A single injection of N-methyl-D-aspartate receptor (NMDAR) antagonists produces a rapid antidepressant response. Lasting changes in the synapse structure and composition underlie the effectiveness of these drugs. We recently discovered that rapid antidepressants cause a shift in the γ-aminobutyric acid receptor (GABA<sub>B</sub>) signaling pathway, such that GABA<sub>B</sub> receptor (GABA<sub>B</sub>) activation shifts from opening inwardly rectifying potassium channels (Kir3.2) to increasing resting dendritic calcium signal and mammalian Target of Rapamycin (mTOR) activity. However, little is known about the molecular and biochemical mechanisms that initiate this shift. Herein, we describe a mechanism by which NMDAR antagonists promote the rapid antidepressant efficacy of NMDAR antagonists (herein referred to as the GABA<sub>B</sub> shift in function).<sup>20</sup> The present study examines the molecular events upstream of the shift in function: (1) are dynamic changes in dendritic protein expression required for the shift in GABA<sub>B</sub> function, and (2) how is the GABA<sub>B</sub> shift in function? The present study examines the molecular events upstream of the shift in GABA<sub>B</sub> function by asking: (1) are dynamic changes in dendritic protein expression required for the shift in function, and (2) how is the GABA<sub>B</sub> shift in function? The present study examines the molecular events upstream of the shift in function: (1) are dynamic changes in dendritic protein expression required for the shift in GABA<sub>B</sub> function, and (2) how is the GABA<sub>B</sub> shift in function? Herein, we describe a mechanism by which NMDAR antagonists cause GABA<sub>B</sub> receptors to decouple from their primary inhibitory target, Kir3 channels. Intraperitoneal (i.p.) injection of an NMDAR antagonist, D(-)-2-amino-5-phosphonopentanoic acid (AP5) or Ro-25-6981, induces rapid changes in protein expression in vivo to favor the shift in GABA<sub>B</sub> signaling such that the protein levels of γ-aminobutyric acid (GABA) are the major inhibitory neurotransmitter in the brain. Metabotropic GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) are G-protein coupled receptors that mediate slow synaptic inhibition by increasing outward potassium current through inwardly rectifying potassium channels (Kir3.2).<sup>17</sup> The role of GABA<sub>A</sub>R signaling in MDD is beginning to emerge.<sup>18</sup> We recently demonstrated that GABA<sub>B</sub>Rs are necessary for the rapid antidepressant efficacy of NMDAR antagonists.<sup>19</sup> Contrary to GABA<sub>B</sub>Rs' established function, NMDAR blockade promotes a shift in GABA<sub>B</sub>R signaling such that activation of the receptor increases resting dendritic calcium in a manner that requires L-type Ca<sup>2+</sup> channels. The rise in dendritic calcium activates hotspots of mammalian Target of Rapamycin (mTOR) kinase activity in the dendrites. mTOR activity promotes the syntheses of plasticity-related proteins, a necessary step for the initiation and sustainment of rapid antidepressant efficacy of NMDAR antagonists (herein referred to as the GABA<sub>B</sub>R shift in function).<sup>20</sup> The present study examines the molecular events upstream of the shift in GABA<sub>B</sub>R function by asking: (1) are dynamic changes in dendritic protein expression required for the shift in GABA<sub>B</sub>R signaling, and (2) how is the GABA<sub>B</sub>R shift in function? Herein, we describe a mechanism by which NMDAR antagonists cause GABA<sub>A</sub>Rs to decouple from their primary inhibitory target, Kir3 channels. 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Kir3.2 (GIRK) decrease and GABA<sub>R</sub>2 increase in the hippocampus through mRNA translation. 14-3-3<sub>η</sub> is an adaptor protein that regulates GABA<sub>R</sub> coupling to Kir3.X channels<sup>21,22</sup> and is implicated in diseases in which NMDAR signaling is dysregulated.<sup>23</sup> We show that 14-3-3<sub>η</sub> expression increases with acute NMDAR antagonism and decreases in socially defeated rodents, a model of depression. NMDAR blockade unexpectedly slows both protein synthesis and degradation of 14-3-3<sub>η</sub>, elevation total levels of 14-3-3<sub>η</sub>. Blocking the functional interaction between 14-3-3<sub>η</sub> with a dominant-negative form of 14-3-3<sub>η</sub> (DN-14-3-3<sub>η</sub>) restores GABA<sub>R</sub>-mediated inhibition in the presence of NMDAR blockade. Moreover, stereotactic injection of a virus coding for DN-14-3-3<sub>η</sub> prevents the rapid antidepressant efficacy of AP5 and Ro-25-6981. Our findings altogether suggest that removing GABA<sub>R</sub>-Kir3 activity may be the initial step required for the shift in GABA<sub>R</sub> function that is necessary for the efficacy of rapid antidepressants.

**MATERIALS AND METHODS**

Isolation of synaptoneurosomes (SNs) and neuronal lysates

SNs were isolated from day in vitro (DIV) 14–18 cortical cultures or from rat or mouse prefrontal cortex and hippocampus by a modified method previously described.<sup>24</sup> Briefly, neurons were harvested in buffer B (20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.4; 5 mM EDTA, pH 8.0; protease inhibitor cocktail (Complete, Roche, Indianapolis, IN, USA); phosphatase inhibitor) and homogenized. Homogenate was filtered first through a sterile 100-μm nylon filter followed by a 20-μm filter. SNs were pelleted at 14 000 <i>g</i> for 20 min at 4 °C. SN pellet was solubilized with radio-immunoprecipitation assay buffer (150 mM NaCl; 10 mM Tris, pH 7.4; 0.1% SDS (sodium dodecyl sulfate); 1% Triton-X-100; 1% deoxycholate; 5 mM EDTA; protease inhibitor cocktail tablet and phosphatase inhibitor) for 20 min on ice. The insoluble fraction was then pelleted at 14 000 <i>g</i> for 20 min at 4 °C, and the supernatant was used for immunoblot analysis.

Western blottings

Equal amounts of SN (25 μg) sample in radio-immunoprecipitation assay (RIPA) buffer and SDS loading buffer were run on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane for 1 h in 20% transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol). Membranes were then blocked for 1 h at room temperature (RT) while shaking in TBST (TBS and 0.1% Tween-20) with 5% nonfat milk. Membranes were incubated overnight (O/N) at 4 °C in primary antibody and for 45 min at RT in secondary antibody. After each incubation step, membranes were washed for 6 × 10 min in TBST. Membranes were imaged with the Odyssey immunoblot software (Lincoln, NE, USA). For 14-3-3<sub>η</sub> blots, membranes were probed by enhanced chemiluminescence. ImageJ (Bethesda, MD, USA) was used for densitometry analysis. The antibodies used were as follows: GABA<sub>R1</sub> (AB55051, Abcam, Cambridge, UK), GABA<sub>R2</sub> (NeuroMab, Davis, CA, USA, N81/2), Kir3.2 (Alomone, Jerusalem, Isreal, APC-006), 14-3-3<sub>η</sub> (sc-17286, Santa Cruz, Dallas, TX, USA), and α-tubulin (ab15246, Abcam, Cambridge, UK).

Cloning of 14-3-3<sub>η</sub>

14-3-3<sub>η</sub> was cloned by RT-PCR from rat brain cDNA primers 5′-AT GGGGGACCGAGGAGCGCTG-3′ and 5′-GCGGACATCATATAATTAAA GAGGACAGGC-3′ amplifying nucleotides 176–1446 (Genbank accession no.: D17445). The remaining 3′ untranslated region (UTR) was cloned by sequential ligations of annealed oligonucleotides (1447–1689). The DN construct was made by site-directed mutagenesis replacing the CGA with GCA at amino acid 56 and the AGG with a GCG at amino acid 60 to replace the arginine to an alanine.<sup>25</sup> Primers used for recombinant adeno-associated virus (rAAV) insert are: Forward primer: 5′-GAGCTCGCCCACATGGGGGAGAGACGACGACG TGCTTACGCGCAGCG-3′ and Reverse primer: 5′-CTGCACTGATGCTCTCCCGGCTTCTCATC-3′.

Kaede was initially cloned into the EGFP-N1 vector (Clontech, Mountain View, CA, USA) by replacing the EGFP cDNA inserted at the BamHI and Not I (blunt) sites. Kaede-14-3-3<sub>η</sub> was generated by PCR using a primer specific for the EGFP-N1 vector in the forward direction (5′-AGGCGTGTTACGTTGGAGGCTCTTATA-3′) and a primer that contained the last 15 nucleotides of the cDNA before the stop codon, of Kaede, with the addition of a Nhe I site at the 3′ end (Kaede: 5′-CCCCCGCCTAGCTGACGTGTTCC-3′) in the reverse direction. The PCR product was digested with Nhe I and inserted into a Nhe I site of the 14-3-3<sub>η</sub> cDNA at the start codon that was previously engineered into the sequence by the Quick Change Site Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA). Kaede-14-3-3<sub>η</sub> 3′UTR cDNA was then subcloned into the Stu site of the SinRep5 virus vector (Invitrogen, Carslbad, CA, USA), and pseudovirions were produced according to the manufacturer's directions.

Adeno-associated viral vectors

The DN-14-3-3<sub>η</sub> and tdTomato proteins<sup>26</sup> were cloned into separate adeno-associated viral vectors containing a mouse synapsin promoter, a woodchuck posttranscriptional regulatory element and SV40 poly-adenylation sequence between flanking AAV2 inverted terminal repeats.<sup>27</sup> rAAVs were assembled using a modified helper-free system (Stratagene) as serotype 2/1 (rep/cap genes) viruses and harvested and purified over sequential cesium chloride gradients as previously described.<sup>28</sup> Viral titers were > 1×10<sup>10</sup> infectious particles per microliter.

Neuronal cultures and transgene expression

Primary neurons were prepared as previously described by Ma et al.<sup>29</sup> For DN-14-3-3<sub>η</sub> and tdTomato co-infections, rAAV(DN-14-3-3<sub>η</sub>)rAAV(tdTomato) ratio was 4:1. For tdTomato controls, rAAV (tdTomato) was diluted 1:5 with phosphate-buffered saline (PBS).14-3-3<sub>η</sub>-Kaede in Sindbis was injected 24–36 h prior to live imaging. For imaging experiments, hippocampal neurons were plated at 50–75 000 cells/12 mm coverslip and used at DIV 14–18. Transfections were performed on DIV 12–14 hippocampal neurons using 0.4 μg DNA according to the manufacturer’s instructions (Invitrogen), but in Neurobasal media (Invitrogen), and returned to conditioned media 4–6 h posttransfection. Kaede and Mermaid cDNA was a kind gift from Dr Atsushi Miyawaki at the Riken Brain Institute, Japan. rAAV infections were performed using 1 μl viral mix per coverslip.

Pharmacology

Drug treatment was done in media. All live-imaging experiments were done in HEPES-based artificial cerebral spinal fluid (aCSF (in mM): 100 NaCl, 10 HEPES (pH 7.4), 3 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 glucose) adjusted to match the osmolarity of cell culture media. For in vitro manipulations, neurons were pretreated with the NMDAR antagonist AP5 (50 μM, D-AP5; aCSF+AP5) for 90 min. For GABA<sub>R</sub> activation, neurons were treated with baclofen (50 mM, in vitro) for 5 min. To block Kir3 channels, tertiapin-Q (50 nM, 2 min) was applied. KCl (25 mN) was used to depolarize neurons. All drugs were purchased from Tocris (Bristol, UK).

Microscopy

Images were acquired with a Leica SP5 disk confocal microscope (Wetzlar, Germany) using an oil-immersion ×63 lens or a ×63 water immersion lens for live imaging. For co-localization assays, a 2048 × 2048 frame was sampled at the Nyquist rate (~50 nm per pixel), 400 Hz for 2-dimensional co-localization analysis. For
surface expression, bioorthogonal noncanonical amino acid tagging and proximity ligation assay (BONCAT–PLA) new translation imaging and immunostaining, a 6-µm Z-stack of 1024 × 1024 pixels was obtained using a scan rate of 400 Hz. Max projections of the stack were used for analysis.

Immunofluorescence
For cultured neurons, cells were fixed in 4% paraformaldehyde with 4% sucrose for 20 min at RT, permeabilized with 0.25% Triton X-100 in PBS (PBS) and then blocked in PBS with 8% goat serum for at least 30 min at RT. Primary and secondary antibody incubations were done in blocking buffer O/N at 4 °C and 2 h at RT, respectively. Between incubation steps, neurons were washed 3 × 10 min with PBS. For surface staining, neurons were fixed in 4% paraformaldehyde with 4% sucrose for 20 min on ice and blocked in PBS with 8% goat serum for at least 30 min at RT. Surface primary antibody incubation was completed in blocking buffer O/N at 4 °C. After washing 3 × 10 min with PBS, the cells were permeabilized, and total antibody was probed as above. The following antibodies were used: surface GABAR R1 (AB55051, Abcam), and total GABAR R1 (SC-14006, Santa Cruz), GABAR R2 (Neuromab, N81/2), and Kir3.2 (Alomone APC-006).

Live labeling of surface GABAR R s
Live labeling was performed using a modified method from Hayashi et al. 30 DIV 14–20 hippocampal neurons were incubated in 3% bovine serum albumin aCSF with surface GABAR R1 antibody for 1 h. Following two rinses with bovine serum albumin aCSF, neurons were returned to conditioned media and incubated with AP5 (50 µm) or vehicle (H2O) for 90 min. Neurons were then fixed in 4% paraformaldehyde and stained as above. The following antibodies were used: surface GABAR R1 (AB55051, Abcam), and Kir3.2 (Alomone APC-006).

BONCAT–PLA for protein-specific new translation
BONCAT was performed using a method similar to that described by Dieterich et al. 31–33 and using the Click-iT Metabolic Labeling AHA and Reaction Buffer Kit (Life Technologies, Carlsbad, CA, USA). Coverslips were moved to a methionine free media, aCSF, for 30 min at 37 °C. Drug (AP5, 50 µm or vehicle, H2O) and azide-linked non-canonical amino acid, azidohomoalanine (AHA) were then spiked into the media, and cells were incubated for 1 h at 37 °C. Following this, Click-iT Metabolic Labeling was performed according to the manufacturer’s instructions using a biotin-alkyne to tag the newly synthesized proteins. Coverslips were then blocked and incubated in primary antibody O/N at 4 °C. The following day, the PLA secondary staining was performed using the Duolink Kit according to the manufacturer’s instructions (Duolink, Sigma). Antibodies used include: GABA R1 (Santa Cruz, sc-14006), GABA R2 (Neuromab, N81/2), 14-3-3 (sc-17286, Santa Cruz), biotin/o-rabbit (Sigma, St. Louis, MO, USA, SAB3700857) and biotin/o-mouse (Abcam, ab791111), and MAP2 (Aves, Tigard, OR, USA, MAP).

Live imaging
Live imaging experiments were done in aCSF in a 35-mm culture dish. Single plane images of 512 × 512 pixels were collected at a rate of 400 Hz for 1 min during each acquisition period for mermaid imaging. For imaging of protein stability using Kaede, a Z-stack of 1024 × 1024 pixels was obtained using a scan rate of 400 Hz stacks for the full extent of each neuron used (12–15 µm). Laser intensity, exposure time and image size were held constant within each experiment to allow for comparison.

Live mermaid (voltage) imaging
All live-imaging experiments were done as previously described. 19 Mermaid-transfected neurons were incubated with vehicle (H2O) or AP5 for 90 min and washed three times in aCSF or aCSF+AP5 before image acquisition. Measurements are reported as a ratio (R) of the acceptor (red) to donor (green) fluorescence, where an increase in the ratio indicates depolarization and a decrease indicates hyperpolarization. 34 The baseline ratio (R0) was established by taking one image every 5 s for 1 min. All drugs were allowed to equilibrate for 2–5 minutes prior to measuring the fluorescence during the next 1-min imaging period (RDrug).
Cumulative data are expressed as ΔR/Δt. Kir3 channels were blocked with tertiopin-Q (50 nm, 2 min). All drugs were purchased from Tocris.

Live imaging of protein stability
Live imaging of 14-3-3-µKaede was performed similar to Raab-Graham et al. 36 and Kosanya et al. 35 Coverslips were infected with a Sindbis virus coding for 14-3-3-µKaede. 24 to 36 h later, coverslips were washed three times with aCSF and moved to the imaging chamber. An initial Z-stack of the full volume of the neuron (12–18 µm) was acquired (pre-ultraviolet (pre-UV)). Kaede protein was then converted from green to red using UV light for 1 min. Another Z-stack was acquired for the t = 0 time point (F0), after which AP5 (50 µm) or Vehicle (H2O) was applied to the bath. A Z-stack was acquired every 15 min for 1 h (F15, F30, F45, F60) after the addition of AP5 or H2O.

Image analysis
For co-localization analysis, the full visible extent of each region of interest (ROI) within a primary dendrite was boxed in a 4 × 4 µm2 area. These ROIs were then analyzed using the JaCOP plugin to determine the Pearson coefficient. 37 Thresholds were set according to the computer-generated value for each ROI.

For intensity and punctal analyses, ROIs were demarcated manually by tracing the full visible extent of dendrites that extend out at least 60 µm from the cell body. Background was subtracted by determining the signal in a region close to the dendrite but void of all processes. For dendritic intensity, the average intensity value for the full extent of each dendrite is reported. For surface staining, the average ratio of surface GABAR R1 intensity to total GABAR R1 intensity is reported. For BONCAT–PLA analysis, the PLA intensity values for each protein are reported as a ratio of the average PLA intensity to map2 intensity. For punctal analysis, puncta were defined as pixel values within each dendritic ROI where the signal intensity for Kir3.2 exceeded the mean signal intensity plus two standard deviations for untreated, control neurons. Non-zero values were then totaled to determine the total number of hotspots and plotted as a function of distance from the soma to visualize areas of increased Kir3.2 signal.

Live-imaging analysis
For live imaging of protein stability using 14-3-3-µKaede, the average intensity for each fluorophore was calculated for 25-µm ROIs that began 10 µm from the cell body and continued consecutively for the full extent of the dendrite for each time point. The change in intensity was then calculated as a percentage of change from t = 0 time point (ΔF = F0 – F0/F0) for both old/red protein and new/green protein.

For Mermaid voltage imaging, the fluorescence resonance energy transfer (FRET) signal was analyzed by defining an ROI that started ~5 µm from the cell body and extended to the first visible branch point. The ROI included the entire visible portion of the dendrite. Neurons were background subtracted, and intensity (red/acceptor and green/donor) values for each fluorophore at each time point were determined using ImageJ. The acceptor:
donor ratio (red/acceptor:green/donor intensity) for each time point in the first imaging period were averaged as the baseline \((R_0)\). The ROI ratio values obtained for each time point after the bacofoen incubation were averaged \((R \text{ or } R_{BAC})\) in Figure 3). The ROI ratio values obtained for each time point after the Tertiapin-Q/ GIRQ blocker incubation were averaged \((R \text{ or } R_{BAC(T)})\). To calculate the change in signal, the following equations were used: (1) for application of one drug \(\Delta R/R = (R - R_0)/R_0\) was used and (2) to calculate the change in fluorescence following Tertiapin-Q/Kir 3 blocker after application of bacofoen, \(\Delta R/R = (R_{BAC(T)} - R_{BAC})/R_{BAC}\) was used. The acceptor:donor ratio values at each time point was also averaged and plotted as a percentage of the baseline.

**Stereotaxic virus injections**

All procedures were performed in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and approved by The University of Texas at Austin Institutional Animal Care and Use Committee using sterile technique. The animals were anesthetized using inhaled isoflurane in \(O_2\) and fixed in a stereotaxic frame using non-perforating ear bars. The surgical site was prepared by clipping the hair on the scalp and sterilizing the scalp with 70% ethanol. A 5–10-mm incision was made to expose the skull. A 1-mm hole was then drilled at predetermined coordinates using an F/G dental drill bit. A Nanoject II microinjector (Drummond Scientific, Broomall, PA, USA) fitted with a pulled-glass injection pipette (5–10-μm tip diameter) containing the viral suspension was inserted into the brain using a stereotoxic manipulator (Sutter Instruments, Novato, CA, USA). Injection coordinates for the dorsal hippocampal CA1 (from bregma) were −2.2 mm A/P, +/− 1.5 mm M/L; −2.5 mm A/P, and +/− 1.6 mm M/L. Thirty nanoliters of virus was injected at depths of −1.2, −1.1 and −1.0 mm from pia at each site.

**In vivo injections**

I.p. injections of APS (2.5 mg kg\(^{−1}\)) or Ro-25-6981 (10 mg kg\(^{−1}\)) were administered to 20–30-day-old Sprague-Dawley male rats and 6–10-week-old mice for behavioral analyses (forced swim test (FST), tail suspension test (TST) and splash test) and biochemistry. Hippocampi were harvested 45 min postinjection for immunoblot analysis. Behavioral testing was completed in uninjected naive and rAAV (tdTomato+vector or tdTomato+DN-14-3-3-Flag) injected 8–10-week-old mice.

**Behavioral testing**

FST was performed using a method similar to Porsolt et al.\(^{38}\) Mice were injected with drug or vehicle and 45 min later subjected to the FST. Immobility was scored blindly the last 4 min of 6 min past the movement necessary to keep its head above water. A training session was conducted for mice injected with Ro-25-6891 2 h prior to injections. TST was completed 24 h postinjection as described in Can et al.\(^{39}\) Mice were scored for the struggling the entire 6 min of the test. Mice were filmed for the entire session and scored blindly for mobility. Mobility was defined as any movement of the mouse but not movement due to oscillations or swing resulting from earlier movement.

Splash test was assessed approximately 30 min after the TST. Grooming frequency was assessed for 5 min after 200 μl of 10% sucrose was squirted on the dorsal coat of the mouse.\(^{40–42}\) Grooming was defined as any behavior in which a mouse licked its fur, groomed with its forepaws and scratched with any limb.

**Social defeat**

Vasectomized 12-week-old male Sprague-Dawley rats (Harlan, Houston, TX, USA) were selected for aggressive behavior by assessing those males that displayed aggressive grooming, pinning or biting towards the probe rat within 1 min of placement into the resident cage. These animals were used for multiple rounds of social defeat. Female cage mates were rotated among residents. Five-week-old male Sprague-Dawley (Harlan) were housed in groups of 2–3 and were utilized as the intruders for the stress paradigm.

Residents and intruders were taken to a behavioral procedure room in their home cages at the end of the dark cycle (0700 hours). The room was lit with red light to encourage activity. Females were removed from residents’ cages to a holding cage. Intruders were introduced to residents’ home cages, and the latency to display of aggressive behavior by the resident (aggressive grooming, pinning, or biting) was recorded. After 5 min of direct contact, a perforated Plexiglass barrier was inserted to physically separate animals while maintaining sensory contact for an additional 25 min. After the defeat session, all animals were returned to their home cages and taken back to the colony, where residents and intruders were examined for injuries. For repeated social defeat, one defeat session occurred daily for 5 days, and intruders were exposed to a different resident each session.

**Statistical analysis**

All statistical analyses were performed using the Graphpad Prism software (La Jolla, CA, USA). For all experiments: significance was calculated at \(a = 0.05\) level. For both one-way and two-way analysis of variance, significance is represented by: \(*P < 0.05, **P < 0.01, ***P < 0.001; and NS indicates no significant difference. For Figure 1c, significance (\#) was calculated with a one-sample T-test where \(\mu = 0\). Error bars represent s.e.m.

**RESULTS**

Rapid antidepressants are NMDAR antagonists that increase synaptic efficacy by affecting key regulators of protein synthesis.\(^{19,43,44}\) Rapid antidepressant efficacy requires GABA\(_B\) activation of mTOR-dependent protein synthesis in dendrites.\(^{19}\) It is unclear if the events prior to the shift in GABA\(_B\)R require new protein synthesis. Therefore we independently tested two NMDAR antagonists, APS and Ro-25-6981. First, we determined whether these NMDAR antagonists produced a behavioral phenotype consistent with antidepressant efficacy. AP-7, an NMDAR antagonist similar in structure, selectivity and potency to APS, has demonstrated rapid antidepressant properties.\(^{35}\) Ro-25-6981, which blocks GluN2B-containing NMDARs, produces rapid antidepressant effects in animal models and is being tested clinically for MDD.\(^{15}\) Few studies suggest that APS may not cross the blood–brain barrier;\(^{46}\) however, many report its effectiveness in the central nervous system following peripheral administration.\(^{47–54}\) To compare the two NMDAR antagonists, we i.p. injected mice with either APS or Ro-25-6981 and subsequently measured their behavior during the FST. FST is a well-established measure of antidepressant efficacy, requiring rodents to swim without escape and eliciting an immobile response.\(^{55,56}\) Treatment with antidepressants reduces the length of immobility induced by FST (that is, the animals swim longer).\(^{55}\) Similar to Ro-25-6981, AP5 reduced immobility time by ~ 40 s at 45 min and ~ 50 s at 24 h post-i.p. injection (Figures 1a and b, Supplementary Figure S1A; 45 min: Control, 140 ± 6 s; APS, 97 ± 4 s, \(P < 0.005\); Figure 1b; Control, 146 ± 13 s; Ro-25-6981, 76 ± 18 s, \(P < 0.02\)). These results demonstrate that a single i.p. injection of APS produces antidepressant behavioral effects, similar to other NMDAR antagonists.\(^{19,20,45}\) NMDAR antagonists reduce Kir3.2 and correspondingly increase GABA\(_A\)R2 levels

We next asked whether GABA\(_A\)R coupling to Kir3 channels changes with NMDAR antagonism. We have previously determined that
NMDAR antagonists increase the surface expression of GABA$_B$R1; however, it is unclear whether this elevated surface expression requires an increase in total protein. GABA$_B$R is an obligate heteromultimer, requiring the assembly of GABA$_B$R1 and R2 to make a functional receptor. Activation of the GABA$_B$R releases the βγ subunits of its associated trimeric G protein, consequently opening Kir3 channels. The extent of GABA$_B$R-Kir3 coupling could vary with the expression levels of Kir3.2, GABA$_B$R1 and R2.

Figure 1. Bidirectional regulation of the γ-aminobutyric acid receptor (GABA$_B$R) pathway by N-methyl-D-aspartate receptor (NMDAR) antagonists, D-(-)-2-amino-5-phosphonopentanoic acid (AP5) and Ro-25-6981. Compared with control, (a) AP5 (2.5 mg kg$^{-1}$, intraperitoneal (i.p.)) or (b) Ro-25-6981 (10 mg kg$^{-1}$, i.p.) reduced immobility time by ~40 s at 45 min postinjection. N = 3–4 animals. Representative western blottings and quantification of (c–e) Kir3.2, (d and g) GABA$_B$R1 and (e and h) GABA$_B$R2 show that NMDAR antagonists decrease Kir3 expression, whereas increasing GABA$_B$R2 expression in hippocampal synaptoneurosomes (SN) of rats i.p. injected with (c–e) AP5 or (f–h) Ro-25-6981 compared with vehicle. Tissue harvested 45 min postinjection. N = 4 animals. (i–l) AP5 does not change (i and j) GABA$_B$R1 levels, but increases translation of (k and l) R2 using bioorthogonal noncanonical amino acid tagging and proximity ligation assay. N = 10 neurons. Scale bar = 25 μm. (m–o) Social defeat does not change (m) Kir3.2 levels but significantly decreases expression of (n) GABA$_B$R1 and (o) GABA$_B$R2 in hippocampal SN harvested at 36 h after last defeat session. N = 8 animals. Significance assessed by Student’s t-test.
Thus we examined the protein levels of Kir3.2, GABA\(_{B1}\) and R2 at 45 min postinjection in the hippocampal synaptoneurosomes (SN, presynaptic and postsynaptic nerve endings) of Control (Vehicle, saline) and AP5 (2.5 mg kg\(^{-1}\)) or Ro-25-6981 (10 mg kg\(^{-1}\)) mice. Western blotting analysis revealed that AP5 reduced total Kir3.2 expression by \(\sim 65\%\) compared with vehicle (Figure 1c; Kir3.2: 0.35 ± 0.04, \(P < 0.02\), relative to Control), complementing the report that NMDAR activity stabilizes Kir3.2 surface expression.\(^{59}\)

Though GABA\(_{B1}\)R1 expression did not increase at 45 min (Figure 1d), AP5 increased GABA\(_{B2}\)R levels by 20% (Figure 1e; R1: 1.25 ± 0.04, \(P < 0.01\), relative to Control). These findings were consistent with Ro-25-6981 injection (Figures 1f–h; Kir3.2: 0.69 ± 0.05, \(P < 0.01\); R1: 1.13 ± 0.19; R2: 1.47 ± 0.1, \(P < 0.01\); relative to Control). GABA\(_{B1}\)R1 and GABA\(_{B2}\)R levels were elevated at 2 h postinjection of Ro-25-6981 (Supplementary Figures S1B and C; R1: 1.6 ± 0.20, \(P < 0.03\); R2: 1.43 ± 0.05, \(P < 0.04\) relative to Control). Taken together, NMDAR antagonists alter protein expression that may favor the shift in GABA\(_{B}\)R function.

NMDAR antagonism increases GABA\(_{B}\)R2 expression through new protein synthesis

An increase in GABA\(_{B}\)R1 and R2 expression with NMDAR blockade may occur through new protein synthesis. We measured new translation of GABA\(_{B}\)R1 or GABA\(_{B}\)R2 during NMDAR blockade using BONCAT in cultured hippocampal neurons.\(^{31–33}\) BONCAT incorporates an AHA-tagged non-canonical amino acid by utilizing an alkyl-azide reaction to label newly synthesized proteins with biotin. To detect new GABA\(_{B}\)R1 or GABA\(_{B}\)R2 protein specifically, we subsequently performed a proximity ligation assay (PLA) on BONCAT-treated neurons. PLA emits a fluorescent signal only when two antibodies (anti-biotin and anti-GABA\(_{B}\)R1 or -GABA\(_{B}\)R2) are within 30–40 nm of each other. The combination of these methods stringently identifies newly translated GABA\(_{B}\)R1 or GABA\(_{B}\)R2. NMDAR blockade by AP5 significantly increased newly synthesized dendritic GABA\(_{B}\)R2 protein by \(\sim 60–70\%\) but not GABA\(_{B}\)R1 (Figures 1i–l; 0–100 μm: R1: 0.97 ± 0.27; R2: 1.70 ± 0.28, \(P < 0.01\); 100–150 μm: R1: 1.04 ± 0.34; R2: 1.63 ± 0.32, \(P < 0.01\); relative to Control). These results demonstrate that NMDAR inhibition can induce rapid protein synthesis and that the elevated GABA\(_{B}\)R2 levels observed during NMDAR blockade may be through new mRNA translation.

GABA\(_{B}\)R levels are reduced in a rat model of depression

Depression models seek to mimic the physical or emotional stresses that may alter normal molecular signaling and lead to depression or other mood disorders.\(^{50,61}\) Therefore, we hypothesized that social defeat, a depression model, downregulates GABA\(_{B}\)R and upregulates Kir3 channel expression, consistent with a bidirectional regulation of the pathway.\(^{3,62}\) In the social defeat model of depression, experimental rats are placed in the home cage of a novel, aggressive rat for 30 min day\(^{-1}\), including 5 min of direct contact for 5 days.\(^{63–65}\) To assess the effect of social defeat on the GABA\(_{B}\)R pathway, we isolated hippocampi from socially defeated and control rats 36 h after their last encounter with the aggressor.\(^{64}\) Western blotting analysis showed that rats exposed to social defeat have significantly lower levels of GABA\(_{B}\)R1 and GABA\(_{B}\)R2 relative to controls, while Kir3.2 levels remained unchanged in hippocampal SNs (Figures 1m–o, Supplementary Figures S1D–F; Kir3.2: 0.99 ± 0.10; R1: 0.79 ± 0.04, \(P < 0.009\); R2: 0.75 ± 0.04, \(P < 0.004\); relative to Control). These data suggest that rapidly altering GABA\(_{B}\)R2 and Kir3.2 expression may favor the rapid shift in GABA\(_{B}\)R function, as these changes coincide with the earliest time point assayed for rapid antidepressant efficacy. In contrast, an increase in GABA\(_{B}\)R1 may be required for long-lasting effects with NMDAR antagonists.

Blocking NMDARs reduces GABA\(_{B}\)R–Kir3.2 co-localization

As NMDAR antagonism reduces total Kir3.2 expression but increases GABA\(_{B}\)R2 expression, we asked whether co-localization between GABA\(_{B}\)R and Kir3.2 decreases. In contrast to the increase in new GABA\(_{B}\)R2 protein and consistent with what we observe in vivo (Figure 1), Kir3.2 expression levels measured by puncta number and intensity decrease with acute AP5 treatment (Supplementary Figures S2A and B). We previously demonstrated that the surface expression of GABA\(_{B}\)R1 increases with NMDAR blockade.\(^{13}\) Thus a reduction in co-localization between GABA\(_{B}\)Rs and Kir3 channels may occur because of (1) an increase in the number of surface GABA\(_{B}\)Rs or (2) a decoupling of GABA\(_{B}\)R and Kir3 channels (Figure 2a). To examine whether NMDAR blockade affects GABA\(_{B}\)R coupling to Kir3 channels in dendrites, we used a live-labeling protocol that allows us to specifically mark the population of GABA\(_{B}\) surface receptors present on the membrane prior to APS exposure (Figure 2a, old receptors coupled to Kir3 channels are pink).\(^{30}\) After antibody feeding to live cells, we applied APS for 90 min and fixed the cells for immunocytochemistry to detect Kir3.2 co-localization with the labeled, surface GABA\(_{B}\)Rs (pink receptors, Figure 2a). In APS-treated neurons, GABA\(_{B}\)R co-localization with Kir3.2 decreased by \(\sim 34\%\) relative to vehicle-treated neurons (Figures 2b and c; 0.66 ± 0.05; relative to Control). These results demonstrate that NMDAR antagonism reduces GABA\(_{B}\)R coupling to Kir3 channels.

Blocking NMDARs decreases GABA\(_{B}\)R-induced hyperpolarization mediated by Kir3/GIRK in the dendrites

As APS reduced GABA\(_{B}\)R–Kir3.2 co-localization, we suspected that GABA\(_{B}\)R-mediated hyperpolarization decreases in the dendrites. Unlike in vivo, NMDAR antagonists in culture require exogenous GABA\(_{B}\)R activation to increase resting dendritic calcium and mTOR kinase activity.\(^{19,20}\) This difference allows us to separate activation from downstream signaling, an event that is indistinguishable in vivo due to endogenous GABA.\(^{19}\) Using Memraid, a voltage-sensitive genetic FRET sensor, we measured the relative changes in membrane potential within the primary dendrite, where the shift in GABA\(_{B}\)R function is localized.\(^{9,34}\) Although fluorescent sensors are slow,\(^{66}\) the shift in GABA\(_{B}\)R function with NMDAR blockade occurs 3–5 min after APS application, increases resting dendritic calcium signal that lasts at least 5 min and requires L-type calcium channels.\(^{19}\) Accordingly, Memraid is an appropriate tool to visualize site-specific changes in dendritic membrane potential.\(^{66}\) An increase in FRET signal indicates depolarization (Supplementary Figures S3C and D, Supplementary Methods), whereas a decrease indicates hyperpolarization relative to the starting membrane potential.\(^{34}\)

Using cultured hippocampal neurons expressing Memraid, we determined the effect of GABA\(_{B}\)R activation in the presence or absence of APS (Figures 2d–g). As expected, application of GABA\(_{B}\)R agonist, baclofen, significantly hyperpolarized the control dendrites as indicated by a decrease in FRET ratio (Figures 2d and e; \(ΔR/ R = 0.09 ± 0.03, P < 0.02\); one-way T-test). However, when NMDARs are blocked, baclofen negligibly changed the membrane potential of the primary dendrite (Figures 2d and e; \(ΔR/ R = 0.003 ± 0.01\)) without altering the somatic response (Supplementary Figures S2E and F). These results suggest that inhibiting NMDAR activity abrogates GABA\(_{B}\)R-induced hyperpolarization in the dendrites.

To assess the contribution of Kir3 activity to GABA\(_{B}\)R-induced hyperpolarization during NMDAR blockade, we treated Mermaid-transfected cells with baclofen in the presence or absence of APS. After baclofen treatment, we measured the FRET signal after blocking Kir3 channels with tetraptian Q (tet-Q, 50 mM; \(ΔR/ R = R_{\text{TACQ}} − R_{\text{BAC}}/R_{\text{BAC}}\)). As expected, baclofen hyperpolarized the dendritic membrane (Figure 2f, middle panel unfilled circles; Figure 2g, white bar). Addition of tet-Q to vehicle-treated neurons increased the FRET signal, indicating a significant depolarization.
Upon NMDAR antagonism, the stability of 14-3-3 η, an adaptor protein that decouples GABABR and Kir3,2 Several reports link 14-3-3 η to the regulation of GABABR signaling.21,22 Thus 14-3-3 η could potentially regulate GABABR response to NMDAR blockade. First, we determined whether NMDAR antagonism increases the stability of 14-3-3 η, an adaptor protein that decouples GABABR and Kir3.

(Figure 2f, right panel; Figure 2g, hatched bar ΔR/R = ±1%). In contrast, APS-treated neurons showed no observable change in FRET signal with the Kir3 blocker (Figure 2f, filled circles right panel; Figure 2g, hatched bar). These data suggest that blocking NMDARs reduces GABABR-coupled Kir3 activity.
NMDAR antagonism altered 14-3-3η expression. Western blotting analysis of hippocampal SNs indicated that NMDAR blockade (AP5 or Ro-25-6891) increased 14-3-3η expression relative to control (Figures 3a and b; AP5: 1.26 ± 0.09, P < 0.03; Ro-25-6891: 1.46 ± 0.13, P < 0.02). As seen with GABABR, social defeat significantly decreased 14-3-3η (Figure 3c; 0.68 ± 0.04, P < 0.001; relative to Control).

To determine whether the increase in 14-3-3η expression was due to new protein synthesis, as observed with GABABR2 (Figure 1), we used BONCAT–PLA to detect new 14-3-3η protein.

Figure 3. N-methyl-D-aspartate receptor antagonism slows both degradation and new protein synthesis of 14-3-3η. (a–c) Representative western blotting of hippocampal synaptoneurosomes show 14-3-3η protein significantly increases 45 min postinjection of (a) D-(-)-2-amino-5-phosphonopentanoic acid (AP5) or (b) Ro-25-6891 and (c) significantly decreases with social defeat. N = 4 animals in panels (a and b). N = 8 animals in panel (c). (d and e) APS decreases new translation of 14-3-3η in dendrites. N = 10 neurons. Scale bar = 25 μm. (f–h) APS slows the rate of degradation of 14-3-3η. (f) Representative images of 14-3-3η-Kaede expressing neurons before and after ultraviolet conversion (from green to red) following 60-min incubation with APS or vehicle (H2O, control). (g and h) Summary graphs show that APS significantly increases old (red) protein present in the dendrite and significantly decreases new (green) protein in distal dendrites after 1 h (see also Supplementary Figure S3). N = 10 neurons. Significance assessed by Student’s T-test.
Unexpectedly, NMDAR antagonism reduced the detectable levels of new, biotinylated 14-3-3η protein by ~56% (Figures 3d and e; 0–100 μm: 0.44 ± 0.04; 100–150 μm: 0.38 ± 0.04, P < 0.005; relative to Control). These results were puzzling as the overall 14-3-3η expression levels increased with NMDAR antagonists (Figures 3a and b).

We hypothesized that NMDAR blockade increases the stability of the 14-3-3η mRNA to test this prediction, we expressed the photobleachable fluorescent protein Kaede fused to 14-3-3η in cultured hippocampal neurons. UV light converts the fluorescence Kaede from green to red irreversibly. After a brief UV exposure, detection of the green signal indicates a ‘new’ protein synthesis, which can be differentiated from the ‘old’ red protein. Thus Kaede can distinguish changes in protein synthesis from changes in protein stability (that is, increase in detectable green protein indicates new translation versus no change in red protein indicates protein stability). Changes in ‘new’ green and ‘old’ red protein are measured as ΔF/F0 (ΔF/F = F0 – F/F0, where F0 is the signal intensity at time point 0 after the initial photoconversion, and F is the signal intensity 60 min later). Similar to the BONCAT–PLA assay (Figures 3d and e), the new increase in 14-3-3η was smaller in neurons where NMDARs were blocked (Figures 3f–h; green bars; 0–100 μm: Control = 16 ± 3%; AP5 = 10 ± 3%; Supplementary Figure S3B). Albeit, the decrease in ‘new’ green protein was not as dramatic, as detected by BONCAT–PLA, in dendritic regions close to the cell body. However, at distances > 100 μm from the soma, there was significantly less ‘new’ green protein, indicating that AP5 may significantly slow new translation of 14-3-3η in more distant dendrites (Figures 3f–h; green bars; 100–150 μm: Control = 21 ± 4%; AP5 = 6 ± 2%; P < 0.003). In contrast, NMDAR blockade completely prevented the decrease in ‘old’ red protein both proximally and at distances > 100 μm from the cell body, 60 min postconversion (Figures 3f–h; red bars; 0–100 μm: Control = −17 ± 4%; AP5 = 4 ± 7%; P = 0.009; 100–150 μm: Control = −18 ± 2%; AP5 = 3 ± 8%; P < 0.03). These results suggest that during basal NMDAR activity, 14-3-3η is rapidly synthesized and degraded. NMDAR blockade slows down both 14-3-3η synthesis and protein degradation. Thus the enhanced protein stability outweighs the reduced protein synthesis, resulting in a net increase in 14-3-3η expression levels.

14-3-3η reduces the dendritic GABA<sub>A</sub>R and Kir3.2 co-localization and the surface GABA<sub>A</sub>R expression in NMDAR-blocked neurons To determine whether the increase in 14-3-3η protein with NMDAR blockade is critical to the decoupling of GABA<sub>A</sub>R and Kir3 channels, we constructed a DN form of 14-3-3η (DN-14-3-3η) in which two arginines (R56 and R60) are mutated to alanines at the interface between the two subunits. These mutations significantly reduce dimerization of 14-3-3 proteins and hence interaction with its substrates. We expressed DN-14-3-3η in cultured hippocampal neurons using a rAAV. A second rAAV was used to mark the infected neurons with tdTomato red fluorescent protein. To ensure that all red neurons also expressed DN-14-3-3η, the DN-14-3-3η:tdTomato ratio was set at 4:1. We then performed the live antibody-feeding assay described above to assess the co-localization of GABA<sub>A</sub>R and Kir3.2 after the addition of AP5. As previously observed (Figures 2b and c), NMDAR blockade reduced the co-localization of GABA<sub>A</sub>R with Kir3.2 in neurons expressing tdTomato alone (Figures 4a and b). In DN-14-3-3η-expressing cells, AP5 did not reduce GABA<sub>A</sub>R–Kir3.2 co-localization (Figures 4a and b; DN + AP5, 0.74 ± 0.06; AP5: 1.08 ± 0.08; both relative to Control). These data suggest that 14-3-3η may decouple GABA<sub>A</sub>Rs from Kir3 channels upon NMDAR antagonism.

How does 14-3-3η promote the reduced GABA<sub>A</sub>R–Kir3 co-localization with NMDAR blockade? 14-3-3η prevents the
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interaction between the C-terminal domains of GABA_B1R and GABA_B2R, thereby disrupting the heterodimer. Thus a reduction in GABA_B-R-Kir3 co-localization in AP5 might occur due to reduced stability of surface GABA_BRs present on the membrane prior to NMDAR inhibition. Because it is unknown whether heterodimerization is required for GABA_BR stabilization on the membrane, we next asked whether preventing 14-3-3 co-localization with GABA_BRs affects dendritic surface expression. After co-expressing DN-14-3-3η and dsRed in hippocampal neurons, we measured surface GABA_B1R in non-permeabilized neurons. As previously observed, AP5 increased surface GABA_BRs. Expression of the DN-14-3-3η enhanced the AP5-induced increase on surface GABA_B1R by ~55% above AP5-treated, control neurons (Figures 4c and d; DN-AP5: 1.96 ± 0.26; AP5: 1.40 ± 0.16; both relative to Control; reported as the percentage of Surface/Total). These results argue that 14-3-3η reduces the membrane stability of GABA_BRs, which may govern the reduction in GABA_B-R-Kir3 channel co-localization with NMDAR blockade.

Blocking the functional interaction of 14-3-3η with GABA_BRs restores GABA_BR-mediated inhibition in neurons with reduced NMDAR activity

GABA_B-R-Kir3 activity decreases in the presence of NMDAR antagonists (Figure 2). Moreover, 14-3-3η reduces surface GABA_BRs and GABA_B-R-Kir3 co-localization (Figures 4a–d). Thus we hypothesized that blocking 14-3-3η with its DN would restore the baclofen-induced hyperpolarization during NMDAR blockade. As previously observed using the voltage-sensor Mermaid (Figure 2), AP5 significantly reduced the hyperpolarization induced by baclofen compared with vehicle-treated cells. Expression of DN-14-3-3η completely restored GABA_BR-mediated inhibition in AP5-treated neurons (Figures 5a and b; control: 3 ± 0.5%; AP5: 0.3 ± 0.1%; DN+AP5: 3 ± 0.8%). These data indicate that, during NMDAR blockade, 14-3-3η is necessary to decouple NMDAR from Kir3.

In vivo expression of DN-14-3-3η in hippocampal CA1 pyramidal neurons prevents the rapid antidepressant efficacy of NMDAR antagonists

We have shown in neuronal cultures that during NMDAR inhibition GABA_BR function shifts from opening Kir3.2 channels to facilitating an increase in dendritic calcium. We have also demonstrated that the GABA_BR shift in function requires 14-3-3η. In vivo, we have determined that blocking GABA_BRs prevents the positive effects mediated by rapid antidepressants. Hence, we sought to examine the requirement of 14-3-3η in mediating the antidepressant effects of NMDAR antagonists. We assessed the rapid antidepressant efficacy of AP5 and Ro-25-6981 in animals expressing DN-14-3-3η-Flag+tdTomato or empty vector+GFP. The expression of construct was confirmed by western blotting analysis of isolated hippocampi (Supplementary Figure SSA). Stereotoxically injected controls performed similarly to non-injected animals on the FST for saline (black), AP5 (2.5 mg kg⁻¹; red); Ro-25-6981 (10 mg kg⁻¹; pink) treated mice (Figures 1a and b, Figure 5c). AP5 reduced immobility time by ~40 s at 45 min post-injection and ~45 s at 24 h postinjection. Similar to AP5, Ro-25-6981-infected mice reduced immobility by ~60 s at 45 min postinjection. However, mice expressing DN-14-3-3η (blue) were unresponsive to AP5 or Ro-25-6981 injection and had similar immobility times to saline-injected mice (Figure 5c; Control = 137 ± 4 s, DN+AP5 = 134 ± 11 s; Control = 136 ± 15 s, DN+Ro-25-6981, 134 ± 11 s; Supplementary Figure S4A).

To further validate the role of 14-3-3η in rapid antidepressant efficacy, we subjected stereotaxically injected mice to two additional behavioral readouts, the TST and the splash test, for grooming behavior. TST is commonly used to screen antidepressants, and grooming frequency in the splash test assesses self-care and motivational behavior. Consistent with the FST, Ro-25-6981 significantly increased the time spent struggling of vector-injected, control mice but not of DN-14-3-3η-Flag-injected animals (Figure 5d; Control = 125 ± 7 s, Ro-25-6981 = 176 ± 10 s, DN+Ro-25-6981 = 118 ± 11 s). Following the TST, animals were assessed for grooming behavior using the splash test (30–40 min after TST). We predicted that Ro-25-6981-treated, vector-injected mice will display increased rate of grooming over vehicle-treated (10% dimethyl sulfoxide), vector-injected mice. Furthermore, expression of DN-14-3-3η will prevent Ro-25-6981-induced increase in grooming. A 10% sucrose solution was squirted on the dorsal coat of the mouse, and grooming frequency was measured. Indeed, Ro-25-6981-treated, control mice displayed augmented grooming behavior relative to vehicle-treated, control mice (Figure 5e; Control = 4 ± 0.4, Ro-25-6981 = 9 ± 1). Importantly, Ro-25-6981 in mice expressing the DN-14-3-3η did not increase the grooming frequency compared with control (Figure 5e; DN +Ro-25-6981 = 5 ± 1). These data altogether suggest that 14-3-3η is necessary for the rapid antidepressant effect of NMDAR antagonists in vivo.

**DISCUSSION**

Model for molecular changes mediating rapid antidepressant efficacy

We describe a mechanism that addresses how rapid antidepressants affect GABA_BR-mediated inhibition. We provide evidence that 14-3-3η facilitates the decoupling of GABA_BR from Kir3 channels. By decoupling GABA_BR and Kir3, GABA_BR function shifts from opening potassium channels to increasing resting dendritic calcium, a mechanism required to activate mTOR-dependent protein synthesis and mediate the rapid antidepressant effects of NMDAR antagonists (Supplementary Figure SS). Intriguingly, our results reveal an overall remodeling of dendritic proteins with rapid antidepressants. In a rat model of depression, GABA_B1R, GABA_B2R and 14-3-3η levels are decreased. In contrast, treatments with rapid antidepressants reduce Kir3.2 levels, whereas 14-3-3η and GABA_B2R concurrently increase. As R1 total protein does not initially change (Figure 1) and requires R2 to traffic to the membrane, the increase in R2 may be necessary for new receptors to assemble and target the membrane. An increase in new surface GABA_BRs that facilitate L-type Ca²⁺ channel activity, in combination with removal of GABA_B-R-Kir3 channels via 14-3-3η, favors the rapid shift in GABA_BR function to mediate calcium entry (Supplementary Figure SS). Furthermore, the drop in Kir3.2 expression in the presence of NMDAR antagonists likely ensures that GABA_BR activation supports an immediate increase in resting dendritic calcium, as hippocampal Kir3.2 levels do not change between socially defeated and control rats.

14-3-3η is linked to diseases whereby NMDA-dependent changes in protein expression are dysregulated, such as schizophrenia, bipolar disorder and now rodent models of depression. Our results indicate that NMDAR signaling increases the rate of 14-3-3η mRNA translation and protein degradation, arguing that precise synthesis and degradation of 14-3-3η are imperative for normal neuronal function. Consistent with this idea, a seven-base pair repeat and a single-nucleotide polymorphism within the 5’ and 3’ UTRs of 14-3-3η are associated with schizophrenia and bipolar disorder, respectively. As most translational regulation through RNA-binding proteins and microRNAs occur at sites within the UTRs, it will be medically informative to determine whether these polymorphisms result in aberrant 14-3-3η protein levels and GABA_BR signaling. Interestingly, 14-3-3η is detectable in cerebrospinal fluid of patients with Alzheimer’s disease and is being considered as an early biomarker. Our results provide a mechanistic insight into how 14-3-3η reduces inhibition that is beneficial for the treatment of depression, but if left unchecked...
may lead to excitotoxicity and neurodegeneration. Future work on how NMDAR signaling balances the mRNA translation and protein degradation of 14-3-3η will expand our understanding on the nature of mental health disorders. In summary, we have identified 14-3-3η as a critical player in the removal of GABABR-Kir3 signaling that is required for the efficacy of rapid antidepressants. Notably, molecular changes induced by NMDAR antagonists in animals subjected to depression paradigms parallel those observed in naive animals.20,77–80 Consistent with these reports, we have shown that the protein levels of GABABR1, GABABR2 and 14-3-3η drop in a rat model of depression. However, a single i.p. injection of a NMDAR antagonist results in elevated expression of these proteins. NMDAR antagonists exert their effects by altering the rate of protein synthesis and degradation of GABABR2 and 14-3-3η, respectively. The exact signaling pathways that facilitate protein synthesis and degradation of these proteins are yet to be defined. Be that as it may, through careful dissection of the molecular changes that mediate the shift in GABABR signaling with rapid antidepressants, we have identified 14-3-3η as a promising target for the treatment of MDD.

CONFLICT OF INTEREST

PCGH is employed by Genentech/Roche and may own shares in the company. This does not alter the authors’ adherence to all the journal’s policies on sharing data and materials. The remaining authors declare no conflict of interest.
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