INTRODUCTION

A single, subanesthetic dose of the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine exerts a rapid antidepressant action in patients with depression, and has been associated with a number of adaptations throughout the brain. However, ketamine acts as a psychotomimetic and has abuse potential, so understanding which specific adaptations are contributing to its positive behavioral effects is crucial for safer treatment options for depression. In this study, we propose that GluN2B subunit-containing NMDARs within the bed nucleus of the stria terminals (BNST) contribute to the actions of ketamine in a novelty-induced hypophagia (NIH) paradigm. First, we verified that ketamine, as well as the GluN2B antagonist Ro25–6981, decreased the latency to consume food in a novel environment in a version of the NIH test. We then hypothesized that GluN2B-containing receptors within the BNST may be a target of systemic ketamine and contribute to behavioral effects. Through the combination of a GluN2B-selective mouse line and stereotaxic delivery of lentiviral Cre recombinase, we found that targeted knockdown of this subunit within the BNST mimicked the decrease in affective behavior observed with systemic ketamine or Ro25–6981 in the NIH test. These data suggest a role for GluN2B-containing NMDARs within the BNST in the affective effects of systemic ketamine.

MATERIALS AND METHODS

Animals and treatment

All animals were housed 2–5 per cage and were provided with rodent chow and tap water ad libitum. The temperature- and humidity-controlled animal facilities are maintained on a 12:12-h light:dark cycle (lights on 0600–1800 hours). All experiments took place during the light phase of the cycle.

For ketamine and Ro25–6981 experiments, male wild-type C57Bl/6J mice (Jackson Laboratory, Bar Harbor, ME, USA; 8 weeks) were acclimated to the vivarium for a minimum of 1 week prior to experimentation. Following acclimation, mice were handled for 5 days and given habituating saline injections for the last 3 days of handling. Ketamine (3 mg kg$^{-1}$ body weight, Ketaved; Webster Veterinary Supply, Sterling, MA, USA), Ro 25–6981 maleate (5 mg kg$^{-1}$; Tocris, Minneapolis, MN, USA) and 0.9% saline were administered intraperitoneally (i.p.).

Floxed Grin2b mice were generated by Eric Delpire as previously described, and floxed Nr3c1 (GR) mice were generated by Louis Muglia as previously described. Both lines were bred homozygous for the floxed allele in our facility and underwent surgery (described below) at 1–4 months of age. Prior to behavioral testing, mice were handled for 5 days.

All protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Surgical procedure

Mice were anesthetized with 1.5% isoflurane. Then, 200 nl LV-CRE (UNC Vector Core; Titer = 1.3 $\times$ 10$^{6}$) or LV-GFP (UNC Vector Core; Titer = 1.3 $\times$ 10$^{10}$)
was injected into the dorsal BNST (coordinates: AP: 0.14 mm; L: ±0.88 mm; D: −4.24 mm) at a rate of 50 nl/min using an AngleTwo stereotax at a 15.03° angle to avoid ventricles. Postoperatively, mice were administered ketoprofen (5 mg kg$^{-1}$ subcutaneously) once per day for 72 h, then p.r.n. for 1 week. Mice were given at least 2 weeks recovery time following surgery before behavioral experimentation.

Novelty-induced hypophagia (NIH)

The NIH test consisted of 4 training days followed by a testing day. On all days, mice were given at least 1 h acclimation to the testing room under low red light (~40 lux), and all mice had access to rodent chow throughout behavioral testing. During training, mice were given 30 min per day access to a highly palatable food (liquid Ensure, home-made vanilla shake flavor) in the testing room while group-housed in their home cages under low red light. By the second training day, all mice had consumed Ensure, so no mice were excluded from the study. On the test day in the ketamine and Ro25–6981 study, half of the mice were given a 1-h restraint stress in 50-ml conical tubes, while the other half were allowed to remain in their home cages. Half an hour following the termination of restraint stress, all mice were given i.p. injections of ketamine, Ro25–6981 or equal-volume saline, yielding six groups (no restraint-saline, no restraint-ketamine, no restraint-Ro, restraint-saline, restraint-ketamine and restraint-Ro). On the testing day, each mouse was transferred to an individual novel cage devoid of bedding under bright lighting (~200 lux) immediately prior to 30 min Ensure access. Cages were cleaned with 30% EtOH before and after each animal.

Data and statistical analysis

Latency (s) to the first sip of Ensure and amount (g) consumed were measured in the NIH test. For ketamine and Ro25–6981 studies, statistical significance was calculated via two-way analysis of variance (ANOVA) for treatment x restraint with a post-hoc Bonferroni multiple comparison test in the NIH test. For all studies using transgenic animals, statistical significance was calculated via t-test. All data were analyzed using GraphPad Prism 5 (La Jolla, CA, USA). Data are represented as mean ± s.e.m. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

We first validated a version of the NIH test for analysis of the antidepressant efficacy of NMDAR manipulation. In brief, the latency to consume food in a novel environment is quantified as a measure of the affective state. Previous studies used chronically stressed rodents and/or food restriction in similar paradigms. We used a protocol similar to that described by Dulawa and Hen, in which satiated mice seek a highly palatable food reward. This test, thus, is thought to rely on hedonic drive to consume rather than hunger, which may be more closely aligned with depression-associated anhedonia.

Mice received ketamine (3 mg kg$^{-1}$, i.p.), Ro25–6981 (5 mg kg$^{-1}$, i.p.) or equivalent volume saline for half an hour following a 1-h restraint stressor or no stress. The amount of Ensure consumed and the latency to consume the first sip of Ensure were measured, with increased latency indicating increased negative affective behavior. We found that ketamine and Ro25–6981 each significantly reduced this latency ($F_{(2, 72)} = 12.46$, $P < 0.0001$) in mice, regardless of whether the mice had been previously treated with a 1-h restraint stressor or not (Figure 1a). No difference in consumption was observed with ketamine or Ro25–6981 administration (Supplementary Figure 1). We examined the effect of Figure 1. Viral deletion of GluN2B from the bed nucleus of the stria terminalis (BNST) phenocopies systemic treatment with ketamine or Ro25–6981. (a) Timeline above. Decreased latencies with systemic ketamine (3 mg kg$^{-1}$, i.p.), Ro25–6981 (5 mg kg$^{-1}$, i.p.) or equivalent volume saline for half an hour following a 1-h restraint stressor or no stress. The amount of Ensure consumed and the latency to consume the first sip of Ensure were measured, with increased latency indicating increased negative affective behavior. We found that ketamine and Ro25–6981 each significantly reduced this latency ($F_{(2, 72)} = 12.46$, $P < 0.0001$) in mice, regardless of whether the mice had been previously treated with a 1-h restraint stressor or not (Figure 1a). No difference in consumption was observed with ketamine or Ro25–6981 administration (Supplementary Figure 1). We examined the effect of
 Knockdown of BNST GluN2B-containing NMDA receptors

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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**REFERENCES**

1. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS et al. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* 2000; 47: 351–354.
2. Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H et al. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol Psychiatry* 2011; 69: 754–761.
3. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng FF et al. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* 2011; 475: 91–95.
4. Sink KS, Walker DL, Freeman SM, Flandreau EI, Ressler KJ, Davis M. Effects of continuously enhanced corticotropin releasing factor expression within the bed nucleus of the stria terminals on conditioned and unconditioned anxiety. *Mol Psychiatry* 2013; 18: 308–319.
5. Wills TA, Klug JR, Silberman Y, Baucum AJ, Weitlauf C, Colbsan RJ et al. GluN2B subunit deletion reveals key role in acute and chronic ethanol sensitivity of glutamate synapses in bed nucleus of the stria terminals. *Proc Natl Acad Sci USA* 2012; 109: E278–E287.
6. Regev L, Neufeld-Cohen A, Tsoory M, Kuperman Y, Getselter D, Gil S et al. Prolonged and site-specific over-expression of corticotropin-releasing factor reveals differential roles for extended amygdala nuclei in emotional regulation. *Mol Psychiatry* 2011; 16: 714–728.
7. Kim SY, Adhikari A, Lee SY, Marshel JH, Kim CK, Mallory CS et al. Diverging neural pathways assemble a behavioural state from separable features in anxiety. *Nature* 2013; 496: 219–223.
8. Bigman JL, Wright T, Talani G, Prasad-Mulcare S, Jinde S, Sebold GK et al. Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. *J Neurosci* 2010; 30: 4590–4600.
9. Brewer JA, Kbor B, Vogt SK, Muglia LM, Fujiwara H, Haegele KE et al. T-cell glucocorticoid receptor is required to suppress COX-2-mediated lethal immune activation. *Nat Med* 2003; 9: 1318–1322.
10. Dulawa SC, Hen R. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neuropsychopharmacology* 2005; 29: 771–783.
11. Kaiser M, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ et al. Predicting new molecular targets for known drugs. *Nature* 2009; 462: 175–181.
12. Kolber BJ, Roberts MS, Howell MP, Wozniak DF, Sands MS, Muglia LJ. Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. *Proc Natl Acad Sci USA* 2008; 105: 12004–12009.

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