cellular resiliency, which have been postulated to contribute to the development of classical neurodegenerative disorders, may also exist in mood disorders. It remains unclear whether these impairments correlate with the magnitude or duration of the biochemical perturbations in mood disorders, reflect an enhanced vulnerability to the deleterious effects of these perturbations (eg, due to genetic factors and/or early life events), or indeed represent the fundamental etiological process in mood disorders. Nevertheless, it is noteworthy that there is growing evidence from preclinical and clinical research that the glutamatergic system is involved in the pathophysiology and treatment of mood disorders.

Over the last few years, an impressive amount of information has been gathered regarding the mechanisms underlying the regulation of AMPA receptor localization at synapses. The findings that mood stabilizers—in therapeutically meaningful paradigms—regulate AMPA receptors at synapses opens new potential avenues for new drug development in regards to regulating glutamatergic synaptic strength in critical neuronal circuits *(Figure 2)*. The development of new modulators of AMPA receptor signaling for the treatment of mood disorders may lead to improved therapeutics for these devastating disorders. ❑

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Regulación de la plasticidad celular y de la resiliencia mediante estabilizadores del ánimo: el papel del tráfico del receptor AMPA

Diversas fuentes aportan una evidencia creciente acerca de la asociación entre los trastornos del ánimo severos y reducciones regionales del volumen cerebral, como también del número, tamaño y densidad de la glía y de las neuronas en distintas áreas cerebrales. Aunque la fisiopatología específica que está a la base de estos cambios morfométricos no está totalmente aclarada, los datos sugieren que los trastornos del ánimo severos están asociados con un deterioro en la plasticidad estructural y en la resiliencia celular. En este contexto es destacable que una información creciente sugiere que el sistema glutamatérgico (que se sabe que juega un papel importante en la plasticidad neuronal y en la resiliencia celular) puede estar involucrado en la fisiopatología y en el tratamiento de los trastornos del ánimo. El tráfico de la subunidad GluR1 del receptor AMPA (ácido-3-hidroxi-5-metil-4-isoxazol propiónico) juega un papel decisivo en la regulación de varias formas de plasticidad neural. Es de destacar que estudios recientes han mostrado que estabilizadores del ánimo, estructuralmente disímiles, como el litio y el valproato regulan el tráfico de la subunidad GluR1 y su localización en las sinapsis. Estos estudios sugieren que la regulación de la plasticidad sináptica mediada por el sistema glutamatérgico puede jugar un papel en el tratamiento de los trastornos del ánimo y se aumenta la posibilidad que agentes que afecten más directamente la subunidad GluR1 sináptica se transformen en nuevas terapias para estas devastadoras enfermedades.

Régulation de la plasticité et de la résilience cellulaires par les stabilisateurs de l'humeur : le rôle du trafic neuronal du récepteur AMPA

Il existe de plus en plus d'arguments provenant de sources différentes en faveur de l'association des troubles de l'humeur sévères avec des réductions régionales du volume cérébral, ainsi qu'avec des réductions en nombre, taille et densité de la glie et des neurones dans des zones cérébrales discrètes. Bien que la physiopathologie exacte sous-tendant ces modifications morphométriques soit incomplètement élucidée, les données suggèrent que les troubles de l'humeur sévères sont associés à des déficits de la plasticité structurale et de la résilience cellulaire. Dans ce contexte il faut remarquer qu'un nombre croissant de données est en faveur d'une intervention du système glutamatergique (connu pour jouer un rôle majeur dans la plasticité neuronale et la résilience cellulaire) dans la physiopathologie et le traitement des troubles de l'humeur. Le trafic neuronal de la sous-unité GluR1 du récepteur AMPA (acide glutamate α-amino-3-hydroxy-5-méthyl-4-isoxazole propionique) et les changements morphologiques qui en résultent jouent un rôle crucial dans la régulation des différentes formes de plasticité neuronale. À ce titre il faut également noter que de récentes études ont montré que des stabilisateurs de l'humeur structurellement différents comme le lithium et le valproate régulent le trafic neuronal des sous-unités GluR1 et leur localisation dans les synapses. Ces études suggèrent que la régulation de la plasticité synaptique médiée par le glutamate peut jouer un rôle dans le traitement des troubles de l'humeur et évoquent la possibilité pour ces maladies invalidantes d'un nouveau traitement par l'intermédiaire de molécules affectant plus directement le GluR1 synaptique.

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