GLYX-13, a NMDA Receptor Glycine-Site Functional Partial Agonist, Induces Antidepressant-Like Effects Without Ketamine-Like Side Effects

Jeffrey Burgdorf¹, Xiao-lei Zhang², Katherine L Nicholson³, Robert L Balster³, J David Leander¹, Patric K Stanton², Amanda L Gross¹, Roger A Kroes¹ and Joseph R Moskal^{*,1}

¹Department of Biomedical Engineering, Falk Center for Molecular Therapeutics, McCormick School of Engineering and Applied Sciences, Northwestern University, Evanston, IL, USA; ²Department of Cell Biology & Anatomy, New York Medical College, Valhalla, NY, USA; ³Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, USA

Recent human clinical studies with the NMDA receptor (NMDAR) antagonist ketamine have revealed profound and long-lasting antidepressant effects with rapid onset in several clinical trials, but antidepressant effects were preceded by dissociative side effects. Here we show that GLYX-13, a novel NMDAR glycine-site functional partial agonist, produces an antidepressant-like effect in the Porsolt, novelty induced hypophagia, and learned helplessness tests in rats without exhibiting substance abuse-related, gating, and sedative side effects of ketamine in the drug discrimination, conditioned place preference, pre-pulse inhibition and open-field tests. Like ketamine, the GLYX-13-induced antidepressant-like effects required AMPA/kainate receptor activation, as evidenced by the ability of NBQX to abolish the antidepressant-like effect. Both GLYX-13 and ketamine persistently (24 h) enhanced the induction of long-term potentiation of synaptic transmission and the magnitude of NMDAR-NR2B conductance at rat Schaffer collateral-CA1 synapses *in vitro*. Cell surface biotinylation studies showed that both GLYX-13 and ketamine led to increases in both NR2B and GluR1 protein levels, as measured by Western analysis, whereas no changes were seen in mRNA expression (microarray and qRT-PCR). GLYX-13, unlike ketamine, produced its antidepressant-like effect when injected directly into the medial prefrontal cortex (MPFC). These results suggest that GLYX-13 produces an antidepressant-like effect without the side effects seen with ketamine at least in part by directly modulating NR2B-containing NMDARs in the MPFC. Furthermore, the enhancement of 'metaplasticity' by both GLYX-13 and ketamine may help explain the long-lasting antidepressant effects of these NMDAR modulators. GLYX-13 is currently in a Phase II clinical development program for treatment-resistant depression.

Neuropsychopharmacology (2013) **38**, 729–742; doi:10.1038/npp.2012.246; published online 30 January 2013

Keywords: NMDA receptor; GLYX-13; ketamine; depression; medial prefrontal cortex

INTRODUCTION

NMDA receptor (NMDAR) modulation has therapeutic potential for the treatment of depression (Danysz and Parsons, 1998; Machado-Vieira *et al*, 2009; Skolnick *et al*, 2009). There are great unmet needs in the treatment of major depressive disorder: major depressive disorder affects ~10% of the adult population and is the second leading cause of global burden of disease (Kessler *et al*, 2005; Mathers and Loncar, 2006). Human clinical studies with the NMDAR antagonist ketamine have demonstrated antidepressant effects within 2 h and duration of effect lasting

several days following a single dose in patients with treatment-resistant depression and bipolar depression (aan het Rot *et al*, 2010; Berman *et al*, 2000; Diazgranados *et al*, 2010a, b; Price *et al*, 2009; Zarate *et al*, 2006a; Zarate *et al*, 2012). These results, along with postmortem data showing that NMDAR protein expression is altered in the prefrontal cortex of depressed patients, make the NMDAR a target of high interest in major depressive disorder (Feyissa *et al*, 2009; Skolnick *et al*, 1996).

GLYX-13 is a glycine-site modulator at the NMDAR (Burgdorf *et al*, 2011b; Haring *et al*, 1991; Moskal *et al*, 2005; Thompson *et al*, 1992; Zhang *et al*, 2008) and has been shown to preferentially modulate NR2B-containing NMDARs, given that the facilitation of NMDAR current by GLYX-13 at rat Schaffer collateral-CA1 synapses *in vitro* is completely blocked by the NR2B antagonist ifenprodil (Zhang *et al*, 2008). GLYX-13 is an amidated tetrapeptide (threonine-proline-proline-threonine) with an ~7-min half life in plasma, which was derived from a hypervariable

*Correspondence: Dr JR Moskal, Department of Biomedical Engineering, Director, The Falk Center for Molecular Therapeutics, Northwestern University, 1801 Maple Ave, Suite 4300, Evanston, IL 60201, USA, Tel: +1 847 491 4802, Fax: +1 847 491 4810, E-mail: j-moskal@northwestern.edu

Received 1 November 2012; accepted 8 November 2012; accepted article preview online 5 December 2012

region cloned and sequenced from the monoclonal antibody B6B21 (Moskal *et al*, 2005). GLYX-13 has been shown to readily cross the blood-brain barrier, showing a brain uptake index of 80% (Oldendorf, 1970; Moskal *et al*, 2005). GLYX-13 has been reported to: (1) enhance the magnitude of LTP of synaptic transmission while reducing long-term depression, which differentiates GLYX-13 from D-cycloserine (Zhang *et al*, 2008), (2) enhance learning in a variety of hippocampus-dependent learning tasks including trace eyeblink conditioning and the Morris water maze in both young adult and learning-impaired aging rats (Burgdorf *et al*, 2011b), (3) markedly reduce CA1 pyramidal neuronal cell death 24 h after bilateral carotid occlusion in Mongolian gerbils when administered up to 5 h after induction of occlusion ischemia (Stanton *et al*, 2009), and (4) produce analgesic effects in the rat formalin and Bennett models of sustained pain (Wood *et al*, 2008).

The experiments reported here were designed to determine if GLYX-13 exhibits antidepressant-like effects similar to ketamine without ketamine-like side effects. First, antidepressant-like effects of GLYX-13, ketamine, and fluoxetine were examined in the rat Porsolt, novelty-induced hypophagia (NIH), and learned helplessness (LH) tests, using doses and routes of administration of ketamine and fluoxetine as positive controls at time points previously shown to produce an antidepressant response in these tests (Autry *et al*, 2011; Burgdorf *et al*, 2009; Detke, Rickels and Lucki, 1995; Dulawa and Hen, 2005; Li *et al*, 2010; Page *et al*, 1999). Second, we also tested for ketamine-like side effects of GLYX-13 using the drug discrimination, conditioned place preference, open field, and prepulse-inhibition tests using doses and routes of administration of ketamine previously shown to produce behavioral effects in these tests (de Bruin *et al*, 1999; Burgdorf *et al*, 2009, 2010; Nicholson and Balster, 2009). Third, we determined if GLYX-13 and ketamine induced metaplasticity, defined as activity-dependent alterations in the cellular properties of synapses that alter their threshold for inducing long-term changes in synaptic plasticity (Abraham 2008; Huang *et al*, 1992), and measured by increases in the threshold for the induction of hippocampal LTP 24 h post-dosing. Finally, in order to determine if the antidepressant-like effects of GLYX-13 and ketamine were associated with a LTP metaplasticity-like mechanism, we also determined if these compounds increased cell surface expression of the NR2B and GluR1 subunits of the NMDA and AMPA receptor, and if the antidepressant-like effects of GLYX-13 were blocked by the AMPA/kainate receptor antagonist NBQX (Maeng *et al*, 2008).

MATERIALS AND METHODS

Animals

Adult male (2–3 month-old) Sprague-Dawley rats were used, except for the dose-finding study for medial prefrontal cortex (MPFC) injection of GLYX-13 in the Porsolt test (Figure 3a) in which adult male (2–3 month-old) Fisher Brown Norway (FBNF1) rats were used. All animals in this study were purchased from Harlan (USA), except for animals used in the drug-discrimination studies that were purchased from Charles River (USA). All rats were

housed in Lucite cages with corn cob or sawdust bedding, maintained on a 12:12 light:dark cycle (lights on at 0600 or 0800 hours), and given *ad libitum* access to Purina lab chow and tap water throughout the study. All experiments were approved by the Northwestern University, Virginia Commonwealth University, or New York Medical College Animal Care and Use Committees.

Drugs

GLYX-13 (molecular weight: 413.5) was purchased from Sai Advantium (India) and was administered in sterile saline vehicle at a volume of 1 ml/kg IV and SC, 5 µl/rat for intranasal, or 0.5 µl/rat for intracranial injection. Scrambled peptide (proline-threonine-threonine-proline-NH₂) was purchased from Bachem (USA). Ketamine HCl (molecular weight: 274.2) was purchased from Butler (USA) or JHP Pharmaceuticals (USA) as a 100 mg/ml stock solution and was administered in sterile saline vehicle (Baxter, USA) at a final volume of 1 ml/kg IV, SC and IP, or in 0.5 µl for intracranial injection. Fluoxetine HCl (molecular weight: 345.8) was purchased from Spectrum Chemical Manufacturing Corporation (USA) and was administered in Milli-Q water (Millipore, USA) at a volume of 4 ml/kg, as described in Detke, Rickels & Lucki (1995). Isoflurane used for intracranial surgeries (1–5%) was purchased from Butler. Ifenprodil and NBQX were purchased from Sigma (USA) and NVP-AAM077 was obtained from Novartis (USA).

Porsolt Test

The Porsolt forced-swim test, adapted for use in rats, was performed as previously described (Burgdorf *et al*, 2009; Page *et al*, 1999). Animals were placed in a 46 cm tall × 20 cm in diameter clear glass tube filled to 30 cm with tap water (23 ± 1 °C) for 15 min on the first day (habituation) and 5 min on the subsequent test day. Water was changed after every other animal. Animals were videotaped, with floating time defined as the minimal amount of effort required to keep the animal's head above water, and was scored offline by blind experimenters with high inter-rater reliability (Pearson's $r > 0.9$).

Animals received a single dose of GLYX-13 before a single test session, following IV dosing (1, 3, 10, 32, 56 mg/kg, 20 min or 24 h before testing), SC dosing (1, 3, 10, 32, 56, 100 mg/kg; 60 min before testing), intranasal (79, 141, 250, 2500 µg/rat; 0.32, 0.56, 1, 10 mg/kg; 1 h before testing). Animals were given intranasal injections with two calibrated 2-µl Pipetman (Gilson, USA), delivering half the total dose to each nostril, as described in Gozes *et al* (2000). Intra-MPFC injections of GLYX-13 and ketamine were also administered (0.1, 1, 10 µg/site; 1 h before testing), on the basis of a previous report that the antidepressant-like effects of ketamine (10 mg/kg, IP) were blocked by MPFC injections of an mTOR inhibitor (Li *et al*, 2010). Sterile saline was used as the vehicle for all routes of administration.

A single dose of a scrambled peptide containing the identical four amino acids as GLYX-13, but in a different sequence was administered IV (3 mg/kg) and compared with 3 mg/kg GLYX-13, IV 1 h before testing or by intra-MPFC

injection of 1 μ g of the scrambled peptide or 1 μ g GLYX-13, 20 min before testing. Sterile saline was used as the vehicle. A single dose of ketamine IP (10 mg/kg, 1 h before testing; Li *et al*, 2010) was also used. Fluoxetine SC (20 mg/kg, 23.5, 5, and 1 h before testing; Detke, Rickels & Lucki, 1995) was also used as a positive control.

For peripheral administration studies that were conducted 24 h post dosing, GLYX-13 (3 mg/kg, IV) and ketamine (10 mg/kg, IV) were used. We also examined the effect of fluoxetine (20 mg/kg, SC, as described above) 24 h after the last dose. The GLYX-13 dose was chosen, given that this dose was the lowest dose that produced a significant antidepressant-like effect in the Porsolt test 1 h post dosing, and this dose was used for all efficacy studies. The ketamine dose was chosen, given that it produced a robust increase in protein levels of pS845 form of the obligatory AMPA GluR1 subunit in the MPFC to a similar extent as GLYX-13 (see Results section), which is consistent with a previous report showing that the antidepressant-like effects of ketamine are blocked by the AMPA/kainate receptor antagonist NBQX (Maeng *et al*, 2008).

Open-Field Test

Open-field testing was performed as previously described (Burgdorf *et al*, 2009). Testing consisted of placing an animal in a 40 $\times$ 40 $\times$ 20 cm high, opaque Plexiglas open-field cage divided into nine equal-sized 13.3 $\times$ 13.3 cm sections under red lighting for 10 min. Between animals, feces and urine were removed from the apparatus. Animals were videotaped, and line crosses were scored offline by blind experimenters with high inter-rater reliability (Pearson's $r > 0.9$).

A single dose of GLYX-13 IV (10 mg/kg, 1 h before testing) or ketamine positive-control SC (20 mg/kg, 1 h before testing) was given. The dose of GLYX-13 (10 mg/kg, IV) was chosen, given that it was the highest maximally effective dose of GLYX-13 in the Porsolt test. The dose of ketamine (20 mg/kg, SC) was chosen given our unpublished observation that this dose produces sedation/ataxia in the rotarod test 1 h post-dosing.

Novelty-Induced Hypophagia (NIH) test

A version of the NIH test was used that has previously been shown to detect the acute antidepressant-like effect of ketamine (Li *et al*, 2010). Animals were food deprived overnight before testing, and lab chow was placed into the center chamber of the open field (40 $\times$ 40 $\times$ 20 cm) for 10 min under dim red lighting. Between animals, feces and urine were removed from the apparatus. After NIH testing, the latency to eat in the animal's home cage was determined as a control. Animals were videotaped, and latency (sec) for the animal to take the first bite of food was scored offline by a blind experimenter.

A single dose of GLYX-13 IV (3 mg/kg, 1 h before testing) or ketamine IV (10 mg/kg, 1 h before testing) was given. Sterile saline was used as the vehicle. Fluoxetine SC (20 mg/kg, 23.5, 5, and 1 h before testing; Detke, Rickels & Lucki, 1995) was administered as a reference compound. Milli-Q water (Millipore) was used as the vehicle for fluoxetine.

Learned Helplessness (LH) Test

A version of the LH test was used that can detect the antidepressant-like effect of ketamine 24 h post-dosing (Li *et al*, 2010). Experimental animals received pre-shock, which consisted of 60 inescapable shocks (0.8 mA; 15 s in duration; average intertrial interval of 45 s), 24 h before dosing. Animals received LH testing 24 h post dosing, consisting of 30 escapable shocks (0.6 mA; 30 s max duration; inter-trial interval 60 s). Animals could escape by shuttling through an open door to the no-shock side of the chamber. Naive control animals did not receive pre-shock or injection before LH testing. Escape latency was recorded by a blind experimenter. Animals received a single IV injection of GLYX-13 (3 mg/kg), fluoxetine (20 mg/kg SC; 23.5, 5, and 1 h before testing), or vehicle, 24 h before testing.

Conditioned Place Preference

Conditioned place preference testing was conducted, as described previously (Burgdorf *et al*, 2001b, 2007). Animals were trained with GLYX-13 (10 mg/kg, IV), ketamine (10 mg/kg, IV), or saline vehicle (1 ml/kg, IV), utilizing an unbiased, two-chamber conditioned place preference apparatus. Animals received three drug-environment pairings and three vehicle pairings counterbalanced across 6 consecutive days. Immediately after injection, animals were placed into either the drug (white side) or vehicle (black side) paired chamber for 20 min. On the day before and after the conditioning sessions, animals received 15 min free access to both chambers. Habituation and post conditioning preferences for the drug-paired side were calculated from video recordings by a blind experimenter.

Prepulse Inhibition

Testing was conducted as previously described (de Bruin *et al*, 1999). Animals were given injections of GLYX-13 (10 mg/kg) or saline (IV) 15 min before testing, or ketamine (10 mg/kg; de Bruin *et al*, 1999) or saline vehicle (IP) and tested immediately post injection for prepulse inhibition. Following a 5 min habituation period in the test chambers (Med Associates, USA) with background white noise (70 dB), animals were given 15 trials of each of the following stimuli: startle alone (120 dB white noise, 20 ms in duration), prepulse (73 dB white noise, 40 ms in duration followed by the startle stimulus 100 ms later), or no stimulus (70 dB white noise background). Presentation of the stimuli was randomized with the inter-trial interval varied between 10 and 20 s.

Drug Discrimination

Testing was conducted as described previously (Nicholson and Balster, 2009). Adult male Sprague-Dawley rats were trained to discriminate ketamine (10 mg/kg, IP) from saline. Animals were then tested following administration of various doses of GLYX-13 (3–156 mg/kg, SC) and the percent of ketamine lever responding and rates of responding were recorded. Various doses of ketamine were

tested as a positive control by both the IP and SC routes. Food (Harlan Teklad Rodent Diet, USA) access beyond those obtained during behavioral sessions was restricted to ~15 g, given post session in order to increase lever-pressing for food. The subjects were trained daily (Monday to Friday) in 15 min sessions in standard two-lever operant conditioning chambers (Med Associates, USA) under a double-alternation schedule of ketamine or saline (K, K, S, S, K, K, ect.) administered IP 15 min prior to session start. Completion of a fixed ratio (FR) 10 on the correct lever resulted in delivery of a 45 mg food pellet (P.J. Noyes Company, USA). During sessions, a white stimulus light located centrally above each lever was illuminated. Incorrect responding reset the FR for correct-lever responding. Test sessions were conducted on Tuesday and Friday when the subjects met the following criteria on the preceding training sessions: (1) first FR completed on the correct lever, and (2) >85% correct lever responding over the entire session. During test sessions, completion of a FR on either lever resulted in the delivery of food reinforcement. Switching levers before completion of a consecutive FR resulted in resetting the FR count. Training continued under the double alternation of 10 mg/kg ketamine and saline injections between test sessions. Illumination of lights, recording of responses, and pellet delivery were performed using a personal computer programmed with MED-PC operant conditioning software (version 1.1, USA).

Microinjection Surgery

Unilateral 22-gauge guide cannulae (Plastic Products, USA) were stereotactically implanted into the infralimbic/prelimbic cortex regions of the medial prefrontal cortex (MPFC; +2.7 mm anterior, $\pm$ 0.5 mm lateral, 3.0 mm ventral to bregma; flat brain) under isoflurane anesthesia. All animals were allowed 1 week to recover from surgery before the start of testing. GLYX-13 dose finding and localization studies were conducted in 2–3 month-old FBNF1 rats with bilateral cannulae (MPFC; +2.7 mm anterior, $\pm$ 0.7 mm lateral, 3.1 mm ventral to bregma; cannulae angled 12° away from the midline) or dorsal control primary/secondary motor cortex (+2.7 mm anterior, $\pm$ 1.2 mm lateral, 1.0 mm ventral to bregma; cannulae angled 12° away from the midline) under isoflurane anesthesia, as previously described (Burgdorf et al, 2010). Cannulae were secured to the skull with jeweler's screws and dental cement. Microinjections (0.5 μ l) were made with an injection cannulae that extended 1 mm past the guide cannulae. Injections were made across 1 min with a Harvard apparatus syringe pump (USA) and the cannulae were left in place for an additional 30 s. After the completion of behavioral testing, histology was conducted for cannulae tip location as described previously (Burgdorf et al, 2001a), and sections stained with H&E, with a representative section shown in Figure 3e. For medial prefrontal cannulae, all tips were located within the infralimbic or prelimbic cortex 2.2–3.2 mm anterior to bregma. For motor cortex cannulae, all tips were located within the primary or secondary motor cortex 2.2–3.2 mm anterior to bregma.

Protein Determinations

Whole-cell lysates. Animals were injected with GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV) or saline vehicle (1 ml/kg, IV), and 24 h post dosing, their brains were rapidly removed, frozen on dry ice, and the MPFC (frontal pole 3.0 mm anterior to bregma and medial to the forceps minor of the corpus callosum) and the hippocampus were dissected and stored at -80°C . Samples were extracted in ice cold RIPA buffer (1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM PMSF, 2 mM Na_3VO_4 , 20 mM tetrasodium pyrophosphate, protease and phosphatase inhibitor cocktail), protein concentration was determined by the BCA assay (Pierce, USA), and aliquots stored at -80°C until assay.

Sulfo-NHS-SS-Biotinylation of cell surface protein.

Twenty-four hours post-dosing with GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV), or sterile saline vehicle (1 ml/kg IV), rat brains were rapidly removed following decapitation (~90 s), the MPFC and hippocampus were dissected on an ice cold platform and MPFC and hippocampal sections (~300 micron) were washed with ice cold ACSF (pH 7.4) supplemented with phosphatase inhibitors (NaF and Na_3VO_4) saturated with 95% O_2 /5% CO_2 . Sections were then incubated in ice cold Sulfo-NHS-SS-Biotin (1 mg/ml) in ACSF (in mM): 126 NaCl, 2.5 KCl, 1.3 MgCl_2 , 10 glucose, 2.4 CaCl_2 , 1.24 NaH_2PO_4 , 26 NaHCO_3 , pH 7.4) gassed with 95% O_2 /5% CO_2 , for 30 min, with the reaction terminated with ice cold glycine (1 mM) + ACSF and ACSF alone washes. Tissue was then frozen on dry ice and stored at -80°C until assay. Tissue was sonicated in ice cold RIPA buffer (50 mM Tris-HCl pH8, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% TX100, 0.5% deoxycholate) supplemented with protease and phosphatase inhibitors (Sigma, USA), centrifuged at $25\,000 \times g$ for 10 min at 4°C , supernatant removed, and protein content determined by the BCA assay. Protein (3 mg) was precipitated with NeutraAvidin Ultralink resin (Pierce, USA) overnight at 4°C with agitation. Following three washes with ice cold RIPA, samples were boiled in Laemmli buffer (containing 200 mM DTT).

Protein samples were separated by SDS-polyacrylamide gel electrophoresis, transferred onto PVDF membranes (Millipore, USA) and blocked in 1% NFDM, 1% BSA, TBS for 1 h at 23°C . Membranes were incubated with GluR1 antibody (SC-13152, 1:200, Santa Cruz, USA; or MAB2263, 1:500, Millipore), anti-phosphoserine 845 GluR1 antibody (PRB-509p, 1:1000, Covance, USA), NR2B antibody (4207S, 1:500, Cell Signaling, USA) in 1% NMDF, 1%BSA, TBS overnight at 4°C , followed by a 1-h incubation at 25°C with a HRP-conjugated secondary antibody (1:2000–1:5000 Santa Cruz Biotechnology, USA). Immunoreactive bands were visualized by enhanced chemiluminescence (Immun-Star HRP, Bio-Rad, USA) and exposed to film (BioMax, Kodak, USA) for appropriate times. Membranes were re-probed with β -actin (5125, 1:5000–1:20 000, Cell Signaling) or stained with Reactive Brown 10 (Sigma, USA) to ensure equal protein loading. All images were within the linear range of the film and were quantified by ImageJ software (NIH, USA).

Microarray

Microarray and data analysis were conducted as previously described (Burgdorf et al, 2010, 2011; Kroes et al, 2006). Triplicate microarray analyses were performed using the MPFC isolated from individual animals injected with GLYX-13 (3 mg/kg IV), ketamine (10 mg/kg IV) or saline vehicle (1 ml/kg, IV). At 1 or 24 h post dosing their brains were rapidly removed, frozen on dry ice, and the MPFC dissected and stored at -80°C until assay ($n = 5$ per group).

Individual 45-mer oligonucleotides complementary to sequences of 1178 cloned rat CNS mRNAs were synthesized on a PolyPlex 96-well oligonucleotide synthesizer (GeneMachines, USA) and spotted in triplicate onto epoxy coated slides (Telechem, USA) using an OmniGrid robotic microarrayer (GeneMachines, USA). Total RNA was extracted (RNeasy, Qiagen, USA) and used as the substrate for RNA amplification and labeling using the Eberwine protocol (Van Gelder et al, 1990). Two micrograms of Cy5-labeled (experimental) and Cy3-labeled (universal rat reference, Stratagene, USA) amplified RNA (aRNA) were cohybridized on individual arrays at 46°C for 16 h. Arrays were scanned using two lasers (633 and 543 nm) at $5\text{ }\mu\text{m}$ resolution on the ScanArray 4000XL (Packard Biochip Technologies, USA). Raw image files were normalized using locally weighted scatter plot smoothing (LOWESS) curve-fitting (GeneTraffic, USA). Data were analyzed using the Significance Analysis of Microarrays (SAM) algorithm followed by data mining with Gene Ontology Miner (GoMiner) as described in (Burgdorf et al, 2011a; Kroes et al, 2006).

Quantitative Real-time PCR Analysis (qRT-PCR)

qRT-PCR was conducted as previously described (Burgdorf et al, 2011a; Kroes et al, 2006). qRT-PCR was performed on MPFC and hippocampi of individual rats dosed with GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV), or sterile saline vehicle (1 ml/kg IV) sacrificed 1 and 24 h post-dosing. The sequences of the qRT-PCR primers used in the study were as follows: NR1 (NM_017010), forward 5'-ATGGCTTCTGCATAGACC-3' and reverse 5'-GTTGTTTACCCGCTCCTG-3'. NR2A (NM_012573), forward 5'-AGTTCACCTATGACCTCTACC-3' and reverse 5'-GTTGATAGACCACTTACCT-3'. NR2B (NM_012574), forward 5'-AAGTTCACCTATGACCTTTACC-3' and reverse 5'-CATGACCACCTCACCGAT-3'. GluR1 (X17184) forward 5'-TGAAAGTGGGAGGTAACCTG-3' and reverse 5'-TAACACTGCCAGGTTTACTG-3'. GluR2 (NM_017261) forward 5'-GACTCTGGCTCCACTAAAGA-3' and reverse 5'-AGTCCTCACAAACACAGAGG-3'. GluR3 (NM_032990) forward 5'-GCAGCAAGGATGTGATATTT-3' and reverse 5'-GCAGTGTAAGGAAGAGATTATGA-3'. GluR4 (NM_017263) forward 5'-ACAGCAGCTATTGAGAACAG-3' and reverse 5'-GCCTTCGTACTTGTCATTTCC-3'. 18S (NR_046237) forward 5'-CCTGCGGCTTAATTTGACTC-3' and reverse 5'-GGCCTCACTAAACCATCCAA-3'.

Electrophysiology

Twenty-four hours post-dosing with GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV), or sterile saline vehicle (1 mg/kg, IV)

animals were deeply anesthetized with isoflurane and decapitated. Brains were removed rapidly, submerged in ice-cold artificial cerebrospinal fluid (ACSF, $2-4^{\circ}\text{C}$), which contained: 124 mM NaCl, 4 mM KCl, 2 mM MgSO_4 , 2 mM CaCl_2 , 1.25 mM NaH_2PO_4 , 26 mM NaHCO_3 , 10 mM glucose; at pH 7.4, gassed continuously with 95% O_2 /5% CO_2 . Brains were hemisected, the frontal lobes removed, and individual hemispheres glued using cyanoacrylate adhesive onto a stage immersed in ice-cold ACSF gassed continuously with 95% O_2 /5% CO_2 during slicing. 400- μm thick coronal slices were cut using a Vibratome (Leica VT1200S, Germany), and transferred to an interface holding chamber for incubation at room temperature for a minimum of 1 h before transferring to a Haas-style interface recording chamber continuously perfused at 3 ml/min with oxygenated ACSF at $32 \pm 0.5^{\circ}\text{C}$.

Extracellular recordings for LTP studies. Low-resistance recording electrodes were made from thin-walled borosilicate glass (1–2 M Ω after filling with ACSF) and inserted into the apical dendritic region of the Schaffer collateral termination field in stratum radiatum of CA1 to record field excitatory postsynaptic potentials (fEPSPs). A bipolar stainless steel stimulating electrode (FHC, USA) was placed on Schaffer collateral-commissural fibers in CA3 stratum radiatum, and constant current stimulus intensity adjusted to evoke approximately half-maximal fEPSPs once each 30 s (50–100 pA; 100 μs duration). fEPSP slope was measured before and after induction of LTP by linear interpolation from 20–80% of maximum negative deflection, and slopes confirmed to be stable to within $\pm 10\%$ for at least 15 min before commencing an experiment. LTP was induced by stimulation of Schaffer collateral axons with four high-frequency theta burst stimulus trains of $10 \times 100\text{ Hz}$ /five pulse bursts each, applied at an inter-burst interval of 200 ms. Each train was 2 s in duration, and trains were applied 15 s apart. Signals were recorded using a Multiclamp 700B amplifier and digitized with a Digidata 1322 (Axon Instruments, USA). Data were analyzed using pClamp software (version 9, Axon Instruments) on an IBM-compatible personal computer.

All external recording pipette solutions were made with deionized distilled water (resistance $> 18\text{ M}\Omega\text{ cm}^{-2}$; Milli-Q system, Millipore, USA). Chemicals for making extra- and intracellular solutions were purchased from Sigma or Fluka (USA). Electrophysiological data were analyzed initially with Clampfit (v9) (Axon Instruments, USA), and further processed and presented with Origin 6.1 (Microcal Software, USA) and CorelDraw 10.0 (Corel, Canada) programs. Statistical analyses were performed with SPSS (v11, USA). Statistical data are presented as mean $\pm$ SEM.

Intracellular whole-cell recordings of NMDAR currents. Whole-cell patch pipettes were pulled from borosilicate glass (1B150F-4, World Precision Instruments, USA) using a flaming/brown micropipette puller (P-97, Sutter Instruments, USA). The composition of the patch pipette solution for NMDAR current recordings was: 135 mM CsMeSO₃,

8 mM NaCl, 10 mM HEPES, 2 mM Mg-ATP, 0.3 mM Na-GTP, 0.5 mM EGTA, 1 mM QX-314, pH adjusted to 7.25 with CsOH, resulting in an osmolarity of 280 ± 10 mOsm and tip resistances of 5–6 M Ω .

Whole-cell patch clamp recordings were obtained from CA1 pyramidal neurons in slices in a fully submerged recording chamber at 30°C, and 95% O₂/5% CO₂ was passed over the perfusate to ensure an oxygenated local environment. The submerged recording chamber was mounted on a Zeiss Axioskop 2 FS upright microscope equipped with infrared differential interference contrast (DIC) optics. Pyramidal neurons of the hippocampal CA1 region were visualized with a 63 $\times$ water immersion lens, and patched in the voltage-clamp configuration. Excitatory postsynaptic currents (EPSCs) were recorded using a MultiClamp 700B (Axon Instruments, USA), with the low-pass filter setting at 1–3 kHz, series resistance was compensated in the voltage-clamp mode, and patched cells whose series resistance changed by >10% were rejected from analysis. Data were acquired with a 16-bit D/A interface (Digidata 1322A, Axon Instruments, USA) stored on a PC-compatible computer and analyzed using PCLAMP software (v9, Axon Instruments, USA).

Statistics

All data were analyzed by ANOVA, followed by Fisher's PLSD *post hoc* test (Statview), except for microarray analysis, which was analyzed by Significance Analysis of Microarrays (SAM), followed by data mining with Gene Ontology Miner (GoMiner) as described in Burgdorf et al, (2010, 2011a).

RESULTS

GLYX-13 Produces an Antidepressant-like Response in Multiple Rat Models

As shown in Figure 1a, GLYX-13 (3–10 mg/kg, IV) significantly reduced floating times in the Porsolt test 20–60 min post dosing as compared with vehicle ($F(5, 52) = 2.4$, $P < 0.05$, Fisher's PLSD *post hoc* test GLYX-13 3 mg/kg, GLYX-13 10 mg/kg, vs vehicle $P < 0.05$). The positive-control ketamine (10 mg/kg, IP), and fluoxetine (administered three times at 20 mg/kg, SC), but not the negative-control GLYX-13 scrambled peptide, decreased floating times as compared with vehicle ($F(3,30) = 12.2$, $P < 0.05$;

Figure 1 GLYX-13 produces an antidepressant-like effect in multiple rat models of depression. (a) Mean $\pm$ SEM floating time in the rat Porsolt test in 2–3 month-old Sprague Dawley rats treated with GLYX-13 (TPPT-NH₂; 1–56 mg/kg, IV), scrambled GLYX-13 (PTTP-NH₂; 3 mg/kg, IV), ketamine (10 mg/kg, IP), fluoxetine (20 mg/kg, SC), or sterile saline vehicle (1 ml/kg, IV) 30–60 min before testing, or (b) GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV) or fluoxetine (20 mg/kg, SC) or saline vehicle-treated rats tested 24 h post dosing. (c) Mean $\pm$ SEM latency to eat in the novelty induced hypophagia (NIH) test in rats dosed with GLYX-13 (3 mg/kg, IV), ketamine (10 mg/kg, IV) or saline and tested 1 h post dosing. (d) Mean $\pm$ SEM escape failures in the footshock-induced learned helplessness test in 2–3-month-old male SD rats dosed with GLYX-13 (3 mg/kg, IV), fluoxetine (20 mg/kg, SC), or sterile saline vehicle (1 ml/kg IV; tail vein) 24 h before testing. Naïve control animals did not receive pre-shock or injection before LH testing. $N = 7$ –21 per group. * $P < 0.05$ Fisher's PLSD *post hoc* test vs vehicle.

Fisher's PLSD *post hoc* test ketamine, fluoxetine vs vehicle $P < 0.05$, scrambled vs vehicle $P > 0.05$). The sample sizes for each group were: vehicle ($n = 8$), GLYX-13 1 mg/kg ($n = 9$), 3 mg/kg ($n = 11$), 10 mg/kg ($n = 11$), 32 mg/kg ($n = 11$), 56 mg/kg ($n = 8$), scrambled GLYX-13 3 mg/kg IV ($n = 12$), ketamine 10 mg/kg, IP ($n = 7$), fluoxetine 20 mg/kg (three doses), SC ($n = 7$).

GLYX-13 also reduced floating time in the Porsolt test following SC administration (10 and 30 mg/kg) at 1 h post-dosing as compared with vehicle ($F(6, 97) = 2.8$, $P < 0.05$, Fisher's PLSD *post hoc* test 10 and 30 mg/kg GLYX-13 vs vehicle $P < 0.05$, data not shown) and intranasal administration (79–2500 $\mu\text{g}/\text{nare}/\text{rat}$) 1 h post dosing as compared with vehicle ($F(4, 68) = 24.5$, $P < 0.05$, Fisher's PLSD *post hoc* test 79, 141, 250 and 2500 μg GLYX-13 vs vehicle $P < 0.05$, data not shown). Floating levels for the saline control groups did not significantly differ between IV, SC, and intranasal of administration 20–60 min post dosing ($F(2, 47) = 2.8$, $P > 0.05$, data not shown).

As shown in Figure 1b, GLYX-13 (3 mg/kg, IV, $n = 11$) and ketamine (10 mg/kg, IV, $n = 8$), but not fluoxetine (20 mg/kg, SC, 3 doses; $n = 9$) reduced floating time as compared with vehicle treated animals ($n = 21$) in the Porsolt test 24 h post dosing ($F(3, 45) = 21.3$, $P < 0.05$), Fisher's PLSD *post hoc* test GLYX-13, ketamine vs all other groups $P < 0.05$).

As shown in Figure 1c, GLYX-13 (3 mg/kg, IV, $n = 12$) and the positive-control ketamine (10 mg/kg, IV, $n = 11$) significantly reduced latency to eat in a novel environment in the NIH test 1 h post dosing compared with vehicle treated animals ($n = 14$; $F(2, 34) = 5.7$, $P < 0.05$, Fisher's PLSD *post hoc* test GLYX-13, ketamine vs vehicle $P < 0.05$), whereas feeding latencies were not changed in the homecage ($(2, 34) = 0.3$ $P > 0.05$; data not shown). Neither GLYX-13 (3 mg/kg, IV) nor ketamine (10 mg/kg, IV) altered locomotor activity in the NIH test 1 h post dosing ($F(2, 34) = 1.2$, $P > 0.05$). The mean $\pm$ SEM (sample size) locomotor activity scores (line crosses) are: vehicle-treated group (145.1 ± 9.5 ($n = 14$)); GLYX-13-treated group (114.6 ± 21.7 ($n = 12$)); and ketamine-treated group (111.5 ± 20.0 ($n = 12$)). Fluoxetine (20 mg/kg, SC, 24, 5, and 1 h before testing; $n = 6$) produced a non-specific increase in feeding latency compared with vehicle-treated animals ($n = 6$) in both a novel environment ($F(1, 10) = 21.5$, $P < 0.05$), as well as the animals homecage ($F(1, 10) = 6.5$, $P < 0.05$), and, therefore, these data were not included in the main analysis. However, these data are consistent with a previous report that a single dose of fluoxetine (as well as desipramine and amitriptyline) increased feeding latency in a novel environment (feeding latencies in the home environment were not measured), whereas 21 daily doses of fluoxetine (as well as desipramine and amitriptyline) were required to reduce feeding latencies in a novel environment (Bodnoff *et al*, 1989). Therefore, these results further support that chronic dosing with fluoxetine (but not subchronic dosing employed in the present study) is required to produce an antidepressant-like effect in the NIH test. In contrast, the NIH test can detect the antidepressant-like effects of ketamine after a single dose.

As shown in Figure 1d, GLYX-13 (3 mg/kg, IV; $n = 10$), fluoxetine (administered three times at 20 mg/kg, SC; $n = 9$),

as well as no pre-shock naïve control group ($n = 7$), reduced escape failures compared with vehicle ($n = 11$) ($F(3, 33) = 19.0$, $P < 0.05$, Fisher's PLSD *post hoc* test vehicle vs all other groups $P < 0.05$).

We also found that GLYX-13 (3 mg/kg, IV) and ketamine (10 mg/kg, IV) reduced the floating time in the Porsolt test 1 week post-dosing as compared with vehicle-treated animals ($F(2, 29) = 58.1$, $P < 0.05$, Fisher's PLSD *post hoc* test GLYX-13, ketamine vs vehicle $P < 0.05$). The mean $\pm$ SEM (sample size) floating times (sec) are: 1 week vehicle-treated group (112.6 ± 11.3 ($n = 11$)); 1 week GLYX-13-treated group (17.6 ± 3.9 ($n = 11$)); 1 week ketamine-treated group (12.7 ± 3.9 ($n = 10$)).

The Antidepressant-like Effect of GLYX-13 is Blocked by the AMPA/kainate Receptor Antagonist NBQX

As shown in Figure 2, a silent dose of NBQX (10 mg/kg, IP, 70 min before testing) was able to block the antidepressant-like effects of GLYX-13 (3 mg/kg, IV) 1 h post dosing in the Porsolt test ($F(3, 40) = 19.2$, $P < 0.05$), Fisher's PLSD *post hoc* test GLYX-13 alone vs all other groups, $P < 0.05$, NBQX alone vs vehicle alone $P > 0.05$). $n = 11$ per group.

MPFC injections of GLYX-13, but not ketamine, produced an antidepressant-like effect in the Porsolt test

As shown in Figure 3a, MPFC injections of GLYX-13 (1–10 $\mu\text{g}/\text{side}$, but not 0.1 $\mu\text{g}/\text{side}$), produced an antidepressant-like effect in the Porsolt test 1 h post dosing compared with vehicle ($F(3, 36) = 8.9$, $P < 0.05$, Fisher's PLSD *post hoc* test GLYX-13, 1 and 10 $\mu\text{g}/\text{side}$ vs vehicle $P < 0.05$). However, GLYX-13 injected (1 $\mu\text{g}/\text{side}$) into the motor cortex (dorsal and lateral to the MPFC) failed to produce an

Figure 2 The AMPA/kainate antagonist NBQX blocks the antidepressant-like effect of GLYX-13. Mean ($\pm$ SEM) floating time in the Porsolt test in animals pre-treated with NBQX (10 mg/kg, IP) before GLYX-13 (3 mg/kg, IV) dosing and tested 1 h post-dosing. The antidepressant-like effects of ketamine has also been shown to be blocked by NBQX (Maeng *et al*, 2008) $n = 11$ per group. * $P < 0.05$, Fisher's PLSD vs all other groups.

Figure 3 GLYX-13 but not ketamine produces an antidepressant-like effect in the rat Porsolt test when injected into the medial prefrontal cortex. Mean ($\pm$ SEM) time (sec) spent immobile in the Porsolt test in 2–3-month-old male rats implanted with (a) medial prefrontal or motor cortex (dorsal control) cannulae and injected with GLYX-13 (0.1, 1, 10 μ g/side) or sterile saline vehicle (0.5 μ l/1 min) and tested 1 h post-dosing or rats given MPFC injections of ketamine (0.1, 1, 10 μ g), GLYX-13 (1 μ g), or saline and tested (b) 20 min and (c) 24 h post-dosing. Animals received a 15-min training swim session 1 day before dosing. (d) Mean ($\pm$ SEM) line crosses in the open field 20 min; following MPFC infusion of GLYX-13 (1 μ g), ketamine (0.1 μ g) or sterile saline vehicle. Given that 0.1 μ g dose of ketamine increased locomotor activity, the Porsolt data for that dose were not included in the analysis given that increasing locomotor activity produces a false-positive antidepressant-like response. (e) a representative H&E-stained section depicting MPFC cannulae placement, arrow indicates injection site. $n = 5$ –10 per group. * $P < 0.05$, Fisher's PLSD vs vehicle.

antidepressant-like effect compared with vehicle ($F(1, 16) = 0.1$, $P > 0.05$), consistent with a direct antidepressant action of GLYX-13 in the MPFC.

As shown in Figure 3b and c, MPFC injections of ketamine (1–10 μ g) failed to produce an antidepressant-like effect in the Porsolt test at (b) 20 min ($F(2, 15) = 1.3$, $P > 0.05$) or (c) 24 h ($F(2, 15) = 0.3$, $P > 0.05$) post-dosing, and the antidepressant-like effect of MPFC injections of GLYX-13 (1 μ g) was replicated at both the (b) 20 min ($F(1, 10) = 193.5$, $P < 0.05$) and (c) 24 h ($F(1, 10) = 161.6$, $P < 0.05$) time points.

As shown in Figure 3d, MPFC injections of ketamine (0.1 μ g), but not GLYX-13 (1 μ g), increased locomotor behavior in the open field 20 min post dosing as compared with saline vehicle ($F(2, 15) = 28.9$, $P < 0.05$, Fisher's PLSD *post hoc* test ketamine vs all other groups, $P < 0.05$). Given that MPFC injections of 0.1 μ g of ketamine increased locomotor activity 20 min post dosing, Porsolt data from this dose was not included in the statistical analyses. A representative H&E stained coronal section showing the location of cannulae placement in the MPFC is shown in Figure 3e.

Isoflurane has been shown to produce a rapid and long-lasting antidepressant-like effect in treatment-resistant depressed subjects across six isoflurane anesthesia sessions (Langer *et al*, 1995). We therefore examined if a single isoflurane anesthesia exposure would produce an antidepressant-like effect in rats that may potentially confound the antidepressant-like effects of GLYX-13 or ketamine. The isoflurane exposure (4% isoflurane induction, 2.5% maintenance, 20-min total duration) was identical to animals that received cannulae implantation surgery. A single isoflurane anesthesia session did not produce an antidepressant-like effect in the rat Porsolt test 1 h ($F(1, 10) = 0.1$, $P > 0.05$), 24 h ($F(1, 10) = 0.3$, $P > 0.05$), or 1 week post-dosing ($F(1, 21) = 0.4$, $P > 0.05$) compared with control animals placed into a novel box, with the 1-week time point corresponding to the time when MPFC injections of GLYX-13 or ketamine were given. The mean $\pm$ SEM (sample size) floating times (sec) are: 1 h control group (150.6 ± 4.7 ($n = 6$)); 1 h and 1 week isoflurane group (154.6 ± 4.7 ($n = 6$)), 24-h control group (123.6 ± 7.1 ($n = 6$)); 24 h isoflurane group (128.5 ± 11.1 ($n = 6$)); the 1 h and 1 week control group (121.9 ± 8.5 ($n = 12$)), 1 week isoflurane group (112.6 ± 11.3 ($n = 11$)).

Figure 4 GLYX-13 does not show ketamine-like discriminative stimulus effects. Mean ($\pm$ SEM) (a) percentage ketamine-lever responding and (b) rates of responding for different doses of ketamine (IP and SC) and GLYX-13 (SC) in rats trained to discriminate 10 mg/kg ketamine (Ket), IP, from saline (Sal). Values above Sal and Ket are the results of control tests conducted before testing each dose–response curve. Values above Sal/Sal and Sal/Ket are the results of similar control tests performed, following administration of 2 ml saline SC, 30 min before the session start to mimic conditions of GLYX-13 testing. $n = 7$ –8 per group. * $P < 0.05$ Fisher's PLSD *post hoc* test vs vehicle.

Figure 5 GLYX-13 does not show ketamine-like rewarding, sensory-motor gating, or sedative side effects. (a) Ketamine (10 mg/kg, IV), but not GLYX-13 (10 mg/kg, IV), induced conditioned place preference as measured by % time in drug paired chamber. (b) Ketamine (10 mg/kg, IP), but not GLYX-13 (10 mg/kg, IV), decreased sensory-motor gating as measured by prepulse inhibition. (c) A sedating dose of ketamine (10 mg/kg, SC), but not GLYX-13 (10 mg/kg, IV), reduced locomotor activity in the open field as measured by line crosses. $N = 8$ –11 per group. * $P < 0.05$ Fisher's PLSD *post hoc* test vs vehicle. Data are expressed as Mean ($\pm$ SEM).

GLYX-13 does not Show any Ketamine-like Discrimination Stimulus Effects

As shown in Figure 4, GLYX-13 (3–156 mg/kg, SC) did not (a) substitute for 10 mg/kg ketamine IP ($F(6,49) = 0.6$, $P > 0.05$), or (b) suppress operant response rates ($F(6,49) = 0.2$, $P > 0.05$) across a wide dose range, whereas the positive-control ketamine (SC) showed dose-dependent substitution for the ketamine training dose ($F(5,40) = 18.1$, $P < 0.05$, Fisher's PLSD *post hoc* test 1, 3, 10 mg/kg ketamine vs vehicle $P < 0.05$) and suppressed operant behavior at higher doses ($F(5,40) = 6.3$, $P < 0.05$, Fisher's PLSD *post hoc* test 10 mg/kg ketamine vs vehicle $P < 0.05$). IP ketamine also showed dose-dependent substitution for the ketamine training dose ($F(7,54) = 46.3$, $P < 0.05$, Fisher's PLSD *post hoc* test 5.6, 10, 17, 30 mg/kg ketamine vs vehicle $P < 0.05$) and suppressed operant behavior at higher doses ($F(7,56) = 4.6$, $P < 0.05$, Fisher's PLSD *post hoc* test 17, 30 mg/kg ketamine vs vehicle $P < 0.05$).

GLYX-13 does not Show any Ketamine-like Sedative, Rewarding, or Sensory Gating Side Effects

As shown in Figure 5a, ketamine (10 mg/kg, IV), but not GLYX-13 (3 mg/kg, IV), induced drug reward as measured

by an increased conditioned place preference compared with vehicle ($F(2,24) = 3.8$, $P < 0.05$, Fisher's PLSD *post hoc* test ketamine vs vehicle and ketamine vs GLYX-13 $P < 0.05$).

As shown in Figure 5b, ketamine (10 mg/kg, IP), but not GLYX-13 (10 mg/kg, IV), produced sensory-motor gating deficits in the pre-pulse inhibition test as compared with saline vehicle ($F(2,33) = 4.0$, $P < 0.05$, Fisher's PLSD *post hoc* test ketamine vs vehicle and ketamine vs GLYX-13 $P < 0.05$). % Prepulse inhibition = 100—(mean prepulse trial startle amplitude/mean startle trial amplitude) $\times 100$. * $P < 0.05$, Fisher PLSD *post hoc* test comparing drug vs vehicle.

As shown in Figure 5c, ketamine (10 mg/kg, SC), but not GLYX-13 (10 mg/kg, IV), showed sedative effects in the open field ($F(2,24) = 238.6$, $P < 0.05$, Fisher's PLSD *post hoc* test ketamine vs vehicle and ketamine vs GLYX-13 $P < 0.05$).

Both GLYX-13 and Ketamine Produced a Significant Increase in Cell Surface Protein Expression (but not mRNA levels) of both NMDAR-NR2B and AMPA GluR1 in the MPFC or Hippocampus 24 h Post Dosing

As shown in Table 1, neither GLYX-13 (3 mg/kg, IV), nor ketamine (10 mg/kg IV altered mRNA levels of NR1

Table 1 Neither NMDA nor AMPA Receptor mRNA Expression was Significantly Altered in MPFC or Hippocampus by GLYX-13 (3 mg/kg IV) or Ketamine (10 mg/kg IV), as Measured by Microarray and/or qRT-PCR Analyses

Microarray

Data is expressed as fold change relative to vehicle

	NR1	NR2A	NR2B	NR2C	NR2D	GluR1	GluR2
<i>MPFC, 1 h</i>							
GLYX-13	1.08	0.90	0.95	1.00	0.99	0.97	0.99
Ketamine	1.03	0.85	0.96	0.99	1.00	1.03	0.95
<i>MPFC, 24 h</i>							
GLYX-13	1.10	0.87	0.98	1.07	0.99	0.87	1.18
Ketamine	1.03	0.85	0.93	1.07	0.98	0.95	1.04

qRT-PCR

Data is expressed as mean $\pm$ SEM % of control levels

	NR1	NR2A	NR2B	GluR1
<i>MPFC, 1 h</i>				
Vehicle	100 $\pm$ 8	100 $\pm$ 6	100 $\pm$ 9	100 $\pm$ 12
GLYX-13	82 $\pm$ 3	97 $\pm$ 2	92 $\pm$ 2	88 $\pm$ 6
Ketamine	78 $\pm$ 17	83 $\pm$ 15	73 $\pm$ 13	90 $\pm$ 13
<i>MPFC, 24 h</i>				
Vehicle	100 $\pm$ 19	100 $\pm$ 11	100 $\pm$ 12	100 $\pm$ 23
GLYX-13	93 $\pm$ 10	99 $\pm$ 11	100 $\pm$ 8	75 $\pm$ 10
Ketamine	75 $\pm$ 5	82 $\pm$ 5	80 $\pm$ 5	62 $\pm$ 3
<i>Hippocampus, 1 h</i>				
Vehicle	100 $\pm$ 23	100 $\pm$ 27	100 $\pm$ 23	100 $\pm$ 24
GLYX-13	76 $\pm$ 22	73 $\pm$ 20	84 $\pm$ 24	83 $\pm$ 20
Ketamine	115 $\pm$ 14	103 $\pm$ 18	136 $\pm$ 20	105 $\pm$ 18
<i>Hippocampus, 24 h</i>				
Vehicle	100 $\pm$ 16	100 $\pm$ 14	100 $\pm$ 15	100 $\pm$ 16
GLYX-13	105 $\pm$ 7	87 $\pm$ 7	97 $\pm$ 7	84 $\pm$ 9
Ketamine	94 $\pm$ 8	73 $\pm$ 7	69 $\pm$ 6	87 $\pm$ 8

n = 5–6 per group.

($F(2,49) = 0.8$, $P > 0.05$), NR2A ($F(2,49) = 0.9$, $P > 0.05$), NR2B ($F(2,46) = 0.4$, $P > 0.05$), or GluR1 ($F(2,41) = 1.4$, $P > 0.05$) as compared with vehicle 1 and 24 h post dosing in the MPFC and hippocampus, as compared with vehicle as measured by qRT-PCR. Also, GLYX-13 (3 mg/kg, IV) did not alter NMDAR or AMPAR mRNA, as measured by qRT-PCR in an extended time course (0.5, 1, 2, 4, 8, 24 h, 3, 7, 14 days post dosing; data not shown). Consistent with this observation, neither GLYX-13 nor ketamine led to a significant enrichment in gene expression changes associated with NMDAR (GO Term 4972; $P > 0.05$), or AMPA

Figure 6 GLYX-13 and ketamine increased the expression of cell surface NR2B and GluR1 proteins in the rat MPFC and hippocampus 24 h post dosing. Mean $\pm$ SEM protein levels in the medial prefrontal cortex (MPFC) or hippocampus as measured by Western analyses in 2–3-month-old SD rats treated with GLYX-13 (3 mg/kg, IV) or sterile saline vehicle (1 ml/kg IV; tail vein) 24 h before sacrifice. MPFC or hippocampus slices were incubated with Sulfo-NHS-SS-Biotin to label surface protein, and biotinylated protein was precipitated with avidin-agarose beads. Protein samples were analyzed by SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes probed with NR2B-, GluR1-, and β -actin-specific antibodies. The sample sizes for each group are, NR2B MPFC vehicle $n = 11$, GLYX-13 $n = 11$, ketamine $n = 8$; NR2B hippocampus vehicle $n = 7$, GLYX-13 $n = 6$, ketamine $n = 6$; GluR1 MPFC and hippocampus $n = 6$ per group. * $P < 0.05$, Fisher's PLSD *post hoc* test vs vehicle.

receptor (GO Term 4971; $P > 0.05$) comparing vehicle with GLYX-13 or vehicle to ketamine, 1 and 24 h post dosing in MPFC as measured by GoMiner analysis of microarray data.

In contrast, as shown in Figure 6, GLYX-13 (3 mg/kg, IV) and ketamine (10 mg/kg, IV) increased cell surface protein levels of NR2B in the MPFC and hippocampus 24 h post dosing as compared with vehicle controls ($F(2,46) = 11.1$, $P < 0.05$, Fisher's PLSD *post hoc* GLYX-13 vs vehicle, and ketamine vs vehicle individually for both the brain regions, $P < 0.05$). GLYX-13 (3 mg/kg, IV) and ketamine (10 mg/kg, IV) also increased cell surface protein levels of GluR1 ($F(2,33) = 10.4$, $P < 0.05$, Fisher's PLSD *post hoc* GLYX-13 vs vehicle, and ketamine vs vehicle individually for both brain regions, $P < 0.05$). We also show that both GLYX-13 (3 mg/kg, IV) and ketamine (10 mg/kg, IV) increased pS845 GluR1/total GluR1 ratios in the MPFC 24 h post dosing as compared with vehicle controls ($F(2,20) = 3.8$, $P < 0.05$, Fisher's PLSD *post hoc* GLYX-13, ketamine vs vehicle, $P < 0.05$; data not shown).

As shown in Table 2, although GLYX-13 (3 mg/kg, IV) and ketamine (10 mg/kg, IV) increased NR2B protein levels in MPFC whole-cell lysates 24 h post dosing, no effect was seen in the hippocampus (main effect for drug, $F(2,32) = 6.0$, $P < 0.05$; drug $\times$ brain region interaction, $F(2,32) = 3.3$, $P < 0.05$; Fisher's PLSD *post hoc* GLYX-13 vs vehicle, and ketamine vs vehicle in the MPFC, $P < 0.05$, but not the hippocampus, $P > 0.05$). GLYX-13 and ketamine did not alter GluR1 protein levels in whole-cell lysates 24 h post

dosing in either MPFC or hippocampus (main effect for drug, $F(2, 36) = 0.3$, $P > 0.05$).

Both GLYX-13 and Ketamine Persistently Enhanced the Induction of Long-term Potentiation of Synaptic Transmission in the Hippocampus 24 h Post Dosing

As shown in Figure 7, GLYX-13 (3 mg/kg, IV, $n = 6$) and ketamine (10 mg/kg, IV, $n = 6$) 24 h post dosing increased the proportion of NMDAR synaptic EPSCs gated by NR2B-containing NMDAR in hippocampal slices, as compared with vehicle ($n = 5$) ($F(2, 14) = 8.7$, $P < 0.05$, Fisher's PLSD *post hoc* GLYX-13 vs vehicle, and ketamine vs vehicle,

$P < 0.05$). As shown in Figure 8, GLYX-13 (3 mg/kg, IV) and ketamine (10 mg/kg, IV) 24 h post-dosing increased the magnitude of LTP elicited by a submaximal high-frequency stimulation in hippocampal slices ($F(2, 50) = 5.1$, $P < 0.05$, Fisher's PLSD *post hoc* GLYX-13 vs vehicle, and ketamine vs vehicle individually for T1–T3, $P < 0.05$) 24 h post dosing as compared with vehicle.

DISCUSSION

The results reported here show that the novel NMDAR glycine-site functional partial agonist, GLYX-13, produces an antidepressant-like effect in rats without ketamine-like side effects. Both GLYX-13 and ketamine produced rapid acting (1 h) and long-lasting (24 h) antidepressant-like effects in the Porsolt test and a long-lasting (24 h) antidepressant-like effect in the LH tests. GLYX-13 and ketamine also produced a rapid acting antidepressant-like effect in the NIH test, whereas subchronic fluoxetine failed to show an antidepressant-like effect. Our interpretation of these results would have been strengthened by the inclusion of a chronic fluoxetine positive control, as well as testing GLYX-13 and ketamine 24 h post dosing. GLYX-13 did not show any ketamine-like discriminative stimulus effects, gating, sedative or drug-abuse-related side effects. Of the many different NMDAR-specific modulatory compounds that have been reported (Danysz and Parsons, 1998; Traynelis *et al*, 2010), only a few have been shown to have therapeutic potential in clinical trials. Ketamine, a NMDAR channel blocker, has demonstrated antidepressant effects with onset within 2 h and duration of effect up to several weeks, following a single dose in patients with treatment-resistant depression and bipolar depression (aan het Rot *et al*, 2010; Berman *et al*, 2000; Diazgranados *et al*, 2010a, b; Price *et al*, 2009; Zarate *et al*, 2006a). D-cycloserine,

Table 2 Total Cellular Protein Levels of NR2B and GluR1 Were Differentially Expressed in MPFC and Hippocampus, 24 h Following Treatment with Either GLYX-13 (3 mg/kg, IV) or Ketamine (10 mg/kg, IV), as Measured by Western Blot. Data are Expressed as Mean $\pm$ SEM % of Control Levels

	NR2B	GluR1
MPFC 24 h		
Vehicle	100 $\pm$ 26	100 $\pm$ 10
GLYX-13	242 $\pm$ 38*	104 $\pm$ 17
Ketamine	196 $\pm$ 28*	95 $\pm$ 15
Hippocampus 24 h		
Vehicle	100 $\pm$ 21	100 $\pm$ 7
GLYX-13	129 $\pm$ 19	116 $\pm$ 8
Ketamine	89 $\pm$ 13	116 $\pm$ 2

$n = 4–11$ per group.

* $P < 0.05$, Fisher's PLSD *post hoc* test vs vehicle.

Figure 7 GLYX-13 and ketamine increase hippocampus NR2B current 24 h post dosing. (left panel) Representative Schaffer collateral-evoked EPSCs in a CA1 pyramidal neuron, treated 24 h earlier with vehicle (control), GLYX-13 (3 mg/kg, IV) or ketamine (10 mg/kg, IV). Traces are shown before (black), after bath application of the NR2B-selective NMDAR antagonist ifenprodil (IFE; 10 μ M; red), and after co-application of ifenprodil plus the NR2A-NMDAR-selective antagonist NVP-AM077 (NVP; 100 nM; blue). The scale for the x- and y-axes are an inset in the top panel. (right panel) Mean $\pm$ SEM NMDA receptor-dependent single shock-evoked EPSCs in the presence of the NR2B-selective NMDAR antagonist ifenprodil (10 μ M), in CA1 pharmacologically isolated NMDA current in rats that were dosed with GLYX-13 (3 mg/kg, IV) ketamine (10 mg/kg, IV), or sterile saline vehicle (IV), 24 h before *in vitro* NMDA current measurement. $n = 5–6$ per group. * $P < 0.05$, Fisher's PLSD vs vehicle.

Figure 8 GLYX-13 and ketamine induce metaplasticity 24 h post dosing. GLYX-13 (3 mg/kg, IV) or ketamine (10 mg/kg, IV) 24 h post dosing enhances the magnitude of ex-vivo long-term potentiation (LTP) of synaptic transmission at Schaffer collateral-CA1 synapses. Plot of mean $\pm$ SEM Schaffer collateral-evoked normalized field EPSP slopes in hippocampal slices from rats treated 24 h earlier with vehicle (control; black circles), GLYX-13 (GLYX-13; blue circles) or ketamine (ketamine; red squares). Three submaximal bouts of high-frequency Schaffer collateral stimulation (2×100 Hz/800 ms) were applied at the arrows to elicit incremental LTP. Slices from both GLYX-13 and ketamine pre-treated rats exhibited significantly larger LTP compared with controls. $n=8-10$ per group, * $P<0.05$, ** $P<0.01$, Fisher's PLSD *post hoc* test vs vehicle.

a NMDAR glycine-site partial agonist, has been shown to have therapeutic potential in post-traumatic stress syndrome and schizophrenia but not reliably in depression (Goff *et al*, 1999; Heresco-Levy *et al*, 2002a, b, 2006). Memantine is an activity-dependent, low-affinity NMDAR channel blocker shown to have a therapeutic effect in Alzheimer's dementia (McShane *et al*, 2006). Memantine has not been shown to have antidepressant activity in clinical trials (Zarate *et al*, 2006b), however, at high concentrations, where it might display antidepressant properties, it shows dissociative side effects (Bisaga and Evans, 2004).

The unique mechanism of action of GLYX-13 may explain its lack of ketamine-like side effects. GLYX-13 is a functional partial agonist at the glycine site, whereas ketamine is a non-competitive NMDAR channel blocker, which may help explain the lack of ketamine-like side effects seen with GLYX-13 (Moskal *et al*, 2005). GLYX-13 activates the NMDAR at comparatively low levels of NMDAR activity, but acts as an antagonist at higher levels of NMDAR activity. The doses of GLYX-13 that lead to optimal antidepressant-like effects in rats (3–10 mg/kg, IV) are higher than doses that facilitate increases in hippocampus-dependent learning (1 mg/kg, IV) in both young adult and learning-impaired aging rats (Burgdorf *et al*, 2011a), whereas they are similar to the doses effective in pain models (Wood *et al*, 2008). GLYX-13 (1 mg/kg, IV) also increased positive emotional learning, as defined by an increased acquisition rate of heterospecific play-induced hedonic 50-kHz USVs, and this effect was also seen following MPFC injections (Burgdorf *et al*, 2011b). Ketamine, as a NMDAR open-channel blocker, acutely blocks the induction and expression of LTP (Stringer *et al*, 1983), as well as hippocampus-dependent learning tasks (Goulart *et al*, 2011; Wesierska *et al*, 1990). Moreover, ketamine shows no antidepressant-like effects when directly injected

into the MPFC (Figure 3). In fact, ketamine's antidepressant effects appear to be caused by the facilitation of MPFC glutamate release via blockade of GABAergic interneurons projecting into the MPFC (Li *et al*, 2010).

The long-lasting antidepressant effects of both GLYX-13 and ketamine appear to be due to increases in long-term activity-dependent synaptic plasticity or metaplasticity. An important feature of metaplasticity is that these changes last longer than the triggering mechanism which could be electrical, chemical, or behavioral. NMDAR activation is a key component of both LTP induction and the induction of metaplasticity (Abraham 2008; Huang *et al*, 1992).

Both GLYX-13 and ketamine led to a robust increase in the facilitation of LTP, measured 24 h post-dosing. This effect occurs long after the half-life of either compound in rats (plasma half life of GLYX-13 is 7 min and ketamine is 56 min; Rafael & Abdel-Rahman, 2002). GLYX-13 and ketamine also increased NR2B-specific currents in hippocampal slices, as well as significantly increasing both NR2B and GluR1 surface protein expression in the hippocampus and MPFC. The long-term changes in synaptic strength are dependent on NMDAR activation of AMPA receptors (Lee *et al*, 2000; Malinow and Malenka, 2002; Song and Huganir, 2002; Nong *et al*, 2003) to produce its antidepressant-like actions, consistent with the idea that elevated NMDAR transmission acts via LTP, expressed as enhanced AMPAR transmission. The AMPA/kainate receptor antagonist, NBQX, blocks the antidepressant-like effects of both GLYX-13 (Figure 2) and ketamine (Maeng *et al*, 2008).

We propose that both GLYX-13- and ketamine-induced synaptic plasticity may be due to decreased endocytosis of NR2B-containing NMDARs or increased trafficking from the endoplasmic reticulum. NR2B-containing NMDARs have been shown to have a critical role in the induction of metaplasticity (Yang *et al*, 2011). It has recently been reported that the NMDARs may have a low-affinity glycine-binding site that can modulate their endocytosis (Kew and Kemp, 1998; Nong *et al*, 2003). Our results suggest that both GLYX-13 and ketamine may lead to increased cell surface expression of functional NR2B-containing NMDARs by inhibiting normal endocytotic processes.

ACKNOWLEDGEMENTS

This research was supported by grants from The Ralph and Marian Falk Medical Research Trust (Chicago, IL) to JRM, The Hope for Depression Research Foundation (New York, NY) to JRM and JSB, and NIH grants MH094835 to JSB, NS044421 to PKS, and DA01442 to RLB and KLN. Research and grant support was provided by National Institutes of Health, Virginia Foundation for Health Youth, Sonexa Pharmaceuticals, Neurosearch A/S to RLB. We thank the Northwestern University Behavioral Phenotyping Core and Histology Core for their assistance and Ms Mary Schmidt for her expert technical assistance. We also thank Derek Small and Ronald Burch for their helpful discussions.

DISCLOSURE

JRM is the founder of Naurex, Inc. He has founders' shares of stock in the company. JRM receives financial

compensation as a consultant. PKS, RAK and JB have been a consultants for Naurex, Inc. for the past 3 years, and have received financial compensation and stock. ALG is an employee of Naurex, Inc. She has received financial compensation and stock. Over the past 3 years, KLN has received compensation from Merz Pharma and WIL Research for consultation services unrelated to the current manuscript. Naurex, Inc. had provided funds for conducting the experiments described in the text, Figure 5. RBL has the following sources of personal financial support 2009–2012: Virginia Commonwealth University, National Academy of Sciences, Food and Drug Administration, US Department of State, Consumer Healthcare Products Association, Forest Research Institute, UCB Pharma, Astra-Zeneca, Centre for Mental Health and Addiction, Global Biodevelopment, Jurox Pharmaceuticals, Elsevier Science, Merz Pharmaceuticals, Servier, JG Perpich Company, Reckitt-Benckiser, Kendle Toronto, Abbott Laboratories, Washington University. JDL is a paid consultant for Naurex and also has stock in the company. Over the last 3 years he has received financial compensation and/or stock with the following companies: AgeneBio, Nektar, and CoLucid. XLZ receives salary support from a grant from Naurex to PKS. He receives no consulting fees and has no stock in Naurex. All the authors have no other consultantship and have not received any financial compensation from any other private companies for the last 3 years.

REFERENCES

- aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS *et al* (2010). Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol Psychiatry* 67: 139–145.
- Abraham WC (2008). Metaplasticity: tuning synapses and networks for plasticity. *Nat Rev Neurosci* 9: 387–399.
- Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF *et al* (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* 475: 91–95.
- Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS *et al* (2000). Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* 47: 351–354.
- Bisaga A, Evans SM (2004). Acute effects of memantine in combination with alcohol in moderate drinkers. *Psychopharmacology (Berl)* 172: 16–24.
- Bodnoff SR, Suranyi-Cadotte B, Quirion R, Meaney MJ (1989). A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. *Psychopharmacology (Berl)* 97: 277–279.
- Burgdorf J, Knutson B, Panksepp J, Ikemoto S (2001a). Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behav Neurosci* 115: 940–944.
- Burgdorf J, Knutson B, Panksepp J, Shippenberg TS (2001b). Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs. *Psychopharmacology (Berl)* 155: 35–42.
- Burgdorf J, Wood PL, Kroes RA, Moskal JR, Panksepp J (2007). Neurobiology of 50-kHz ultrasonic vocalizations in rats: electrode mapping, lesion, and pharmacology studies. *Behav Brain Res* 182: 274–283.
- Burgdorf J, Panksepp J, Brudzynski SM, Beinfeld MC, Cromwell HC, Kroes RA *et al* (2009). The effects of selective breeding for differential rates of 50-kHz ultrasonic vocalizations on emotional behavior in rats. *Dev Psychobiol* 51: 34–46.
- Burgdorf J, Kroes RA, Beinfeld MC, Panksepp J, Moskal JR (2010). Uncovering the molecular basis of positive affect using rough-and-tumble play in rats: a role for insulin-like growth factor I. *Neuroscience* 168: 769–777.
- Burgdorf J, Kroes RA, Weiss C, Oh MM, Disterhoft JF, Brudzynski SM *et al* (2011a). Positive emotional learning is regulated in the medial prefrontal cortex by GluN2B-containing NMDA receptors. *Neuroscience* 192: 515–523.
- Burgdorf J, Zhang XL, Weiss C, Matthews E, Disterhoft JF, Stanton PK *et al* (2011b). The N-methyl-D-aspartate receptor modulator GLYX-13 enhances learning and memory, in young adult and learning impaired aging rats. *Neurobiol Aging* 32: 698–706.
- Danysz W, Parsons CG (1998). Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacol Rev* 50: 597–664.
- de Bruin NM, Ellenbroek BA, Cools AR, Coenen AM, van Luijckelaar EL (1999). Differential effects of ketamine on gating of auditory evoked potentials and prepulse inhibition in rats. *Psychopharmacology (Berl)* 142: 9–17.
- Detke MJ, Rickels M, Lucki I (1995). Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology* 121: 66–72.
- Diazgranados N, Ibrahim L, Brutsche NE, Newberg A, Kronstein P, Khalife S *et al* (2010a). A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Arch Gen Psychiatry* 67: 793–802.
- Diazgranados N, Ibrahim LA, Brutsche NE, Ameli R, Henter ID, Luckenbaugh DA *et al* (2010b). Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. *J Clin Psychiatry* 71: 1605–1611.
- Dulawa SC, Hen R (2005). Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neurosci Biobehav Rev* 29: 771–783.
- Feyissa AM, Chandran A, Stockmeier CA, Karolewicz B (2009). Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* 33: 70–75.
- Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA *et al* (1999). A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. *Arch Gen Psychiatry* 56: 21–27.
- Goulart BK, de Lima MN, de Farias CB, Reolon GK, Almeida VR, Quevedo J *et al* (2011). Ketamine impairs recognition memory consolidation and prevents learning-induced increase in hippocampal brain-derived neurotrophic factor levels. *Neuroscience* 167: 969–973.
- Gozes I, Giladi E, Pinhasov A, Bardea A, Brenneman DE (2000). Activity-dependent neurotrophic factor: intranasal administration of femtomolar-acting peptides improve performance in a water maze. *J Pharmacol Exp Ther* 293: 1091–1098.
- Haring R, Stanton PK, Scheideler MA, Moskal JR (1991). Glycine-like modulation of N-methyl-D-aspartate receptors by a monoclonal antibody that enhances long-term potentiation. *J Neurochem* 57: 323–332.
- Heresco-Levy U, Ermilov M, Shimoni J, Shapira B, Silipo G, Javitt DC (2002a). Placebo-controlled trial of D-cycloserine added to conventional neuroleptics, olanzapine, or risperidone in schizophrenia. *Am J Psychiatry* 159: 480–482.
- Heresco-Levy U, Kremer I, Javitt DC, Goichman R, Reshef A, Blararu M *et al* (2002b). Pilot-controlled trial of D-cycloserine for the treatment of post-traumatic stress disorder. *Int J Neuropsychopharmacol* 5: 301–307.
- Heresco-Levy U, Javitt DC, Gelfin Y, Gorelik E, Bar M, Blararu M *et al* (2006). Controlled trial of D-cycloserine adjuvant therapy

- for treatment-resistant major depressive disorder. *J Affect Disord* 93: 239–243.
- Huang YY, Colino A, Selig DK, Malenka RC (1992). The influence of prior synaptic activity on the induction of long-term potentiation. *Science* 255: 730–733.
- Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62: 617–627.
- Kew JN, Kemp JA (1998). An allosteric interaction between the NMDA receptor polyamine and ifenprodil sites in rat cultured cortical neurones. *J Physiol* 512(Pt 1): 17–28.
- Kroes RA, Panksepp J, Burgdorf J, Otto NJ, Moskal JR (2006). Modeling depression: social dominance-submission gene expression patterns in rat neocortex. *Neuroscience* 137: 37–49.
- Langer G, Karazman R, Neumark J, Saletu B, Schönbeck G, Grünberger J et al (1995). Isoflurane narcosurgery in depressive patients refractory to conventional antidepressant drug treatment. A double-blind comparison with electroconvulsive treatment. *Neuropsychobiology* 31: 182–194.
- Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405: 955–959.
- Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M et al (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* 329: 959–964.
- Machado-Vieira R, Manji HK, Zarate CA (2009). The role of the tripartite glutamatergic synapse in the pathophysiology and therapeutics of mood disorders. *Neuroscientist* 15: 525–539.
- Maeng S, Zarate CA Jr., Du J, Schloesser RJ, McCammon J, Chen G et al (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* 63: 349–352.
- Malinow R, Malenka RC (2002). AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* 25: 103–126.
- Mathers CD, Loncar D (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 3: e442.
- McShane R, Areosa Sastre A, Minakaran N (2006). Memantine for dementia. *Cochrane Database Syst Rev* CD003154.
- Moskal JR, Kuo AG, Weiss C, Wood PL, O'Connor Hanson A, Kelso S et al (2005). GLYX-13: a monoclonal antibody-derived peptide that acts as an N-methyl-D-aspartate receptor modulator. *Neuropharmacology* 49: 1077–1087.
- Nicholson KL, Balster RL (2009). The discriminative stimulus effects of N-methyl-D-aspartate glycine-site ligands in NMDA antagonist-trained rats. *Psychopharmacology (Berl)* 203: 441–451.
- Nong Y, Huang YQ, Ju W, Kalia LV, Ahmadian G, Wang YT et al (2003). Glycine binding primes NMDA receptor internalization. *Nature* 422: 302–307.
- Oldendorf WH (1970). Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. *Brain Res* 24: 372–376.
- Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I (1999). Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology (Berl)* 147: 162–167.
- Price RB, Nock MK, Charney DS, Mathew SJ (2009). Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol Psychiatry* 66: 522–526.
- Rofael HZ, Abdel-Rahman MS (2002). The role of ketamine on plasma cocaine pharmacokinetics in rat. *Toxicol Lett* 129: 167–176.
- Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R (1996). Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. *Pharmacopsychiatry* 29: 23–26.
- Skolnick P, Popik P, Trullas R (2009). Glutamate-based antidepressants: 20 years on. *Trends Pharmacol Sci* 30: 563–569.
- Song I, Huganir RL (2002). Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci* 25: 578–588.
- Stanton PK, Potter PE, Aguilar J, Decandia M, Moskal JR (2009). Neuroprotection by a novel NMDAR functional glycine site partial agonist, GLYX-13. *Neuroreport* 20: 1193–1197.
- Stringer JL, Greenfield LJ, Hackett JT, Guyenet PG (1983). Blockade of long-term potentiation by phencyclidine and sigma opiates in the hippocampus in vivo and in vitro. *Brain Res* 280: 127–138.
- Thompson LT, Moskal JR, Disterhoft JF (1992). Hippocampus-dependent learning facilitated by a monoclonal antibody or D-cycloserine. *Nature* 359: 638–641.
- Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK et al (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev* 62: 405–496.
- Van Gelder RN, von Zastrow ME, Yool A, Dement WC, Barchas JD, Eberwine JH (1990). Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc Natl Acad Sci USA* 87: 1663–1667.
- Wesierska M, Macias-Gonzalez R, Bures J (1990). Differential effect of ketamine on the reference and working memory versions of the Morris water maze task. *Behav Neurosci* 104: 74–83.
- Wood PL, Mahmood SA, Moskal JR (2008). Antinociceptive action of GLYX-13: an N-methyl-D-aspartate receptor glycine site partial agonist. *Neuroreport* 19: 1059–1061.
- Yang Q, Liao ZH, Xiao YX, Lin QS, Zhu YS, Li ST (2011). Hippocampal synaptic metaplasticity requires the activation of NR2B-containing NMDA receptors. *Brain Res Bull* 84: 137–143.
- Zarate CA Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA et al (2006a). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry* 63: 856–864.
- Zarate CA Jr., Singh JB, Quiroz JA, De Jesus G, Denicoff KK, Luckenbaugh DA et al (2006b). A double-blind, placebo-controlled study of memantine in the treatment of major depression. *Am J Psychiatry* 163: 153–155.
- Zarate CA Jr., Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A et al (2012). Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. *Biol Psychiatry* 71: 939–946.
- Zhang XL, Sullivan JA, Moskal JR, Stanton PK (2008). A NMDA receptor glycine site partial agonist, GLYX-13, simultaneously enhances LTP and reduces LTD at Schaffer collateral-CA1 synapses in hippocampus. *Neuropharmacology* 55: 1238–1250.