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Bipolar disorder: involvement of signaling cascades and AMPA receptor trafficking at synapses

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Abstract

There is increasing evidence that severe mood disorders are associated with impairment of structural plasticity and cellular resilience. Cumulative data demonstrate that mood stabilizers regulate intracellular signaling cascades, including protein kinase C (PKC), PKA, mitogen-activated protein (MAP) kinase, glycogen synthase kinase 3-β (GSK3-β) and intracellular calcium, which are signaling pathways that regulate synaptic plasticity. In this context, it is noteworthy that a growing body of data indicates that the glutamatergic system, has a major role in neuronal plasticity and cellular resilience, might be involved in the pathophysiology and treatment of mood disorders. AMPA glutamate-receptor trafficking is important in synaptic plasticity and might play crucial roles in maintaining critical neuronal circuits associated with mood. Two clinically effective, structurally dissimilar, antimanic agents, lithium and valproate (VPA), down-regulate synaptic expression of AMPA receptor subunit GluR1 in hippocampus in chronically treated rats. This reduction in synaptic GluR1 by lithium and VPA is due to attenuated phosphorylation of GluR1 at a specific PKA site (residue 845 of GluR1), which is crucial for AMPA receptor insertion. By contrast, imipramine, which can provoke mania, increases synaptic expression of GluR1 in the hippocampus in vivo. Furthermore, there is ample evidence from preclinical and clinical research that the glutamatergic system is involved in the pathophysiology of mood disorders and that many of the somatic treatments used for mood disorders including antidepressants, mood stabilizers, atypical antipsychotic drugs and electroconvulsive therapy have both direct and indirect effects on the glutamatergic system. Given these findings, further research with medications that specifically affect the glutamatergic system is warranted. Recent studies in our lab have shown that riluzole, a FDA approved medicine that regulates the glutamatergic system, shows antidepressant efficacy in unipolar and bipolar depression. These studies indicate that regulation of glutamate-mediated synaptic plasticity might play a role in the treatment of mood disorders, and raise new avenues for novel therapies for this devastating illness.

Keywords

Lithium; valproate; antidepressant; bipolar disorder; glutamatergic system

INTRODUCTION

Bipolar disorder (also known as manic–depressive illness) is a severe, often life-threatening illness, that affects 2.3 million Americans (Goodwin and Jamison, 1990). The costs associated with disability and premature death from major mood disorders represent an economic burden

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of tens of billions of dollars annually in the United States alone (Simon, 2003). Despite the devastating impact of bipolar disorder on millions worldwide, little is known about underlying etiology and neurobiology. Historically, the brain systems that receive the greatest attention in neurobiological studies of mood disorders are the monoaminergic neurotransmitter systems, which are distributed extensively throughout the network of limbic, striatal and prefrontal cortical neuronal circuits that are thought to support the behavioral and visceral manifestations of mood disorders (Drevets, 2001; Manji *et al*., 2001; Nestler *et al*., 2002a). In addition to the growing appreciation that investigation of the pathophysiology of complex mood disorders have focused excessively on monoaminergic systems, there is growing concern that progress in developing truly novel and improved medications has been limited. 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 investigation of the putative roles of intracellular signaling cascades and synaptic plasticity. Recent evidence demonstrating that impairments of neuroplasticity might underlie the pathophysiology of mood disorders, and that antidepressants and mood stabilizers exert major effects on the signaling pathways that regulate cellular plasticity has generated considerable excitement among the clinical neuroscience community and is reshaping views about the neurobiological underpinnings of these disorders (Manji and Lenox, 2000a; Manji *et al*., 2001; D'Sa and Duman, 2002; Nestler *et al*., 2002a; Young, 2002).

Signaling networks regulated by mood stabilizers and antidepressants as key modulators of synaptic plasticity

Recent research into the pathophysiology and treatment of mood disorders has moved from a focus on neurotransmitters and cell surface receptors to intracellular signaling cascades. Multi-component cellular signaling pathways interact at various levels, thereby forming complex signaling networks that allow cells to receive internal and external cues, process these cues and respond to information (Fig. 1). These signaling networks enable the integration of signals across multiple time points and the generation of distinct outputs depending on input strength and duration. Moreover, these signaling networks regulate intricate feed-forward and feedback loops (Bourne and Nicoll, 1993;Bhalla and Iyengar, 1999;Weng *et al*., 1999) (Fig. 1). Given their widespread, crucial role in the integration and fine-tuning of physiological processes, it is not surprising that abnormalities in signaling pathways have been identified in several human diseases.

Signaling pathways also represent major targets for a number of hormones, including glucocorticoids, thyroid hormones and gonadal steroids (Manji, 1992; Speigel, 1998). These biochemical effects might play a role in mediating certain clinical manifestations of altered hormonal levels in mood-disorder subjects (e.g. the frequent onset of bipolar disorder in puberty, triggering of episodes in the postpartum period, association with depression, potentially rapid cycling with hypothyroidism, and triggering of mood episodes in response to exogenous glucocorticoids).

Complex signaling networks might be especially important in the CNS, where they balance and integrate diverse neuronal signals, and transmit them to effectors, thus, forming the basis of a complex information-processing network (Bourne and Nicoll, 1993; Bhalla and Iyengar, 1999; Weng *et al*., 1999). Highly complex signaling networks might be one mechanism by which neurons acquire the flexibility for generating the wide range of responses observed in the nervous system. These pathways are undoubtedly involved in regulating diverse vegetative functions such as mood, appetite and wakefulness. Therefore, they are likely to be involved in the pathophysiology of bipolar disorder. We now describe direct and indirect evidence supporting a role for signaling pathway abnormalities in the pathophysiology and treatment of bipolar disorder.

The Gs protein cAMP-generating signaling pathway

Several laboratories report abnormalities in G-protein subunits in bipolar patients. Data from postmortem studies of the brains of bipolar patients demonstrate increased levels of the stimulatory G protein (Gas), accompanied by increases in post-receptor stimulated adenylate cyclase activity (Young *et al*., 1993; Warsh *et al*., 2000a; Warsh *et al*., 2000b). These observations are supported by the demonstration of increased agonist-activated (Emamghoreishi *et al*., 1997) GTPγS binding to G-protein α subunits in the frontal cortical membranes of patients with bipolar disorder (Wang and Friedman, 1996). Several studies find elevated Gαs protein and mRNA levels in peripheral circulating cells in bipolar disorder, but how these levels depend on clinical mood state is unclear (Manji *et al*., 1995; Mitchell *et al*., 1997; Lenox and Manji, 1998; Spleiss *et al*., 1998; Wang and Friedman, 1999; Warsh *et al*., 2000a).

Recent studies from our laboratory and others show that basal activity of protein kinase A (PKA) is decreased in hippo-campal samples from animals chronically treated animals with either lithium or valproate (VPA) (Du *et al*. 2004a; reviewed by Gould *et al*., 2004b). In addition, Perez *et al*. looked further downstream in this signaling pathway and found higher levels of cAMP-stimulated phosphorylation of an ~22 kDa protein (subsequenty identified as Rap1) in platelets from treated, euthymic, bipolar patients compared with healthy subjects (Perez *et al*., 2000).

The PKC signaling pathway

The PKC family of enzymes is a group of calcium- and phospholipid-dependent enzymes that comprise a family of closely related kinase subspecies and appears to play a crucial role in regulating synaptic plasticity and various forms of learning and memory (Stabel and Parker, 1991; Nishizuka, 1992; Newton, 1995; Nishizuka, 1995). PKC is one of the major intracellular-signal mediators that is generated after external stimulation of cells via several neurotransmitter receptors (including acetylcholine M_1, M_3 and M_5 receptors, α_1 adrenoceptors, metabotropic glutamate receptors and serotonin $5HT_{2A}$ receptors), which induce the hydrolysis of various membrane phospholipids.

Recent evidence indicates that alterations in PKC activity might play a significant role in mood disorders. Friedman *et al*. (1993) investigated PKC activity and PKC translocation in response to serotonin in platelets obtained from bipolar disorder patients before and during lithium treatment. They report that the ratio of platelet-membrane-bound:cytosolic PKC activity is elevated in manic subjects. In addition, serotonin-elicited platelet PKC translocation is enhanced in these subjects. Wang and Friedman (1996) measured PKC isozyme levels, activity and translocation in postmortem brain tissue from bipolar disorder patients. They report increased PKC activity and translocation in bipolar disorder brains compared with controls, accompanied by elevated levels of selected PKC isozymes in the cortex.

Evidence from several groups demonstrates clearly that therapeutically relevant concentrations of lithium exert significant effects on the PKC signaling cascade. Data indicate that chronic treatment with lithium attenuates PKC activity and down-regulates the expression of isozymes PKCα and PKCε in the frontal cortex and hippocampus of patients with bipolar disorder (Manji and Lenox, 1999; Manji and Lenox, 2000b). Chronic lithium also dramatically reduces the hippocampal levels of a major PKC substrate, myristoylated alanine-rich C kinase substrate (MARCKS), which has a role in regulating long-term neuroplastic events.

To validate the therapeutic relevance of these biochemical findings, it is noteworthy that the structurally-dissimilar antimanic agent VPA produces similar effects to lithium on PKC α and PKC ε isozymes and the MARCKS protein (Manji and Lenox, 1999; Manji and Lenox,

2000b). Following chronic treatment with lithium, PKC activation was significantly reduced in rat brains, measured by translocation of cytoplasmic PKC to membrane-bound PKC. Lithium and VPA might bring about their effects on the PKC signaling pathway by distinct mechanisms, consistent with clinical observations that some patients show preferential response to one of these agents, and that additive therapeutic effects are often observed in patients when the two agents are co-administered. A recent study has shown that lithium inhibits phorbol 12-myristate 13-acetate-induced PKC activity in the prefrontal cortex, which might have an impact on learning and memory (Birnbaum *et al*., 2004).

In view of the pivotal role of the PKC signaling pathway in regulating neuronal excitability, neurotransmitter release and long-term synaptic events (Conn and Sweatt, 1994; Hahn and Friedman, 1999), we postulated that the attenuation of PKC activity might play a role in the antimanic effects of lithium and VPA. To test this idea, we piloted a study in seven bipolar manic patients treated with tamoxifen, a non-steroidal anti-estrogen that inhibits PKC inhibitor at higher concentrations (Couldwell *et al*., 1993). Tamoxifen showed antimanic efficacy in this study (Bebchuk *et al*., 2000), but because of the small sample size, these results are considered preliminary. However, this preliminary data suggesting the involvement of the PKC signaling system in the pathophysiology of bipolar disorder indicates that PKC inhibitors might be useful in the treatment of mania, and warrant larger, double-blind, placebo-controlled studies of tamoxifen and novel, selective PKC inhibitors.

Glycogen synthase kinase

Glycogen synthase kinase 3 (GSK-3) is a crucial kinase that functions as an intermediary in many intracellular signaling pathways. Recent research indicates the importance of this enzyme in bipolar disorder (Chen *et al*., 1999; Gould and Manji, 2002). GSK-3 is inhibited directly by the mood stabilizer lithium in concentrations within therapeutical range (~1 mM) (Klein and Melton, 1996). GSK-3 has two, nearly identical isoforms in mammals, the a and b isoforms. GSK-3 is unique among kinases because it is generally constitutively active. Therefore, intracellular signals such as the Wnt pathway, phosphoinositide (PI) 3-kinase pathway, PKA and PKC inactivate this enzyme. Several endogenous growth factors (e.g. nerve growth factor and brain-derived growth factor; BDNF) use the PI 3-kinase signaling cascade as a major effector system. Thus, growth factors might exert many of their neurotrophic and neuroprotective effects in part by inhibiting GSK-3. GSK-3 phosphorylates, and thereby inactivates, many transcription factors, and modulates the function of cytoskeletal proteins such as the Alzheimer's disease protein tau (a previous name for GSK-3 is tau kinase). Thus, inhibition of GSK-3 releases this inhibition and activates multiple cellular targets.

Growing evidence indicates that GSK-3 plays an important role in regulating neuroplasticity and cellular resilience, including synapse formation and axonal growth. Studies indicate that changes in GSK-3-mediated phosphorylation of mitogen-activated protein-1B (a cytoskeletal protein) are associated with the loss and/or unbundling of stable axonal microtubules (Lucas *et al*., 1998). Furthermore, inhibition of GSK-3β results in accumulation of synapsin I, which is involved in synaptic vesicle docking and release of growth cone-like areas (Lucas and Salinas, 1997). Recent data also show that VPA protects cells from endoplasmic reticulum (ER)-stress-induced lipid accumulation and apoptosis by inhibiting GSK-3 (Kim *et al*., 2005).

Amphetamine-induced hyperactivity is the most established rodent model for mania; this hyperactivity is attenuated by a number of mood stabilizers including lithium, anticonvulsants, and antipsychotics. Recently, Beaulieu *et al*. reported that dopamine-dependent activity increases in mice are mediated via a GSK-dependent mechanism, and that lithium and other inhibitors of GSK-3 attenuate the hyperactivity in mice that lack the dopamine transporter (Beaulieu *et al*., 2004). We have also found that peripheral administration of a GSK-3 inhibitor

decreases amphetamine-induced hyperactivity in rats (Gould *et al*., 2004b). Taken together, these data support the possibility that inhibition of GSK-3 might be involved in the antimanic effects of lithium.

ERK MAP kinase pathways

Studies from our lab demonstrate that therapeutically relevant concentrations of lithium and VPA activate the extracellular-signal related kinase (ERK) mitogen-activated protein (MAP) kinase cascade in human neuroblastoma SH-SY5Y cells and in crucial limbic and limbic-related areas of rodent brain (Yuan *et al*., 2000; Einat *et al*., 2003). MAP kinase is involved in neurotrophic factor signaling pathways, promoting cell survival, and might have important roles in neuroprotection and neuroplasticity in mood disorders (reviewed by Du *et al*., 2003).

Calcium-signaling abnormalities

Calcium ions play a crucial role in regulating the synthesis and release of neurotransmitters, neuronal excitability and long-term neuroplastic events and, therefore, it is not surprising that several studies have investigated the role of intracellular Ca^{2+} in peripheral cells in bipolar disorder. Intracellular Ca^{2+} signaling and homeostasis are maintained by an intricate array of processes that act in concert including, for example, inositol trisphosphate (IP_3)- and ryanodine-stimulated release of Ca^{2+} from ER storage pools, voltage- and ligand-gated ion-channel-mediated Ca^{2+} influx, store-operated Ca^{2+} entry, plasma membrane Ca^{2+}–ATPase pumps, sarcoplasmic–ER Ca^{2+}–ATPase pumps, and mitochondrial Ca^{2+} uptake, storage and release (reviewed by Pisani *et al*., 2004). Regardless of the complexity of intracellular Ca^{2+} regulation, impaired regulation of Ca^{2+} cascades is the most reproducible biological abnormality described in bipolar disorder research. For this reason, mechanisms involved in Ca^{2+} regulation are postulated to underlie aspects of the pathophysiology of bipolar disorder. To date, cumulative studies consistently revealed elevations in basal intracellular Ca^{2+} concentrations in platelets, lymphocytes and neutrophils of patients with bipolar disorder (Emamghoreishi *et al*., 1997).

Post-mortem brain studies have also revealed changes that might reflect possible ‘signatures’ of abnormal Ca^{2+} homeostasis in bipolar disorder. These include a marked blunting of G-protein-activated PI hydrolysis (Jope *et al*., 1996) and altered mRNA expression of two candidate proteins that might have important roles in Ca^{2+} homeostasis. These are inositol monophosphatase (IMPase) type II (Yoon *et al*., 2001a) and a transient receptor potential channel, TRPM2 (TRPC7 in earlier nomenclature) (Yoon *et al*., 2001b), which is a ligand-gated plasma membrane ion channel that also mediates Ca^{2+} entry into cells. In addition, lithium and VPA inhibit IMPase (Hallcher and Sherman, 1980), which has been suggested to diminish overactive signaling through PI-linked second messengers and, in turn, Ca^{2+} (Berridge *et al*., 1982).

In summary, mood disorders are associated with alterations in signaling networks that might, subsequently, regulate synaptic plasticity and cell resilience of neuronal circuitry associated with affective disorders. It is noteworthy that modulation of synaptic plasticity by signaling cascades has been studied extensively in the glutamatergic system, specifically for α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor trafficking.

AMPA receptor trafficking has crucial roles in regulating forms of neural plasticity

Surprisingly, the potential role of the glutamatergic system in the pathophysiology and treatment of bipolar disorder has only recently started to be evaluated. Glutamate is the major excitatory synaptic neurotransmitter and regulates numerous physiological functions in the

mammalian CNS such as synaptic plasticity, learning and memory. It is a major neurotransmitter system in the circuitry that is thought to subserve many of the symptoms of severe, recurrent, mood disorders (Drevets, 2001). Three major classes of ionotropic glutamate receptors are expressed throughout the mammalian CNS including AMPA, kainate and *N*-methyl-D-aspartate (NMDA) receptors (Fig. 2). AMPA receptors mediate the majority of excitatory synaptic transmission in the CNS; the AMPA R channel is composed of the combination of GluR1, GluR2, GluR3 and GluR4 subunits. 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 (Fig. 2).

The NMDA receptor is activated by glutamate, a co-agonist (either glycine or D-serine) and depolarization, to open and permit the entry of Na^+ and Ca^{2+} (Fig. 2). The NMDA receptor channel is composed of combination of NR1, NR2A, NR2B, NR2C, NR2D, NR3A and NR3B subunits (Fig. 2). It is generally believed that activation of the AMPA receptor results in neuronal depolarization sufficient to liberate the Mg^{2+} cation from the NMDA receptor, thereby permitting its activation. NMDA receptors play a crucial role in regulating synaptic plasticity (Malenka and Nicoll, 1999), including AMPA receptor trafficking. 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 are characterized extensively and proposed to represent cellular models of learning and memory (Malenka and Nicoll, 1999). 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 (Fig. 2).

Recent data indicate that AMPA receptor trafficking, including receptor insertion, 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 and several signal transduction cascades can produce short- and long-term changes in expression of AMPA receptor subunits at the synaptic surface (Malenka and Nicoll, 1999). Although the mechanisms of LTP and LTD have not been elucidated completely, it is widely accepted that AMPA-receptor trafficking is the key player in these phenomena.

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 either passive or active delivery along microtubules and, finally, fusion with the target membrane (Antonny and Schekman, 2001). This mechanism delivers AMPA receptors to the neuronal membrane surface. Each subunit of the AMPA receptor is composed of an N-terminal extracellular domain, a membrane-spanning domain, and a C-terminal intracellular domain (Bennett and Dingledine, 1995; Wo *et al*., 1995). 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, by preventing constitutive exchange and conferring inducible delivery of the heteromer (Shi *et al*., 2001). Phosphorylation of GluR1at the PKA site *P*845 facilitates the insertion of GluR1 onto the membrane and synapses, and is often associated with LTP (Lee *et al*., 2000). Dephosphorylation of the GluR1 by protein phosphatases (e.g. calcineurin and PP1) target GluR1 to recycling endosomes, where re-phosphorylation by PKA might occur and reinsertion of the receptors onto the membrane (Ehlers, 2000). Phosphorylation of GluR1 at the PKA site is enhanced by synapse associated protein 97 (SAP97)/protein A kinase

anchoring protein (AKAP79) complex, which directs PKA to GluR1 via a PDZ-domain interaction (Colledge *et al*., 2000).

A growing body of data indicates the importance of this PKA phosphorylation site of GluR1 in psychiatric disorders. Very recent studies also demonstrate that chronic administration of antidepressant enhances the membrane expression of GluR1 as well as phosphorylation of GluR1 at this PKA site (*P*845) (Martinez-Turrillas *et al*., 2002; Svenningsson *et al*., 2002). It has also been shown that dopamine, which is a neurotransmitter with psychostimulant effects, binds to D1 receptors and enhances the surface expression of GluR1 receptor through activation of PKA at this site and AKAP79 (Mangiavacchi and Wolf, 2004).

Calcium/calmodulin-dependent kinase II (CaMKII) pathway

Numerous studies demonstrate that CaMKII is required for the proper formation of LTP in slice preparations, and in regulating learning and memory in rodents (Fink and Meyer, 2002). In response to stimulation, CaMKII translocates to postsynaptic sites, where it has two major effects on AMPA receptor activity at the post-synaptic density during the formation of LTP (Fink and Meyer, 2002). First, the AMPA single conductance is increased directly by CaMKII at Ser831 of the GluR1 subunit (Derkach *et al*., 1999). Second, CaMKII is required for delivery of the AMPA receptor to the synapse, which lacks AMPA receptors (Shi *et al*., 1999; Liao *et al*., 2001; Shi *et al*., 2001). Enhancement of synaptic GluR1 levels by activation of CaMKII requires an intact C-terminal domain of GluR1, and might involve interaction with SAP97 (Hayashi *et al*., 2000). Protein phosphatase 1 (PP1), an important modulator of learning and memory, can dephosphorylate the phosphorylation by CaMKII of the *P*831 site of GluR1 (Genoux *et al*., 2002). It is noteworthy that this CaMKII site of GluR1 is phosphorylated after antidepressant treatment (Svenningsson *et al*., 2002) and CaMKII might also be involved in the pathophysiology of mood disorders (Du *et al*., 2004b).

ERK MAP kinase pathway

A recent study reported that the small GTPases Ras and Rap are involved in AMPA-receptor trafficking through a post-synaptic signaling mechanism. Ras mediates activity evoked increases in AMPA receptors that contain GluR1 and GluR4 at the synaptic surface via a pathway that requires p42/44 MAPK activation. By contrast, Rap mediates NMDA-dependent removal of synaptic GluR2/GluR3-containing vesicles via a pathway that involves p38 MAP kinase. Regulation through Ras and Rap, which work as molecular switches, might, in turn, control the AMPA-receptor level at synapses (Zhu *et al*., 2002).

PKC pathways

AMPA GluR2 receptors respond to secondary signals by constitutive receptor recycling. Phosphorylation of Ser880 on GluR2 causes a switch from receptor retention at the membrane by binding to ABP/GRIP, to receptor internalization by binding to PICK 1. Therefore, phosphorylation of GluR2 at Ser880 by PKC might release the AMPA receptor from anchoring proteins and initiate the internalization of receptors (Matsuda *et al*., 2000; Xia *et al*., 2000; Kim *et al*., 2001; Perez *et al*., 2001).

The mechanism of AMPA-receptor trafficking is specific for brain region and type of neuron. For example, the endocytosis of AMPA receptors that mediate LTD is triggered by different signaling cascades in different cell types, despite the fact that a conserved cell-biological mechanism (i.e. clathrin/dynamine-dependent endocytosis) appears to be involved. Specifically, in CA1 pyramidal cells, protein phosphatases are involved in triggering LTD through dephosphorylation of GluR1. Phosphorylation of the PKA site on GluR1 is associated with LTP (Ehlers, 2000). However, in midbrain dopamine cells, activation of PKA appears to trigger LTD and endocytosis of AMPA receptors (Gutlerner *et al*., 2002). Most importantly

for the present discussion, AMPA-receptor trafficking is highly regulated by PKA, PKC, CaMKII and MAP kinase signaling cascades (reviewed by Du *et al*., 2004c). These are the same signaling cascades on which mood stabilizers and antidepressants exert major effects (Gould *et al*., 2004a; Gould *et al*., 2004b; Payne *et al*., in press; Popoli *et al*., 2003). These observations have led to an extensive series of studies that demonstrate that AMPA receptor trafficking is highly regulated by anti-depressants and mood stabilizers (Gray *et al*., 2003; Du *et al*., 2004a).

Mood stabilizers modulate synaptic plasticity by regulating AMPA-receptor trafficking

In view of the crucial role of AMPA-receptor trafficking in regulating various forms of plasticity, our laboratory has sought to determine if two structurally dissimilar anti-manic agents, lithium and VPA, exert effects on AMPA-receptor trafficking. Lithium, a monovalent cation, and VPA, an eight-carbon fatty acid, are the two most commonly used agents in the treatment of mania. Because lithium and VPA require several weeks to exert their therapeutic effects, it is widely believed that adaptive changes in intracellular signaling and/or cellular physiology underlie their beneficial effects. These two agents exert robust effects on the signaling pathways that regulate AMPA-receptor trafficking. Thus, we investigated if lithium and VPA regulate synaptic plasticity and AMPA-receptor trafficking in the hippocampus, a brain region that is presumed to be involved in the circuitry of mood disorders.

We find that lithium and VPA have a common effect of down-regulating AMPA GluR1 synaptic expression in the hippocampus after prolonged treatment with therapeutically relevant concentrations both *in vitro* and *in vivo* (Fig. 3). However, total protein concentrations of GluR1 and synaptophysin remain unchanged after lithium and VPA treatment *in vitro* and *in vivo*. In hippocampal neurons in culture, lithium and VPA attenuate surface expression of GluR1 after long-term treatment (Du *et al*., 2004a) and phosphorylation of a specific PKA site (GluR*P*845) is attenuated significantly by lithium and VPA treatment (by 52 and 33%, respectively). Sp-cAMP treatment reversed the attenuation of phosphorylation by lithium and VPA and returned GluR1s to the surface, which indicates that phosphorylation of GluR*P*845 is involved in the mechanism of GluR1 surface attenuation (Du *et al*., 2004a). To support the therapeutic relevance of these findings, we found the antidepressant imipramine, which provokes mania, has an opposite effect as it up-regulates AMPA synaptic strength in the hippocampus (Fig. 4).

Further supporting our data are recent studies showing that AMPA receptor antagonists attenuate several ‘manic like’ behaviors produced by amphetamine administration. Thus, AMPA antagonists attenuate psychostimulant-induced development or expression of sensitization and hedonic behavior without affecting spontaneous locomotion. Additionally, some studies demonstrate that AMPA receptor antagonists reduce amphetamine/cocaine-induced hyperactivity (Burns *et al*., 1994; Li *et al*., 1997; Mead and Stephens, 1998; Hotsenpiller *et al*., 2001; Backstrom and Hyytia, 2003; Tzschentke, 2003). The need for caution in the appropriate application of animal models to complex neuropsychiatric disorders is well articulated, and it is unlikely we will ever develop rodent models that display the full range of symptoms that are observed clinically in humans (Nestler *et al*., 2002b ; Einat *et al*., 2003). However, one current model of mania that is used extensively 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 induce manic-like symptoms in healthy volunteers, and trigger frank manic episodes in individuals with bipolar disorder (Goodwin and Jamison, 1990). Thus, the best-established animals of mania utilize the administration of either amphetamine or cocaine to produce hyperactivity, risk-taking behavior and increased hedonic drive, which are all important facets of the clinical condition of mania in humans. 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 can attenuate psychostimulant-induced sensitization, hyperactivity and hedonic behavior (Burns *et al*., 1994; Li *et al*., 1997; Mead and Stephens, 1998; Hotsenpiller *et al*., 2001; Backstrom and Hyytia, 2003; Tzschentke, 2003) provides compelling behavioral support for our contention that AMPA receptors have important roles in regulating affective behavior. Taken together, the biochemical and behavioral studies that investigate the effects of antimanic (lithium and VPA) and pro-manic (antidepressants, cocaine and amphetamine) agents on GluR1 indicate strongly that AMPA-receptor trafficking is an important target in the pathogenesis and treatment of some facets of bipolar disorder.

Role of the glutamatergic system in treatment of mood disorders

The noradrenergic and serotonergic hypothesis of mood disorders, which was developed from the pharmacological effects of early drugs, no longer provides satisfactory explanations of the mode of action of all antidepressant agents and the underlying pathophysiology of depression. Several agents that are associated with glutamatergic systems demonstrate antidepressant efficacy in clinical studies. In the 1950s, Dcycloserine (DCS), which is a partial agonist at the NMDA receptor glycine site and used as a part of multidrug antituberculosis treatment, was reported to have mood elevating effects (Crane, 1959; Crane, 1961; Heresco-Levy and Javitt, 1998). Subsequent studies with DCS have reported cases of euphoria and amnestic effects in humans that resemble the effects of subperceptual doses of non-competitive NMDA antagonists (Krystal *et al*., 1994). Early evidence that NMDA antagonism might be important for antidepressant effects in humans comes from case series and blinded trials conducted with the non-competitive NMDA antagonist amantadine. Amantadine has antidepressant effects in patients with Parkinson's disease, and in unipolar and bipolar patients (Parkes *et al*., 1970; Vale *et al*., 1971; Rizzo and Morselli, 1972) (Fig. 5). Recently, Berman *et al*. (2000) reported the first placebo-controlled, double-blind trial to assess the effects of a single dose of the NMDA receptor antagonist ketamine in seven patients with depression (Fig. 5). Ketamine is a high-affinity, NMDA-receptor antagonist with lower, but potentially relevant, affinity for the μ opiate receptor and weak antagonist activity at the dopamine receptor (Wong *et al*., 1996) (Fig. 5). Perhaps the greatest evidence that glutamate modulation might be important in the pathophysiology of mood disorders comes from the clinical use of the anticonvulsant lamotrigine. Recently, several double-blind, placebo-controlled studies, report that lamotrigine is effective in bipolar depression (Calabrese *et al*., 1999). Although the exact mechanism of action of lamotrigine is unknown, inhibition of excessive release of glutamate is postulated as a likely mechanism of action (Leach *et al*., 1986; Calabresi *et al*., 1996; Wang *et al*., 1996).

In addition, a recent study has also suggested the potential of AMPA-receptor modulation in the treatment of mood disorder because an AMPA-receptor potentiator has anti-depressant effects in animal models (Zarate *et al*., 2003; Du *et al*., 2004c). As discussed in this review, the mood stabilizers lithium and VPA appear to attenuate glutamatergic function through multiple mechanisms. Repeated administration of lithium, the prototype mood stabilizer, might promote the uptake of glutamate from the synapse (Dixon and Hokin, 1998), attenuate the function of glutamate receptors (Nonaka *et al*., 1998; Du *et al*., 2004a) and reduce the function of intracellular signaling cascades that are activated by the binding of glutamate to its receptors (Manji and Lenox, 1999).

Given the preclinical and clinical data that supports a role for the glutamatergic system in the pathophysiology of mood disorders, several therapeutic agents that act on this system should be explored as potential treatments for mood disorders. These agents include riluzole, memantine, felbamate and zinc. Riluzole is a neuroprotective agent with anticonvulsant properties that easily crosses the blood–brain barrier (Benavides *et al*., 1985). It inhibits the release of glutamate rather than acting as an NMDA antagonist and is one of the few

antiglutamatergic agents available for use in humans. Currently, it is used in patients with amyotrophic lateral sclerosis, a severe, debilitating condition, and has a favorable side-effect profile. These characteristics make it an ideal candidate to test in patients with mood disorders (Zarate *et al*., 2003). A recent study from our laboratory has demonstrated that riluzole in combination with lithium is effective in bipolar depression (Fig. 6) (Zarate *et al*., 2005), which indicates that riluzole might have antidepressant efficacy in subjects with bipolar depression. Memantine is a potent, non-competitive, voltage-dependent NMDA antagonist that easily crosses the blood–brain barrier. It has a receptor effect that is comparable to MK801 (Bormann, 1989) and inhibits (Jope *et al*., 1996) the binding of MK801 to human hippocampal NMDA receptors (Berger *et al*., 1994). Memantine is one of the few NMDA antagonists available for use in humans and is ideal for testing in mood disorders because it has been used clinically for many years with minimal side-effects (Kornhuber *et al*., 1994) and has a favorable pharmacological profile. Felbamate (2-phenyl-1, 3-propanediol dicarbamate) is a unique, broad-spectrum anticonvulsant (Brown and Aiken, 1998) (Fig. 5). Kass reported substantial improvement with felbamate in a patient with severe bipolar disorder (Kass, 1994) (Fig. 5). However, the use of felbamate is limited by the increased risk of aplastic anemia. Zinc (ZnSO4) is a potent inhibitor of the NMDA-receptor complex; it plays an important role in a wide range of biochemical processes (Harrison and Gibbons, 1994) and might have a role in the treatment of mood disorders.

Concluding remarks

For many years, a considerable body of data has supported the notion that the mono-amine neurotransmitter systems are dysfunctional in bipolar disorder. This explains why these systems are a common target for pharmacological interventions. However, conceptual and experimental evidence indicate that abnormalities in the regulation of signal-transduction cascades and neuroplasticity might be more primary causes of the pathophysiology of bipolar disorder. Thus, it is noteworthy that growing evidence from preclinical and clinical research implicates the glutamatergic system in the pathophysiology and treatment of mood disorders. For a complex disease like bipolar disorder, it is not surprising that many molecules involved in network of signaling cascades and synaptic plasticity are regulated. The finding that mood stabilizers regulate synaptic AMPA receptors in therapeutically meaningful paradigms opens potential avenues for the development of new drugs to regulate glutamatergic synaptic strength in crucial neuronal circuits. The development of new modulators of glutamatergic neurotransmission might lead to improved therapeutics to treat these devastating mood disorders.

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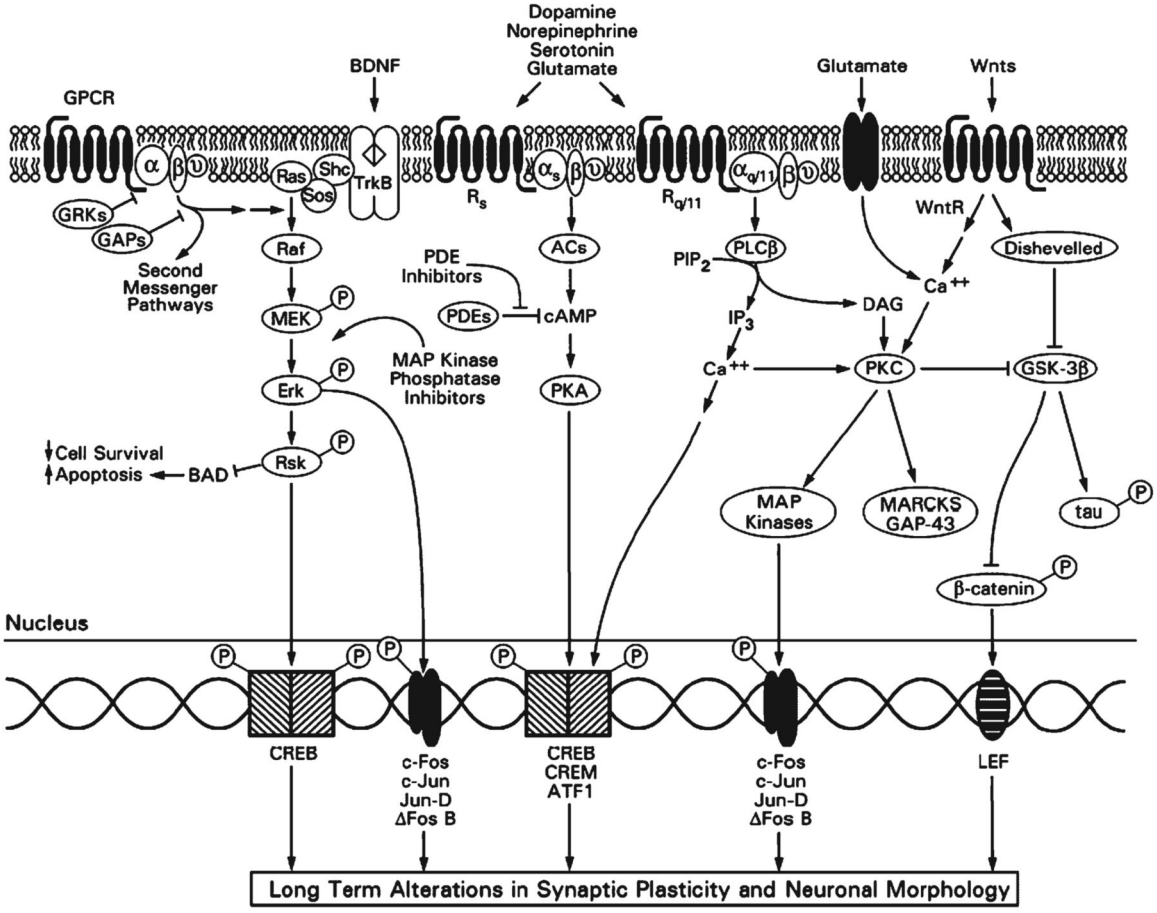
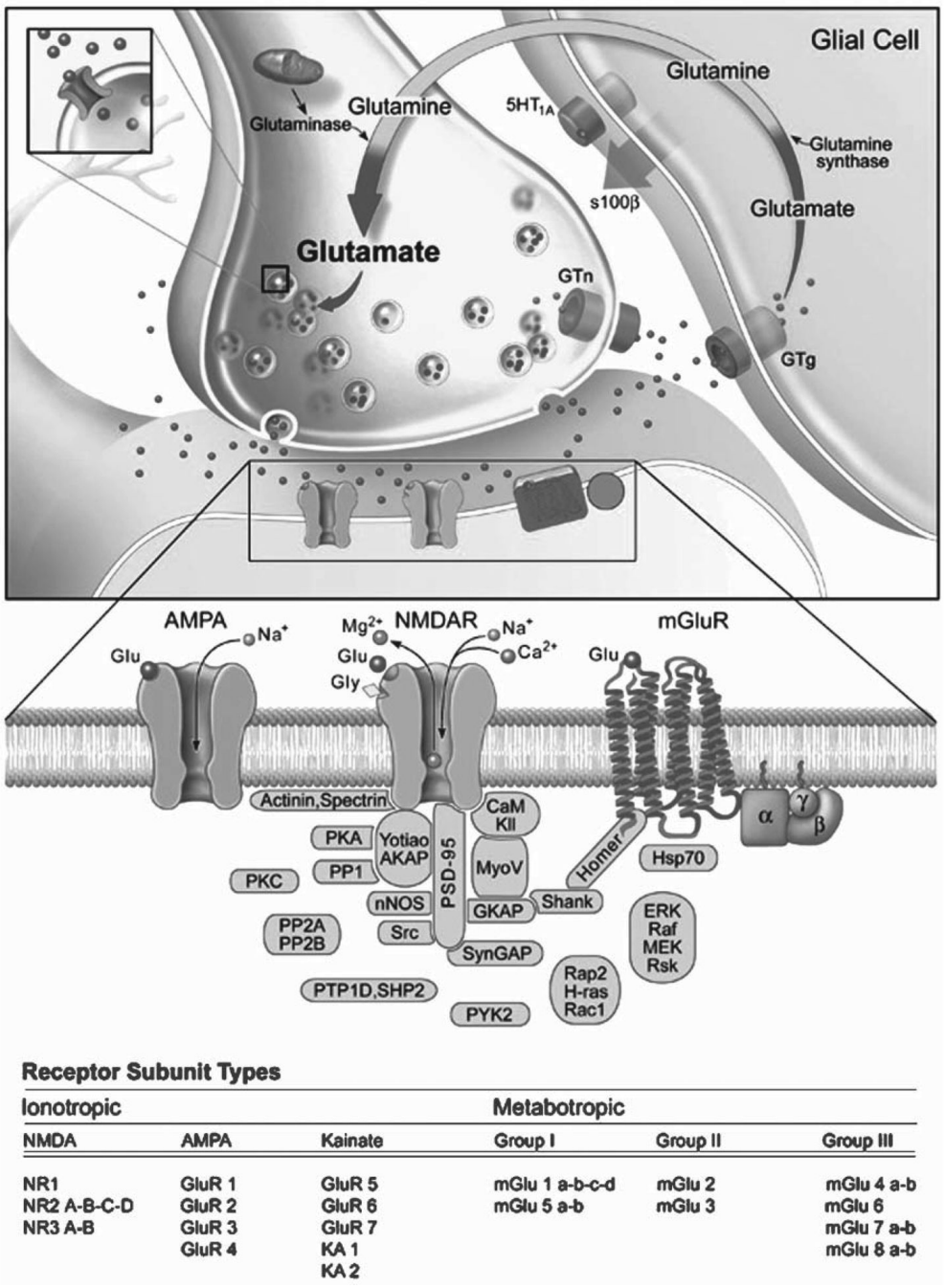


Fig. 1. Major intracellular signaling pathways in bipolar disorder

Cell-surface receptors transduce extracellular signals such as neurotransmitters and neuropeptides into the interior of the cell. Most neurotransmitters and neuropeptides communicate with other cells by activating seven-transmembrane-spanning G-protein-coupled receptors (GPCRs). As their name implies, GPCRs activate G proteins, which are composed of α and βγ subunits. Two families of proteins turn off the GPCR signal and might, therefore, represent attractive targets for new medication development. G-protein-coupled-receptor kinases (GRKs) phosphorylate GPCRs, thereby uncoupling them from their respective G proteins. GTPase-activating proteins (GAPs, also called RGS or regulators of G-protein-signaling proteins) accelerate the G-protein turn-off reaction (an intrinsic GTPase activity). Two major signaling cascades that are activated by GPCRs are the cAMP-generating second-messenger system and the PI system. cAMP activates PKA, a pathway that has been implicated in the therapeutic effects of antidepressants. Among the potential targets for the development of new antidepressants are phosphodiesterases (PDEs) that catalyze the breakdown of cAMP. Thus, PDE inhibitors are expected to sustain the cAMP signal and might represent an antidepressant-augmenting strategy. Activation of receptors coupled to PI hydrolysis results in the breakdown of phosphoinositide 4,5-biphosphate (PIP_2) into two second messengers, inositol 4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP mobilizes Ca^{2+} from intracellular stores, whereas DAG is an endogenous activator of PKC, which is also activated directly by Ca^{2+}. PKC, PKA and other Ca^{2+}-dependent kinases either directly or indirectly activate several transcription factors, including CREB, CREM, ATF-1, c-Fos, c-Jun, Jun-D and ΔFos B. Endogenous growth factors such as BDNF utilize different types of signaling pathways. BDNF binds to and activates its tyrosine kinase receptor (TrkB), which facilitates the recruitment of

other proteins (SHC and SOS) and results in the activation of the ERK–MAP kinase cascade (via sequential activation of Ras, Raf, MEK, Erk and Rsk). In addition to regulating several transcription factors, the ERK–MAP kinase casade, via Rsk, down-regulates BAD, a pro-apoptotic protein. Enhancing the ERK–MAP kinase cascade might have similar effects to endogenous neurotrophic factors; one potential strategy is to utilize inhibitors of MAP kinase phosphatases (to inhibit the turn-off reaction) as potential drugs with neurotrophic properties. In addition to utilizing GPCRs, many neurotransmitters (e.g. glutamate and GABA) produce their responses via ligand-gated ion channels. Although these responses are very rapid, they bring about more stable changes via regulation of gene transcription. One pathway that is gaining increasing attention in adult mammalian neurobiology is the Wnt signaling pathway. Wnts are a group of glycoproteins that are active in development and play important roles in the mature brain. Binding of Wnts to the Wnt receptor (WntR) activates an intermediary protein, Disheveled, which regulates GSK-3β. GSK-3β exerts many cellular effects; it regulates cytoskeletal proteins, including tau, and has an important role in determining cell-survival/cell-death decisions. GSK-3β has been identified recently as a target for the action of lithium. GSK-3β also regulates the phosphorylation of β-catenin, a protein that, when dephosphorylated, acts as a transcription factor at lymphoid enhancer factor (LEF) sites. Additional abbreviations: CREB, cAMP response element binding protein; R_q and R_s, extracellular GPCRs that are coupled to stimulation and inhibition of adenylate cyclases, respectively; $R_{q/11}$, GPCR coupled to activation of PLC; MARCKS, myristoylated alanine rich C kinase substrate, which is associated with several neuroplastic events; PLC, phospholipase C.



Receptor Subunit Types

Ionotropic			Metabotropic		
NMDA	AMPA	Kainate	Group I	Group II	Group III
NR1	GluR 1	GluR 5	mGlu 1 a-b-c-d	mGlu 2	mGlu 4 a-b
NR2 A-B-C-D	GluR 2	GluR 6	mGlu 5 a-b	mGlu 3	mGlu 6
NR3 A-B	GluR 3	GluR 7			mGlu 7 a-b
	GluR 4	KA 1			mGlu 8 a-b
		KA 2			

Fig. 2. The regulatory processes involved in glutamatergic neurotransmission

The biosynthetic pathway for glutamate involves synthesis from glucose and the transamination of α-ketoglutarate; however, a small proportion of glutamate is formed more directly from glutamine by glutamine synthetase. The latter is synthesized in glia and, via an active process (requiring ATP), is transported to neurons where glutaminase converts this precursor to glutamate. In astrocytes, glutamine is oxidized to α-ketoglutarate which can also be transported to neurons and participate in glutamate synthesis. Glutamate is either metabolized or it is sequestered and stored in secretory vesicles by vesicular glutamate transporters (VGluTs). Glutamate is then released by a calcium-dependent excitotoxic process. Once released from the presynaptic terminal, glutamate binds to many excitatory amino acid

(EAA) receptors, including ionotropic (e.g. AMPA and NMDA) and metabotropic receptors. Presynaptic regulation of glutamate release occurs through metabotropic glutamate receptors ($mGluR_{2/3}$), which act as autoreceptors. However, these receptors are also located on the postsynaptic element. The action of glutamate is terminated in the synapse by reuptake mechanisms that utilize distinct transporters (GLUTs) which exist on presynaptic nerve terminals and astrocytes. Current data indicate that astrocytic uptake of glutamate might be more important for clearing excess glutamate, raising the possibility that astrocytic loss (as documented in mood disorders) might contribute to deleterious glutamate signaling. It is known that there several intracellular proteins alter the function of glutamate receptors (see diagram).

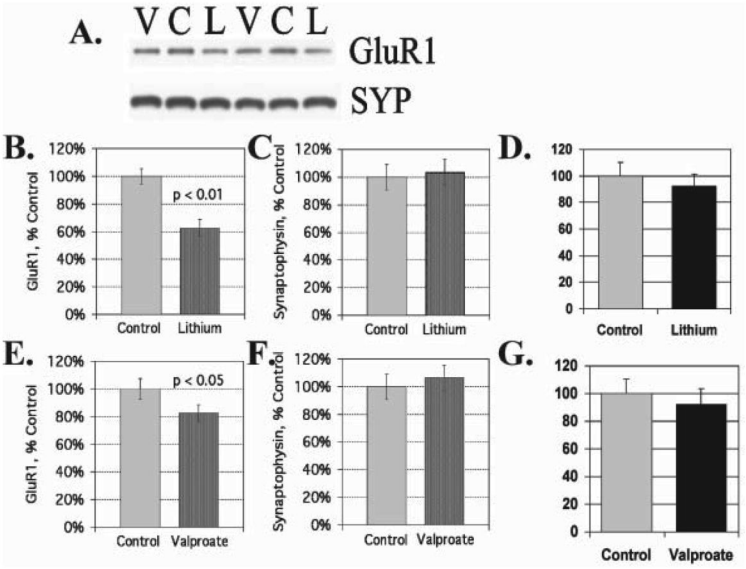


Fig. 3. Attenuation of AMPA receptor subunit GluR1 in synaptosomal preparations from animals treated long-term with lithium and VPA

(A) Western blot analysis of hippocampal synaptosomal preparation from animals treated with either lithium or VPA with anti-GluR1 and anti-synaptophysin antibodies. (B) Quantification of GluR1 in hippocampal synaptosomes from lithium-treated (n = 5) and control animals (n = 6) (T-test, $P < 0.01$). (C) Quantification of synaptophysin in hippocampal synaptosomal preparations from lithium-treated and control animals. (D) Total GluR1 concentration in hippocampal tissue homogenates from lithium-treated and control animals. (E) GluR1 protein in hippocampal synaptosomes from VPA-treated (n = 8) and control animals (n = 7) (T-test, $P < 0.05$). (F) Synaptophysin in hippocampal synaptosomal preparations from VPA-treated and control animals. (G) Total GluR1 concentrations in hippocampal tissue homogenates from VPA-treated and control animals.

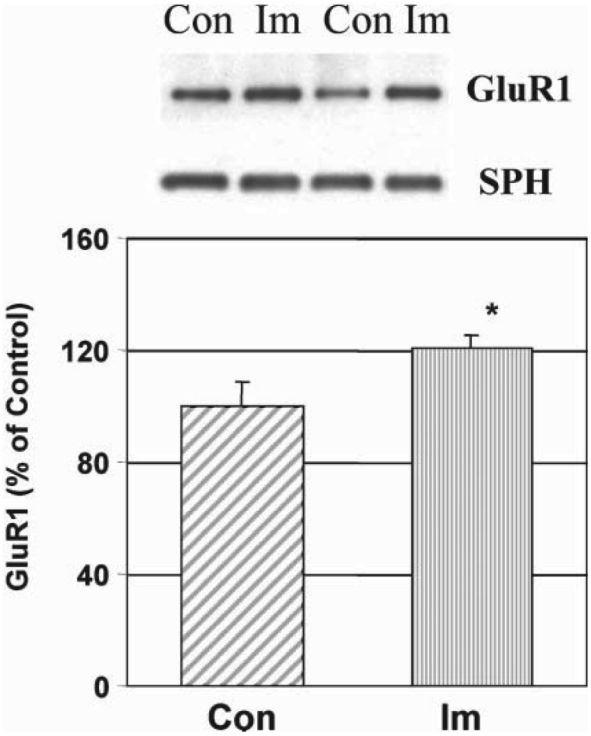


Fig. 4. Imipramine increases synaptic GluR1 *in vivo*

Rats were treated with imipramine (Im; 10 mg kg^{-1}, twice daily, intraperitoneal injection) for 4 weeks and hippocampal tissue isolated and synaptosome preparations obtained using Ficoll gradients. Equal amount of proteins were separated by gel electrophoresis and analyzed with anti-GluR1 antibodies and anti-synaptophysin (SPH) antibodies. The bands were analyzed by Kodak imaging system. $n = 8$, T-test, $P < 0.05$.

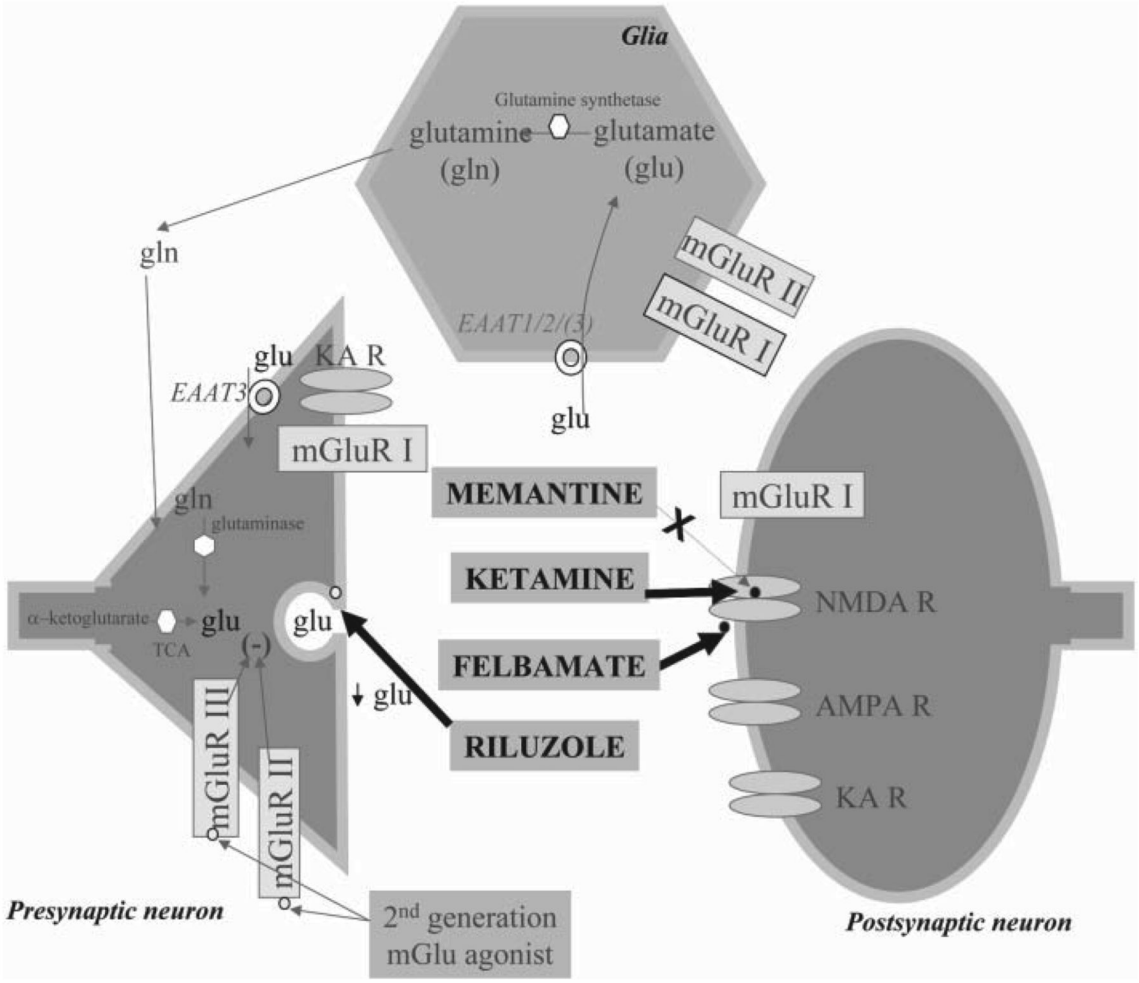


Fig. 5. Antiglutamatergic agents as putative treatments for mood disorders

Activation of the AMPA receptor (AMPA R) by glutamate depolarizes the membrane. When glutamate and glycine are present, depolarization results in the release of magnesium from the NMDA receptor (NMDA R) channel. Calcium also enters through the NMDA R pore. In addition, interchange of cations occurs via NMDA R and kainate receptors (KA R). Activation of group I metabotropic glutamate receptors (mGlu I), which are couped to a G protein (Gq/11), activates phospholipase C-β (PLC-β). Activation of group II metabotropic glutamate receptors (mGlu II), which are coupled to either Gi or Go, inhibit adenylate cyclase (AC) and opening of potassium channels (not shown), respectively. Glutamate is synthesized in neuron from α-ketoglutarate through the tricarboxylic acid cycle (TCA). After release, glutamate is sequestered by glutamate transporters (EAAT1/2/3), shown in glia and a presynaptic neuron (EAAT 3). In glia, the enzyme glutamine synthetase catabolizes glutamate to glutamine, which diffuses to neurons and is metabolized to glutamate through the enzyme glutaminase. The different glutamate receptors and the presumed antiglutamatergic drug site of action is presented. Memantine is a non-competitive antagonist at the NMDA receptor. Felbamate is an anticonvulsant, a non-competitive NMDA receptor antagonist (NR1/NR2B-specific), and inhibits the release of glutamate (acting through blockade of Ca^{2+} and Na^{+} voltage-dependant channels). Riluzole inhibits glutamate release through blockade of Ca^{2+} and Na^{+} voltage-dependant channels. Zinc is an endogenous modulator of ligand- and voltage-gated ion channels. The second generation of mGlu group II and III receptors agonist is also depicted.

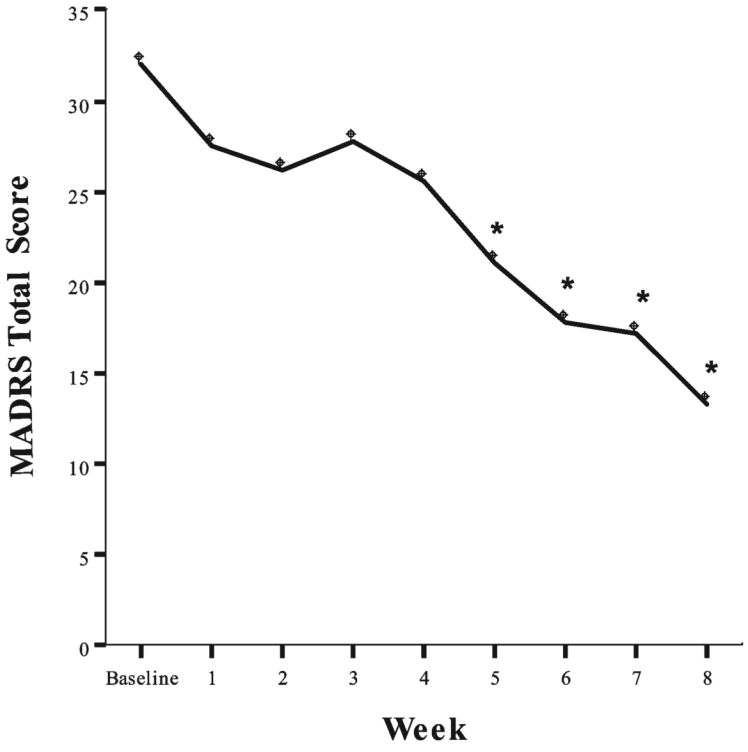


Fig. 6. Effect of riluzole in patients with treatment-resistant bipolar depression

After open treatment with lithium for a minimum of 4 weeks, subjects who continued to have a Montgomery-Asberg Depression Rating Scale (MADRS) rating scale score >20, received riluzole (50–200 mg day^{-1}) for 8 weeks. In all, 14 bipolar depressed patients entered the study. The linear mixed models for total MADRS showed a significant treatment effect ($n = 14$, $^{*}P < 0.05$ compared to baseline). No switch into hypomania or mania was observed and riluzole was well tolerated overall.