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NMDA receptor subunits and associated signaling molecules mediating antidepressant-related effects of NMDA-GluN2B antagonism

Carly Kiselycznyk¹, Nicholas Jury^{1,*}, Lindsay Halladay¹, Kazu Nakazawa², Masayoshi Mishina³, Rolf Sprengel⁴, Seth G.N. Grant⁵, Per Svenningsson⁶, and Andrew Holmes¹

¹Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA

²Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL, USA

³Brain Science Laboratory, The Research Organization of Science and Technology, Ritsumeikan University, Kusatsu, Shiga, Japan

⁴Department of Molecular Neurobiology, Max Planck Institute for Medical Research, Heidelberg, Germany

⁵Genes to Cognition Program, University of Edinburgh, Edinburgh UK

⁶Laboratory of Translational Neuropharmacology, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Abstract

Drugs targeting the glutamate *N*-methyl-D-aspartate receptor (NMDAR) may be efficacious for treating mood disorders, as exemplified by the rapid antidepressant effects produced by single administration of the NMDAR antagonist ketamine. Though the precise mechanisms underlying the antidepressant-related effects of NMDAR antagonism remain unclear, recent studies implicate specific NMDAR subunits, including GluN2A and GluN2B, as well as the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPA) subunit glutamate receptor interacting molecule, PSD-95. Here, integrating mutant and pharmacological in mice, we investigated the contribution of these subunits and molecules to antidepressant-related behaviors and the antidepressant-related effects of the GluN2B blocker, Ro 25-6981. We found that global deletion of GluA1 or PSD-95 reduced forced swim test (FST) immobility, mimicking the antidepressant-related effect produced by systemically administered Ro 25-6981 in C57BL/6J mice. Moreover, the FST antidepressant-like effects of systemic Ro 25-6981 were intact in mutants with global GluA1 deletion or GluN1 deletion in forebrain interneurons, but were absent

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*Correspondence to: Nicholas Jury, Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA, nick.jury@nih.gov.

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in mutants constitutively lacking GluN2A or PSD-95. Next, we found that microinfusing Ro 25-6981 into the medial prefrontal cortex (mPFC), but not basolateral amygdala, of C57BL/6J mice was sufficient to produce an antidepressant-like effect. Together, these findings extend and refine current understanding of the mechanisms mediating antidepressant-like effects produced by NMDAR-GluN2B antagonists, and may inform the development of a novel class of medications for treating depression that target the GluN2B subtype of NMDAR.

Keywords

NMDA; glutamate; PSD-95; prefrontal cortex; GluN2B; GluA1; depression

Introduction

There is growing evidence that drug treatments targeting the glutamate NMDAR may be efficacious in mood and anxiety-related disorders [1]. A single administration of non-specific NMDAR antagonists, such as ketamine, produces rapid antidepressant effects in both clinical [1–5] and relevant preclinical assays, such as the forced swim test (FST) [2–13]. Systemic administration of NMDAR antagonists, including ketamine, has been shown to increase extracellular levels of glutamate and glutamate cycling in the rodent medial prefrontal cortex (mPFC), at doses similar to those producing antidepressant-like actions [14, 15]. The effect of these drugs may be to increase glutamate transmission through synaptic proteins on excitatory cells and exert an excitatory postsynaptic effect on pyramidal neurons – possibly due to antagonism of interneuronal NMDARs that normally exert tonic inhibition over pyramidal cell excitability [16, 17]. This scheme suggests that NMDARs on inhibitory cells, as well as various other synaptic proteins on excitatory cells, are necessary for the antidepressant-like response to NMDAR blockers. However, the precise mechanisms by which NMDAR blockers produce antidepressant-related effects remain to be elucidated.

Another outstanding question relates to the contribution of specific NMDAR subunits to antidepressant-related effects. The NMDAR is a heteromeric receptor complex comprised of a functionally obligatory GluN1 subunit and combinations of GluN2-3 subunits. Constitutive deletion of GluN2A in mice mimics the antidepressant-like profile of NMDAR antagonists [18]. Furthermore, systemic administration of compounds that selectively block GluN2B, such as CP-101-606, ifenprodil and Ro 25-6981, produces antidepressant-like effects in rodents [7, 9, 13, 19–23] and therapeutic efficacy in human depression [24]. In mice, deletion of GluN2B in corticohippocampal pyramidal neurons produce an antidepressant-like phenotype when the deletion extends to the ventral hippocampus [13] but only after repeated stress when deletion is restricted to the dorsal CA1 hippocampal subregion [20]. GluN1 deletion in forebrain interneurons also produces a trend for an antidepressant-like effect in some studies [25, 26]. Together, these data support an antidepressant-related role for the GluN2A and GluN2B subunits, but show that this contribution may be dependent on the brain region and cell type where the subunits are expressed or require additional conditions, such as the concurrent increase in glutamate transmission.

In addition to direct pharmacological effects on NMDARs, NMDAR antagonists cause downstream increases in the synaptic mPFC and hippocampal expression of the AMPAR GluA1 subunit, the NMDAR scaffolding protein, PSD-95, and various plasticity-related proteins including neurotrophins, mTOR and GSK-3 β [3, 4, 9, 11, 23, 27]. These findings have led to a ‘synaptogenic model’ of the antidepressant-related efficacy of NMDAR antagonists, in which the drugs exert their behavioral effects by promoting the formation of synapses in key neural circuits [28]. In support of this model, recent studies have found that blockade or gene deletion of AMPARs [6, 7, 23, 29–31], mTOR [23], GSK-3 β [11], or a loss-of-function brain-derived neurotrophic factor (BDNF) mutation [10] prevent the antidepressant-like effects of ketamine. There is also some evidence that these antidepressant-related effects are localized to the mPFC: an mTOR inhibitor infused into the mPFC is sufficient to occlude ketamine’s antidepressant-like effect [23].

Collectively, the current literature shows that NMDAR antagonists recruit multiple glutamate receptors and downstream signaling pathways to produce their antidepressant-related effects, although a clear and comprehensive model is yet to be defined. The goal of the current study was to build upon these prior findings to explore how loss of GluA1, GluN1, GluN2A, GluN2B, or PSD-95 affects antidepressant-related behavior, and the antidepressant-related effects of an NMDAR antagonist - using a combination of mutant, pharmacological and virus-based approaches. We focused on a selective GluN2B antagonist (Ro 25-6981), given the aforementioned evidence that this class of drugs has antidepressant-like effects in rodents [7, 9, 19–23] and humans [24]. Antidepressant-related activity was assayed in mice using the FST, a simple behavioral assay sensitive to antidepressant drug treatment [32, 33]. We first assessed whether loss-of-function mutation of GluN1, GluA1 and PSD-95 affected baseline FST depression-related behavior or modified the antidepressant-like effects of the GluN2B antagonist, Ro 25-6981. Next, we sought to localize the antidepressant-like effects of GluN2B antagonism by testing the antidepressant-like effects of microinfusing Ro 25-6981 into either the mPFC or another key emotion-mediating region, the basolateral amygdala (BLA).

Material and methods

Subjects

Male C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at ~2 months of age, housed 2 per cage in a temperature- and humidity-controlled *vivarium* under a 12 h light/dark cycle (lights on 0600 h). Mice were given at least 1 week of acclimation to the local housing conditions before testing. For all experiments testing was conducted during the light phase. Mice were at least 8 weeks of age unless stated otherwise.

GluN1^{INTER-KO} mice were generated as described previously [26]. GluN1 was postnatally deleted by crossing floxed-GluN1 mutants with mutants expressing Cre recombinase driven by the Ppp1r2 promoter (protein phosphatase 1, regulatory inhibitor subunit 2). The position of the loxP sites in the floxed mutants (referred to as the ‘Flox B line’ in [26]) causes delayed recombination at a post-adolescent age. There is a resultant loss of GluN1 in 40–50% of, primarily parvalbumin-positive, GABAergic interneurons in corticolimbic regions [26]. GluN1^{INTER-KO} and floxed-GluN1 controls were littermates bred from floxed-GluN1/

Cre-positive dam and floxed-GluN1/Cre-negative sire at the NIH. Mice were tested after 20 weeks of age, when GluN1 deletion is expected to be restricted to interneurons in corticolimbic regions [26]. The mutants were backcrossed onto a C57BL/6J for 5–7 generations. Males and females were used.

GluN2A^{KO} mice were generated as previously described and have a constitutive deletion of GluN2A [34–38]. The mutant line was backcrossed onto a C57BL/6J background for >10 generations. GluN2A^{KO} and non-mutant wild-type (WT) controls were bred from GluN2A heterozygous parents at the NIH. Males and females were used.

GluA1^{KO} mice were generated as previously described and have a constitutive deletion of GluA1 [29, 36, 39–42]. The mutant line was backcrossed onto a C57BL/6J background for >10 generations. GluA1^{KO} and non-mutant WT controls were bred from GluA1 heterozygous parents at the NIH. Males and females were used.

PSD-95^{KO} mice were generated as previously described and have a constitutive deletion of PSD-95 [43–45]. The mutant line was backcrossed onto a C57BL/6J background for >10 generations. PSD-95^{KO} and non-mutant WT controls were bred from PSD-95 heterozygous parents at The Jackson Laboratory (Bar Harbor, ME, USA) and transported to NIH at 8 weeks of age. Males and females were used.

Separate groups of mice were used in each FST experiment. The number of mice used in each experiment is given in the figure legends. Experimental procedures were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the local NIAAA Animal Care and Use Committee.

Depression-related phenotype of loss-of-function NMDAR, AMPAR and PSD-95 mutations

The FST was conducted based on previously described methods [46]. Mice were gently lowered into a 20 cm-diameter cylinder, filled with 24 ± 1.0 °C water, for a 6-minute test. Immobility (cessation of limb movements except minor movement necessary to keep the mouse afloat) was scored every five seconds. Immobility during the final 4 minutes of the test was calculated and converted to a percentage.

The basal FST phenotype of GluN1^{INTER-KO}, GluA1^{KO} and PSD-95^{KO} mutants was tested. In addition, Ro 25-6981-treated C57BL/6J mice, and the GluN1^{INTER-KO}, GluN2A^{KO} and GluA1^{KO} mutants were assessed for responses to repeated forced swim. The mouse was gently lowered into a 30 cm-diameter water-filled cylinder for 10 consecutive days [20]. After 60 seconds elapsed, a platform (plastic wiffle ball) was remotely released and floated up to beneath a 4×4 cm escape hole in the side of the cylinder. The platform sank when the mouse attempted to climb onto it to escape through the hole. Each trial ended 20 seconds after platform release. Immobility during the trial was calculated (i.e., times immobile during 120×5 -second intervals) and converted to a percentage.

Antidepressant-like effects of Ro 25-6981 in NMDAR, AMPAR and PSD-95 loss-of-function mutants

To first confirm that Ro 25-6981 showed an antidepressant-like effect in the FST of non-mutants, mice were intraperitoneally injected (10 mL/kg body weight) with 10 mg/kg Ro 25-6981 (Tocris, Ellisville Missouri) or 0.9% saline vehicle 30 minutes prior to the FST. This dose was chosen based on previous studies in the FST [7, 20, 23]. Using the same procedure, GluA1^{KO}, GluN2A^{KO}, GluN1^{INTER-KO}, and PSD-95 mutants were tested in the FST after injection of Ro 25-6981 or vehicle. One week later, treatment assignments were reversed for each mouse and mice were tested in the open field test 30 minutes after injection of Ro 25-6981 or vehicle. Mice were placed in a 40 × 40 × 35 cm square arena (60 lux) constructed of white Plexiglas for 30 minutes, as previously described [47]. Total distance traveled was measured by the Ethovision videotracking system (Noldus Information Technology Inc., Leesburg, VA) and expressed in meters.

Regionally localized antidepressant-like effects of Ro 25-6981

To localize the FST-effect of GluN2B antagonism to a specific brain region, Ro 25-6981 was infused into one of two structures critical to emotional regulation, the mPFC or BLA. C57BL/6J mice were anesthetized with isoflurane and the head was fixed into a stereotaxic device (Kopf Instruments, Tujunga, CA, USA) and implanted with 26-gauge guide cannula (Plastics One, Roanoke, VA, USA) bilaterally targeting either the mPFC or BLA. The coordinates for mPFC were anteroposterior (AP) +2.00 mm, mediolateral ±0.40 mm, dorsoventral (DV) −2.00 mm relative to bregma, based on our previous studies [48, 49]. The coordinates for BLA were −1.40 mm AP, ±3.30 mm ML, and −3.90 mm DV relative to bregma, again based on our previous studies [50]. Mice were given at least one week of post-surgery recovery before testing.

Mice were tested in the FST 15 minutes following infusion of saline or 1.0 µg/µL Ro 25-6981 (Tocris, Ellisville Missouri) in a volume of 0.30 µL per hemisphere via 33-gauge infusion cannulas (Plastics One, Roanoke, VA, USA) attached with polyurethane tubing to a 2 µL Hamilton syringe (Hamilton, Reno NV). Solutions were slowly infused at a rate of 0.05 µL/min using a microinfusion pump (Harvard Apparatus PHD 22/2000, Holliston, MA, USA) and injectors remained in place for an additional 2 minutes to ensure diffusion into the tissue. The concentration of Ro 25-6981 was based on previous work showing effects of intra-cranial infusion on anxiety-related tasks [51].

To compare the effects of intra-mPFC and intra-BLA Ro 25-6981 infusion in the FST with performance on another behavior known to be modulated by GluN2B [20], we tested mice in the light/dark exploration test for anxiety-like behavior. This test was conducted one week prior to FST to avoid potential effects of the swim stressor on anxiety-like behavior. A Latin square design was used in which drug treatments were switched for each mouse for the two tests. The FST was conducted based on previously described methods [52]. Mice began the test in an opaque black Plexiglas shelter (39 × 13 × 16 cm) with a 13 × 8 cm opening at floor level that opened onto a large white Plexiglas square arena (39 × 39 × 35 cm) illuminated to ~90 lux. Entries into and percent time spent in the light compartment over a fifteen minute session was scored automatically by the Ethovision videotracking system (Noldus

Information Technology Inc., Leesburg, VA), with the first 5 minutes used for analysis, as previously described [53].

On completion of testing, mice were given an overdose of anesthetic, infused with 4% methylene blue through the guide cannula and then transcardially perfused with 4% paraformaldehyde. Coronal sections 50- μ m thick were cut with a vibratome (Classic 1000 model, Vibratome, Bannockburn, IL, USA) and stained with cresyl violet. The localization of cannula tips was estimated with the aid of an Olympus (Center Valley, PA, USA) BX41 microscope.

Statistical analysis

The effects of drug, genotype or virus-group were analyzed using Student's t-test or two-factor analysis of variances (ANOVA), followed by Newman Keuls post-hoc analysis. The effects of drug/genotype and trial were analyzed using two-factor ANOVA, with repeated measures for trial. The statistical threshold was set at $P < .05$.

Results

Depression-related phenotype of loss-of-function NMDAR, AMPAR and PSD-95 mutations

Phenotyping for basal FST behavior found that GluN1^{INTER-KO} mutants did not differ from floxed controls (Figure 1A). The GluA1^{KO} ($t(13)=11.13$, $P < .01$) (Figure 1B) and PSD-95 ($t(19)=3.09$, $P < .01$) mutants showed significantly less immobility than their respective WT controls. (Figure 1C).

In the repeated forced swim test, Ro 25-6981 significantly reduced immobility relative to vehicle controls, in C57BL/6J mice ($t(14)=3.09$, $P < .01$) (Figure S1A), whereas the GluN1^{INTER-KO}, GluN2A^{KO} and GluA1^{KO} mutants showed similar immobility to floxed controls (Figure S1B–D).

Antidepressant-like effects of Ro 25-6981 in NMDAR, AMPAR and PSD-95 loss-of-function mutants

Systemic administration of Ro 25-6981 significantly reduced immobility in C57BL/6J mice, as compared to vehicle ($t(10)=3.64$, $P < .01$) (Figure 2A). In the GluN1^{INTER-KO} mutants, Ro 25-6981 significantly decreased immobility regardless of genotype (drug effect: $F_{1,44}=15.18$, $P < .01$) (Figure 2B). By contrast, in the GluN2A^{KO} mutants, Ro 25-6981 significantly decreased immobility in WT controls, but not the mutant mice, and the vehicle-treated mutants were less immobile than vehicle-treated WT counterparts (drug x genotype interaction: $F_{1,42}=4.74$, $P < .05$, followed by post hoc tests) (Figure 2C). In the GluA1^{KO} mutants, both Ro 25-6981 ($F_{1,20}=6.79$, $P < .05$) and genotype ($F_{1,20}=20.90$, $P < .01$) significantly reduced immobility (Figure 2D). Finally, Ro 25-6981 significantly decreased immobility in WT controls, but not the PSD-95^{KO} mutants, and there was significantly lower immobility in the vehicle-treated mutants than WT controls (drug x genotype interaction: $F_{1,18}=4.87$, $P < .05$) (Figure 2E).

In the open field, Ro 25-6981 increased distance travelled, regardless of genotype, in the GluN1^{INTER-KO} mutants (drug effect: $F_{1,41}=16.70$, $P < .01$) (Figure S2), but had no effect in

the GluN2A^{KO} or GluA1^{KO} mutants (Figure S2B–C), and decreased distance travelled, irrespective of genotype, in the PSD-95^{KO} mutants (drug effect: $F_{1,18}=5.37$, $P<.05$) (Figure S2D). The GluN2A^{KO} (genotype effect: $F_{1,29}=6.27$, $P<.05$) and GluA1^{KO} (genotype effect: $F_{1,19}=6.62$, $P<.05$) mutants travelled farther than their respective WT controls.

Regionally localized antidepressant-like effects of Ro 25-6981

Microinfusion of Ro 25-6981 into the mPFC significantly reduced immobility in the FST, as compared to vehicle-infusion ($t(15)=2.42$, $P<.05$) (Figure 3A,B), but had no effect on light compartment entries or time in the light/dark exploration test (Figure 3C). In contrast, infusion of Ro 25-6981 into the BLA did not significantly alter FST immobility (Figure 3D,E) or light compartment exploration (Figure 3F).

Discussion

The goal of the current study was to illuminate the mechanisms underlying the antidepressant-related effects of GluN2B antagonism by assessing the contribution of various glutamate receptor subunits and the glutamate anchoring protein, PSD-95, to these effects.

Systemic administration of the selective GluN2B antagonist, Ro 25-6981, reliably reduced immobility in C57BL/6J mice. This is in line with multiple prior studies in rats and mice reporting FST antidepressant-like effects of Ro 25-6981 [7, 9, 13, 19–23] or the non-selective NMDAR antagonist, ketamine [2–6, 8–13, 23, 27]. It also resembles the acute FST antidepressant-like phenotype produced by conditional gene deletion of GluN2B on pyramidal neurons throughout the cortex and hippocampus [13], but not by more restricted cortical, plus dorsal CA1 hippocampal, deletion, which only affects FST antidepressant-like behavior after repeated FST [20]. Here, the antidepressant-like effects of Ro 25-6981 were phenocopied by constitutive, global loss of the NMDAR GluN2A subunit or AMPAR GluA1 subunit, as previously reported [18, 29, 39], suggesting the antidepressant-like actions of NMDAR antagonists may be produced by blockade of these subunits. Indeed, the loss of the drug's efficacy in the GluA1 mutants fits the earlier observation that treatment with an AMPAR antagonist (NBQX) occludes the antidepressant-like effects of Ro 25-6981 [7, 31]. However, we cannot exclude the possibility that the failure of Ro 25-6981 to reduce immobility could reflect a 'floor' effect of low baseline immobility in the GluA1 mutant. A floor effect could also have contributed to the apparent loss of Ro 25-6981's effects in the GluN2A mutants.

Restricted deletion of the NMDAR GluN1 subunit in forebrain interneurons did not significantly affect FST behavior, though a modest trend for reduced immobility was evident (similar to that reported with postnatal GluN1 deletion in prior work [25, 26]). The lack of effect of loss of inhibitory GluN1 argue against the hypothesis that antidepressant-like effects are produced by loss of interneuronal NMDAR activity, leading to the disinhibition of pyramidal neuronal firing. However, this conclusion is tempered by the fact that GluN1 is only deleted on a subset of (primarily parvalbumin) interneurons [26] – leaving open the possibility that the remaining interneuronal receptors were sufficient to maintain normal FST behavior in these mutants.

A caveat to the FST data in these the GluA1 and GluN2A mutants is that both lines traveled farther than WT controls in an open field test, suggesting increases in locomotor activity phenotype could have accounted for their reduced FST immobility. Previous work has also found both of these mutants show hyperactivity, albeit in novel and not familiar or low-stress environments [18, 36, 41]. A similar caveat could be levelled at the antidepressant-like effects of Ro 25-6981. However, across experiments, Ro 25-6981 treatment only caused open field locomotor hyperactivity in the GluN1 mutant line (as previously reported with ketamine, but not MK-801 [25, 26, 54]); with no effects evident in the GluA1 or GluN2A lines and a significant decrease in locomotor activity in the PSD-95 mutant line. The variability in this effect echoes previous findings with Ro 25-6981 and ketamine, where increases, decreases and no change [13, 20, 21, 55–57] in open field activity have all been reported. The reason for this variability is currently unclear, but does suggest that locomotor activating effects of these drugs are unlikely to solely account for the ability to produce an antidepressant-like reduction in immobility.

Mutant mice lacking the glutamate receptor interacting protein, PSD-95, showed a basal antidepressant-like phenotype and insensitivity to antidepressant-like effects of Ro 25-6981. A recent study found that pharmacological inhibition of nitric oxide synthase coupling to PSD-95 also produced an antidepressant-like effects in the rat FST [58]. The loss of Ro 25-6981's antidepressant-like effects following PSD-95 deletion is also notable in view of the finding that synaptic PSD-95 is increased in the mPFC after ketamine treatment [9, 23]. Together, these data suggest that dynamic alterations in PSD-95 may be a critical mechanism mediating antidepressant-related behavior in the FST. The precise nature of this contribution remains unclear, however. On the one hand, functional loss/inhibition of PSD-95 could produce an antidepressant-like effect due to disruption of synaptic NMDAR signaling, thereby mimicking the action of NMDAR blockers. On the other hand, increases in synaptic PSD-95 might be expected to support antidepressant-like efficacy of NMDAR antagonism by promoting dendritic spine formation and plasticity in critical circuits within the mPFC [28].

While parsing these potential roles awaits additional work, the current study provides some additional insights into the role of the mPFC and PSD-95 expressed in the mPFC. Infusion of Ro 25-6981 directly into the mPFC, but not the BLA, was sufficient to mimic the antidepressant-like effect of systemic Ro 25-6981 treatment. This effect was behaviorally selective to the FST – given that mPFC-infusion of Ro 25-6981 did not alter behavior in the light/dark exploration test for anxiety-like behavior. These data demonstrate a key contribution of the mPFC to the antidepressant-like effects of Ro 25-6981, consistent with prior evidence that the antidepressant-like effects of Ro 25-6981 and ketamine are associated with synaptogenesis and increases in synaptic excitability and protein expression, including GluA1, PSD-95 and Arc, in the mPFC (and dorsal hippocampus), and with the observation that intra-mPFC inhibition of mTOR blocks the antidepressant-like of systemic ketamine [3, 4, 9, 11, 13, 23]. The current finding that the antidepressant-like effects of Ro 25-6981 are localized to the mPFC, together with the earlier evidence of antidepressant-induced increases in synaptic mPFC PSD-95, suggests that the antidepressant-like effects of Ro 25-6981 may depend on PSD-95 in the mPFC.

In summary, the current study provides further evidence of antidepressant-like activity of GluN2B antagonism and offers novel insight into the glutamate receptor subunits, interacting molecules and brain regions mediating these effects. Loss-of-function mutation of GluA1 and PSD-95 mimicked the antidepressant-related effect of Ro 25-6981 by reducing FST immobility. In addition, the antidepressant-like effects of Ro 25-6981 were unaltered in mutants with global GluA1 deficiency or interneuron-specific GluN1 deletion, but absent in mutants with global deletion of GluN2A or PSD-95. Moreover, pharmacological blockade of GluN2B in the mPFC was sufficient to produce an antidepressant-like action and global PSD-95 deletion prevented the antidepressant-like effects of systemic GluN2B antagonism. These findings help extend and refine current understanding of the mechanisms mediating antidepressant-like effects of NMDAR antagonists, and help inform the development of a novel class of potential medications for treating depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research Highlights

- We studied depressive behaviors with mutant, pharmacological, and viral strategies
- We studied the mechanisms underlying antidepressant activity of NMDAR antagonists
- The effect NMDAR antagonists was not blocked by deletion of interneuronal NMDARs
- Loss of the synaptic protein PSD-95 decreased depression-related behaviors in mice
- Infusion of NMDAR antagonist to the PFC was sufficient for antidepressant response

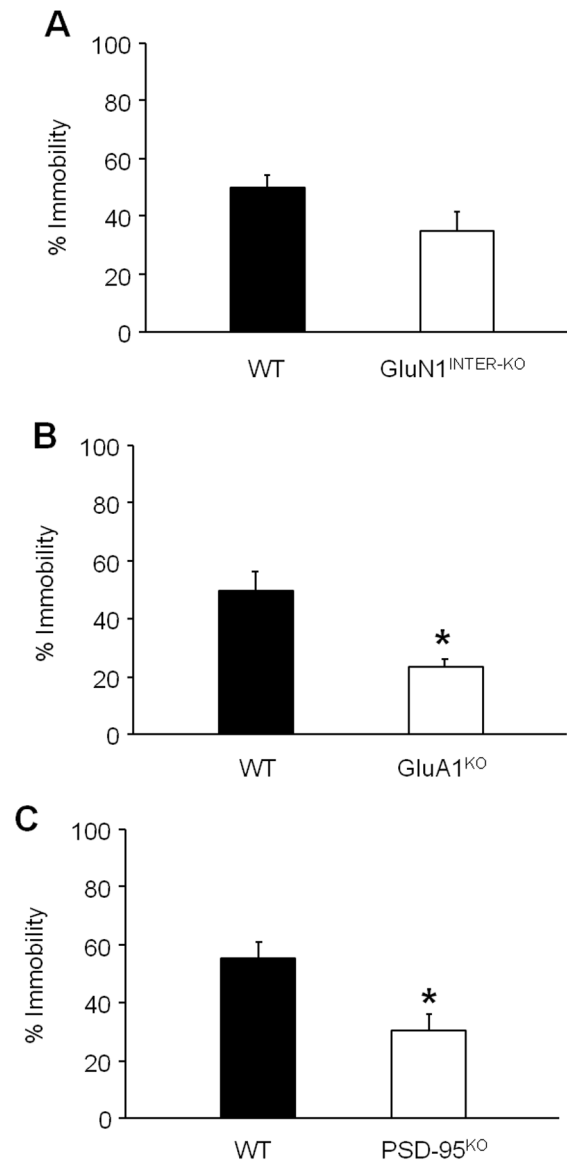


Figure 1. FST phenotype of loss-of-function NMDAR, AMPAR and PSD-95 mutations
 (A) GluN1^{INTER-KO} mutants showed similar immobility to floxed controls (n=7–18 per genotype). (B) GluA1^{KO} mutants showed less immobility than WT controls (n=7–8). (C) PSD-95 mutants showed significantly less than WT controls (n=10–11 per genotype). Data are means \pm SEM. * $P < .05$ WT versus KO

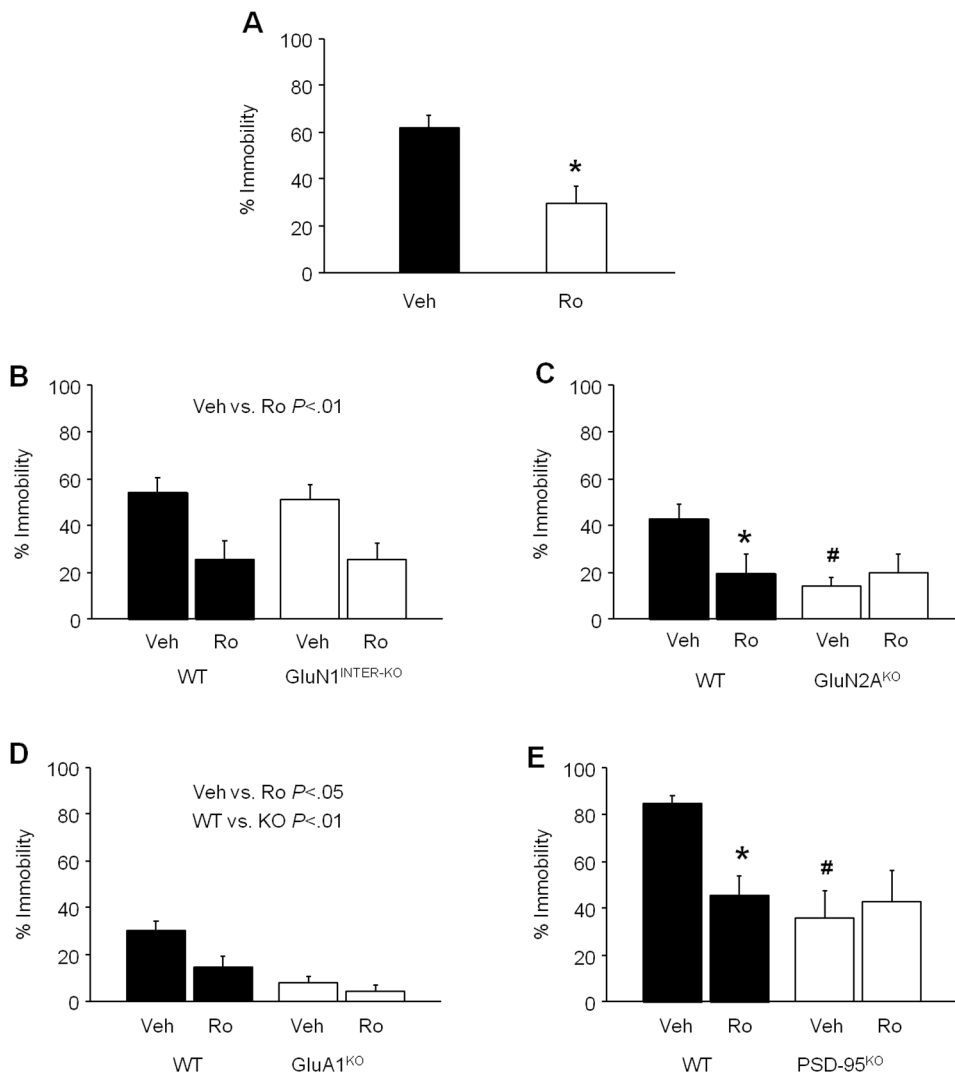


Figure 2. FST antidepressant-like effects of Ro 25-6981 in NMDAR, AMPAR and PSD-95 loss-of-function mutants

(A) Systemic Ro 25-6981 reduced FST immobility in C57BL/6J mice, relative to vehicle (n=6 per treatment). (B) In GluN1^{INTER-KO} mutant mice, Ro 25-6981 decreased FST immobility regardless of genotype (n=12 per genotype/treatment). (C) Ro 25-6981 decreased FST immobility in WT controls but not GluN2A^{KO} mutant mice, and vehicle-treated mutants were less immobile than vehicle-treated WT controls (n=10–14 per genotype/treatment). (D) In GluA1^{KO} mutant mice, Ro 25-6981 decreased FST immobility regardless of genotype, and mutants were less immobile than WT controls (n=6 per genotype/treatment). (E) Ro 25-6981 decreased FST immobility in WT controls but not PSD-95^{KO} mutant mice, and vehicle-treated mutants were less immobile than vehicle-treated WT controls (n=5–6 per genotype/treatment). Veh=vehicle, Ro=Ro 25-6981. Data are means \pm SEM. * $P < .05$ Ro versus Veh, # $P < .05$ KO versus WT

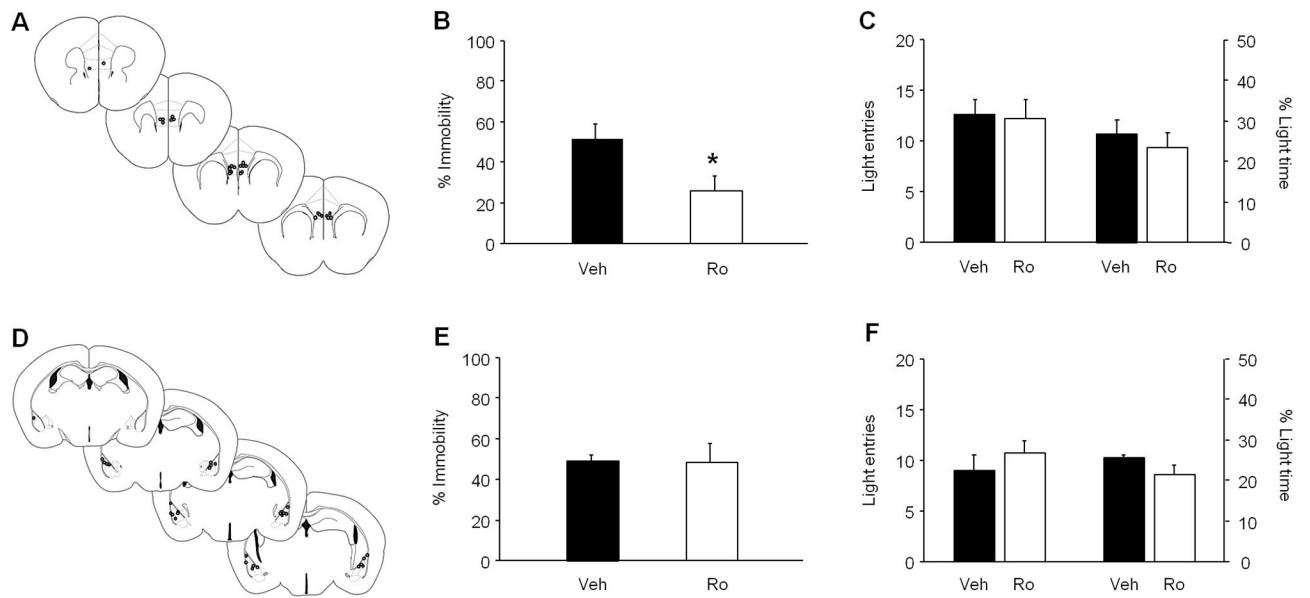


Figure 3. Regionally localized FST antidepressant-like effects of Ro 25-6981

(A) Estimated cannula placements in the mPFC. (B) Microinfusion of Ro 25-6981 into the mPFC reduced FST immobility, as compared to vehicle-infusion (n=8–9 per treatment). (C) Intra-mPFC infusion of Ro 25-6981 did not affect entries or time spent in the light compartment of the light/dark exploration test (n=7–9 per treatment). (D) Estimated cannula placements in the BLA. (E) Microinfusion of Ro 25-6981 into the BLA did not alter FST immobility (n=7–9 per treatment). (F) Intra-BLA infusion of Ro 25-6981 did not affect entries or time spent in the light compartment of the light/dark exploration test (n=8–9 per treatment). mPFC=medial prefrontal cortex, BLA=basolateral amygdala, Veh=vehicle, Ro=Ro 25-6981. Data are means \pm SEM. * P <.05 Ro versus Veh