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# Bidirectional Homeostatic Regulation of a Depression-Related Brain State by Gamma-Aminobutyric Acidergic Deficits and Ketamine Treatment

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## ABSTRACT

**BACKGROUND:** Major depressive disorder is increasingly recognized to involve functional deficits in both gamma-aminobutyric acid (GABA)ergic and glutamatergic synaptic transmission. To elucidate the relationship between these phenotypes, we used $GABA_A$ receptor γ2 subunit heterozygous ($\gamma2^{+/-}$) mice, which we previously characterized as a model animal with construct, face, and predictive validity for major depressive disorder.

**METHODS:** To assess possible consequences of GABAergic deficits on glutamatergic transmission, we quantitated the cell surface expression of *N*-methyl-D-aspartate (NMDA)-type and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors and the function of synapses in the hippocampus and medial prefrontal cortex of $\gamma2^{+/-}$ mice. We also analyzed the effects of an acute dose of the experimental antidepressant ketamine on all these parameters in $\gamma2^{+/-}$ versus wild-type mice.

**RESULTS:** Modest defects in GABAergic synaptic transmission of $\gamma2^{+/-}$ mice resulted in a strikingly prominent homeostatic-like reduction in the cell surface expression of NMDA-type and AMPA-type glutamate receptors, along with prominent functional impairment of glutamatergic synapses in the hippocampus and medial prefrontal cortex. A single subanesthetic dose of ketamine normalized glutamate receptor expression and synaptic function of $\gamma2^{+/-}$ mice to wild-type levels for a prolonged period, along with antidepressant-like behavioral consequences selectively in $\gamma2^{+/-}$ mice. The GABAergic synapses of $\gamma2^{+/-}$ mice were potentiated by ketamine in parallel but only in the medial prefrontal cortex.

**CONCLUSIONS:** Depressive-like brain states that are caused by GABAergic deficits involve a homeostatic-like reduction of glutamatergic transmission that is reversible by an acute, subanesthetic dose of ketamine, along with regionally selective potentiation of GABAergic synapses. The data merge the GABAergic and glutamatergic deficit hypotheses of major depressive disorder.

*Keywords:* Antidepressant drug mechanisms, GABA, Glutamate, Homeostatic synaptic plasticity, Major depressive disorder, Neuroligin

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Major depressive disorder (MDD) is a leading cause of total disability with limited treatment options that are often ineffective (1,2). The etiology of MDD is poorly understood but thought to involve combinations of endogenous vulnerability factors and precipitating life events, such as uncontrollable stress (3). Potential vulnerability factors include diverse defects in gamma-aminobutyric acid (GABA)ergic inhibitory neurotransmission, such as reduced concentrations of GABA (4–9), reduced expression of glutamic acid decarboxylase (10,11), reduced expression of $GABA_A$ receptors ($GABA_A$Rs) (12), and impaired function of GABAergic interneurons (10,13,14) [all reviewed by Luscher *et al.* (15)].

The GABAergic deficit–induced changes in neural excitability (16) and reduced glutamic acid decarboxylase–mediated conversion of glutamate to GABA may conceivably be responsible for increased glutamate concentrations found in the brains of patients with MDD (17,18). However, some studies of MDD point to reduced rather than increased brain content of glutamate [reviewed by Niciu *et al.* (19)], suggesting a dynamic relationship between changes in GABAergic and glutamatergic transmission. Additional evidence suggestive of altered glutamatergic neurotransmission in MDD includes reduced expression and altered function of *N*-methyl-D-aspartate receptors (NMDARs) (20,21) and rapid therapeutic efficacy of subanesthetic doses of ketamine (22–24). Ketamine exerts antidepressant activity as a noncompetitive antagonist of NMDARs by ultimately enhancing glutamatergic synaptic transmission (25,26). Ketamine is especially effective in otherwise treatment-resistant forms of MDD associated with high anxiety (27). However, it is unclear how alterations in glutamatergic transmission and antidepressant efficacy of ketamine are functionally related to GABAergic deficits associated with MDD.

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Stable functioning of neural networks in the face of rapid changes in neural excitability is critically dependent on homeostatic self-tuning mechanisms that, on a slower time course, preserve the balance of excitation and inhibition and the average firing rates of neurons (28). Homeostatic mechanisms have been studied most extensively in cultured neurons. Of particular interest in the context of the present study is a slow form of homeostatic synaptic plasticity whereby pharmacologically induced hyperexcitability of cultured neurons is compensated by global scaling down of glutamatergic synapses and scaling up of inhibitory synapses (29–32).

Mice rendered hemizygous for the γ2 subunit gene (*Gabrg2*) of $GABA_ARs$ ($\gamma2^{+/-}$ mice) have been extensively characterized as a model with construct, face, and predictive validity for anxious MDD (15,33). These mice exhibit a modest impairment of GABAergic transmission characterized by loss of the γ2 subunit in ~15% of $GABA_ARs$ averaged across brain regions, with the most prominent reductions in neocortex and hippocampus (−25% to −35%) (34). The γ2-lacking $GABA_ARs$ are functionally impaired as indicated by their reduced channel conductance (12 vs. 28 pS) and failure to accumulate at synapses (34–36). Behaviorally, $\gamma2^{+/-}$ mice exhibit signs of heightened anxiety, despair, anhedonia, and constitutive stress axis activation, and all these phenotypes are normalized by long-term treatment with the antidepressant desipramine (34,37,38). Cognitive alterations of $\gamma2^{+/-}$ mice in ambiguous cue conditioning tests mimic emotional pattern separation defects associated with MDD (34,39,40). Moreover, a phospho-site mutation that increases the cell surface expression of γ2-$GABA_ARs$ has antidepressant-like behavioral consequences (41). Thus, the $\gamma2^{+/-}$ model lends support for a causative role of GABAergic deficits in the etiology of anxious MDD (15,33).

In this study, we explored the consequences of GABAergic deficits on glutamatergic synapses. We found that $\gamma2^{+/-}$ mice exhibit reduced cell surface expression and function of NMDARs and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs), along with reduced expression of the synaptic adhesion molecule neuroligin 1 (NL1) and defects in the density and function of glutamatergic synapses in the hippocampus and medial prefrontal cortex. Similar defects were observed in $\gamma2^{+/-}$ cultured neurons. Moreover, treatment of $\gamma2^{+/-}$ mice (or cultures) with a subanesthetic dose of ketamine resulted in lasting (≥3 days) enhancement and normalization of glutamate receptor (GluR) expression and glutamatergic synapse function. Thus, depression-related brain states of $\gamma2^{+/-}$ mice involve a homeostatic-like reduction of glutamatergic transmission that can be normalized for a prolonged period by the rapidly acting antidepressant ketamine. Ketamine also potentiated the function of GABAergic synapses but only in anterior cingulate cortex (ACC). These data unite the GABAergic and glutamatergic deficit hypotheses of MDD, suggest that MDD may be caused by aberrant homeostatic plasticity, and provide novel insights into the synaptic mechanisms underlying antidepressant efficacy of ketamine.

## METHODS AND MATERIALS

See the more detailed and complete version in Supplemental Methods and Materials.

### Production and Husbandry of Mice

Two different $GABA_AR$ $\gamma2^{+/-}$ mouse lines were used as part of this study, with virtually identical germline deletions of exon 8 of the *Gabrg2* locus. A first line of $\gamma2^{+/-}$ mice was maintained on a 129X1/SvJ background as previously described (34,38,42). A second line was generated on the C57BL/6J background by mating $\gamma2^{f/f}$ mice (43) with an oocyte-specific Cre line followed by outcrossing of the Cre transgene. Mice used for experimentation were littermates produced by crossing of $\gamma2^{+/-}$ mice and wild-type (WT) mice. The 129X1/SvJ line was used for preparation and analyses of cortical cultures as well as biochemical and electrophysiologic analyses of brain slices. The C57BL/6J line was used for biochemical and behavioral experimentation involving ketamine treatment.

### Drug Treatments

For treatment of cultures, the drugs were diluted or dissolved in culture media to the following final concentrations: ketamine, 10 μmol/L (Ketaject; Phoenix Pharmaceutical, Inc., St. Joseph, MO); D-(-)-2-amino-5-phosphonopentanoic acid (D-APV), 100 μmol/L (Sigma-Aldrich, St. Louis, MO); bicuculline (BIC), 20 μmol/L (R&D Systems, Minneapolis, MN); and Ro 25-6981, 10 μmol/L (Sigma-Aldrich). For treatment of mice (8–9 weeks old), ketamine (Ketaject diluted to 1 mg/mL in 0.9% saline) was administered at 10 mg/kg (biochemical and electrophysiologic analyses) or 3 mg/kg (behavioral analyses) (intraperitoneal injection [i.p.]) as previously described (26,44).

### Cell Surface Biotinylation

Cortical cultures from $\gamma2^{+/-}$ and WT mice were generated from embryonic day 14–15 embryos (129X1/SvJ line) and subjected to cell surface biotinylation at 21 days in vitro and purification using NeutrAvidin agarose beads (Thermo Scientific, Rockford, IL) as described previously (45). For biotinylation of brain slices, we adapted the protocol of Terunuma *et al.* (46). The biotinylated proteins were quantitated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis/western blot using an Odyssey CLx infrared imager (LI-COR Biosciences, Lincoln, NE). Amounts of cell surface biotinylated proteins were normalized to amounts of β-tubulin in total extracts quantitated on parallel gels.

### Immunofluorescent Staining of Cortical Cultures

Immunofluorescent staining of neurons employed glia-free cortical cultures prepared from embryonic day 14–15 embryos as previously described (47). The cells were fixed, permeabilized, and stained at 21 days in vitro as described (47) using rabbit anti-MAP2 (1:1000, Ab5622; EMD Millipore Corp., Billerica, MA), guinea pig anti-VGluT1 (1:500, LV1439669; EMD Millipore Corp.), mouse anti-PSD95 (1:1500, No. 28879; EMD Millipore Corp.), mouse anti-gephyrin (1:500, No. 147111; Synaptic Systems GmbH, Goettingen, Germany), and rabbit anti–vesicular GABA transporter (1:1000, No. 131002; Synaptic Systems GmbH). Synaptic immunoreactivities were developed and quantified as described (47).

### Electrophysiology

Coronal slices (350 μm) containing the dorsal hippocampus or ACC were prepared using a vibratome (Leica VT1200S; Leica Biosystems, Inc., Buffalo Grove, IL) from 7- to 13-week-old