OXFORD

International Journal of Neuropsychopharmacology (2022) 25(8): 688–698

https://doi.org/10.1093/ijnp/pyac035
Advance Access Publication: June 23, 2022
Regular Research Article

REGULAR RESEARCH ARTICLE

Positive Allosteric Modulation of α5-GABA$_A$ Receptors Reverses Stress-Induced Alterations in Dopamine System Function and Prepulse Inhibition of Startle

Alexandra M. McCoy, Thomas D. Prevot, Md Yenus Mian, James M. Cook, Alan Frazer, Etienne L. Sibille, Flavia R. Carreno, Daniel J. Lodge

Department of Pharmacology and Center for Biomedical Neuroscience, UT Health San Antonio, San Antonio, Texas, USA (Mrs McCoy, Dr Frazer, Dr Carreno, and Dr Lodge); South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, Texas, USA (Mrs McCoy, Dr Frazer, Dr Carreno, and Dr Lodge); Campbell Family Mental Health Research Institute of CAMH, Toronto, ON, Canada (Drs Prevot and Sibille); Department of Psychiatry, University of Toronto, Toronto, ON, Canada (Drs Prevot and Sibille); Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA (Dr Mian); Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada (Dr Sibille).

Correspondence: Alexandra M. McCoy, BS, 7703 Floyd Curl Drive, MC 7764, San Antonio, TX, 78229, USA (mccoya1@livemail.uthscsa.edu).

Abstract

Background: Up to 64% of patients diagnosed with posttraumatic stress disorder (PTSD) experience psychosis, likely attributable to aberrant dopamine neuron activity. We have previously demonstrated that positive allosteric modulators of α5-GABA$_A$Rs can selectively decrease hippocampal activity and reverse psychosis-like physiological and behavioral alterations in a rodent model used to study schizophrenia; however, whether this approach translates to a PTSD model remains to be elucidated.
Methods: We utilized a 2-day inescapable foot shock (IS) procedure to induce stress-related pathophysiology in male Sprague-Dawley rats. We evaluated the effects of intra-ventral hippocampus (vHipp) administration GL-II-73, an α5-GABA$_A$R, or viral overexpression of the α5 subunit, using in vivo electrophysiology and behavioral measures in control and IS-treated rats.
Results: IS significantly increased ventral tegmental area dopamine neuron population activity, or the number of dopamine neurons firing spontaneously (n = 6; P = .016), consistent with observation in multiple rodent models used to study psychosis. IS also induced deficits in sensorimotor gating, as measured by reduced prepulse inhibition of startle (n = 12; P = .039). Interestingly, intra-vHipp administration of GL-II-73 completely reversed IS-induced increases in dopamine neuron population activity (n = 6; P = .024) and deficits in prepulse inhibition (n = 8; P = .025), whereas viral overexpression of the α5 subunit in the vHipp was not effective.
Conclusions: Our results demonstrate that pharmacological intervention augmenting α5-GABA$_A$R function, but not α5 overexpression in itself, can reverse stress-induced deficits related to PTSD in a rodent model, providing a potential site of therapeutic intervention to treat comorbid psychosis in PTSD.

Keywords: PTSD, psychosis, α5-GABA$_A$ receptor, ventral hippocampus, positive allosteric modulators

Received: March 4, 2022; **Revised:** May 24, 2022; **Accepted:** June 21, 2022

Significance Statement

Patients with posttraumatic stress disorder (PTSD) often experience psychosis (hallucinations and delusions); however, treatments remain inadequate. This study examined a novel therapeutic approach in a rodent model of stress-related pathophysiology associated with PTSD. Specifically, decreasing ventral hippocampal activity with a novel α5-$GABA_A$ receptor-positive allosteric modulator (GL-II-73) reversed electrophysiological (abnormal dopamine neuron activity) and behavioral (deficits in sensorimotor gating) alterations associated with psychosis. These results suggest that the α5-$GABA_A$ receptors in the ventral hippocampus may be a novel site of intervention for the treatment of psychosis in PTSD.

Introduction

Approximately 8% of the United States general population and 20% of US combat veterans are diagnosed with posttraumatic stress disorder (PTSD), a mental health condition that develops after exposure to a traumatic event (Kilpatrick et al., 2003, 2013). Core symptoms of PTSD can include hyperarousal, avoidance of trauma-related stimuli, cognitive deficits, and sleep dysfunction (Braakman et al., 2009; Battle, 2013), along with the relatively high (approximately 64% patients) comorbid presentation of psychosis symptoms, including hallucinations and delusions (Battle, 2013; Hardy and Mueser, 2017; Compean and Hamner, 2019). Undesirable side effects and limited efficacy of antipsychotic medications often lead to discontinuation of treatment (Lieberman et al., 2005). Therefore, it is critical to understand the pathophysiology of psychosis associated with PTSD to develop more effective therapies.

Imaging studies have provided evidence that dopamine signaling is positively correlated with psychosis, contributing to the long-standing dopamine hypothesis of psychosis (Laruelle and Abi-Dargham, 1999; Howes et al., 2009). Accordingly, antipsychotics (D2-like receptor antagonists) reduce symptoms of psychosis in some patients; however, they are associated with severe side effects, such as metabolic disorders and dyskinesias (Lieberman et al., 2005). To avoid side effects associated with direct modulation of dopamine receptors, targeting upstream brain regions that modulate dopamine neuron activity may provide a more favorable therapeutic approach (Lodge and Grace, 2006b, 2007, 2011). One region of interest known to modulate dopamine neuron activity is the ventral hippocampus (vHipp). This area of the hippocampus is a stress-sensitive region that has been implicated in the pathology of both psychosis (Lodge and Grace, 2007, 2011) and PTSD (Joshi et al., 2020). In rodent studies, pathological activation of the hippocampus can drive aberrant dopamine system function via a multi-synaptic pathway and induce psychosis-like deficits (Lodge and Grace, 2006b, 2007; Perez and Lodge, 2018). Therefore, targeting aberrant hippocampal activity may represent a novel approach for the treatment of psychosis. Furthermore, targeting hippocampal activity may also reverse PTSD-related deficits attributable to pathology in other brain regions (e.g., the thalamus) (Perez and Lodge, 2018).

In both psychosis patients and animal models, hippocampal hyperactivity is suggested to be attributed to loss of gamma aminobutyric acid (GABA) interneuron function (Zhang and Reynolds, 2002; Lodge et al., 2009; Konradi et al., 2011; Perez and Lodge, 2013; Perez et al., 2014, 2019; Heckers and Konradi, 2015). A loss of interneurons may also contribute to decreased hippocampal volumes, one of the most consistent neurological changes observed in PTSD patients (Bremner et al., 1995, 1997, 2003; Stein et al., 1997; Gilbertson et al., 2002; Villarreal et al., 2002). Further, hippocampal volume is negatively correlated with the severity of core PTSD symptoms as well as dissociation severity (Stein et al., 1997; Gilbertson et al., 2002; Villarreal et al., 2002; Bremner et al., 2003). A large postmortem study reported significant downregulation of interneuron-related transcripts in the brains of PTSD participants (Girgenti et al., 2021). Similarly, stress vulnerability has been associated with decreased levels of GABA-related transcripts (Banasr et al., 2017; Sun et al., 2018), whereas increased expression of specific GABA receptors in the hippocampus appears to confer stress resiliency (Ardi et al., 2016; Sun et al., 2018). Additionally, there is substantial evidence to suggest that stress-related deficits in mood and cognition may be attributable to dysfunction of specific interneuron subtypes. Interneurons expressing the neuropeptide somatostatin appear to be vulnerable in stress-related disorders (Lin and Sibille, 2013; Prévot and Sibille, 2020; Tomoda et al., 2022), with reductions in mRNA and protein being noted in postmortem brains of depressed patients (Tripp et al., 2011; Guilloux et al., 2012; Seney et al., 2015). Likewise, silencing somatostatin interneurons induces depressive- and anxiety-like behaviors that can be reversed by administration of a $GABA_A$ receptor-positive allosteric modulator (PAM) (Fee et al., 2021). Based on this evidence, we posit that hippocampal hyperactivity in psychosis is related to deficits in GABAergic function and thus represents a potential site for therapeutic intervention. Indeed, we have repeatedly demonstrated that decreasing activity in the vHipp can reverse psychosis-like alterations in dopamine neuron activity and behavior in a rodent model used to study schizophrenia-like pathophysiology (Perez et al., 2013; Donegan et al., 2017, 2019; Perez and Lodge, 2018); however, whether this approach extends to psychosis in models of stress-induced pathology remains to be established.

Hippocampal activity can be modulated by targeting $GABA_A$ receptors that contain the α5 subunit (α5-$GABA_A$Rs). $GABA_A$Rs typically consist of 2 alpha (α1-6), 2 beta (β1-3), and 1 gamma (γ1-3) or delta (δ) subunits, although 19 genes for $GABA_A$Rs subunits have been identified (Simon et al., 2004; Jacob, 2019). The subunit composition of $GABA_A$Rs confers distinct functional properties as well as unique patterns of expression throughout the brain. Of interest, α5-$GABA_A$Rs are enriched in the hippocampus, where they make up nearly 25% of all hippocampal $GABA_A$Rs (Sur et al., 1999; Olsen and Sieghart, 2009). Further, within the hippocampus, there is higher α5 expression in ventral vs dorsal subregions, making it an ideal target to manipulate activity in the vHipp (Fritschy and Mohler, 1995; Sur et al., 1999). Deficits in α5 expression have been noted in animal models of psychosis (Kiemes et al., 2021), and knockdown of α5-$GABA_A$Rs in otherwise healthy animals has been shown to induce psychosis-like deficits in sensorimotor gating, as measured by prepulse inhibition (PPI) (Hauser et al., 2005). Furthermore, overexpression of the α5 subunit in the vHipp (Donegan et al., 2019) or administration of an α5-$GABA_A$R PAM (Gill et al., 2011; Perez et al., 2022) can reverse dopamine system dysfunction and behavioral deficits associated with psychosis in a model used to study schizophrenia. These studies provide preclinical evidence that targeting α5-$GABA_A$Rs may represent an effective treatment for psychosis. Although aberrant dopamine neuron activity and deficits in PPI are also observed in animal models used to study

PTSD (Elam et al., 2021), the underlying pathology likely differs and whether the efficacy of therapeutic approaches is consistent across diagnosis is not currently known. Nonetheless, we posit that augmenting hippocampal interneuron function by targeting α5-GABA$_A$Rs will correct this deficit. In this study, we investigated if pharmacological increase in function or genetic augmentation of vHipp α5-GABA$_A$R expression could reverse inescapable foot shock (IS)- induced alterations in dopamine system function and deficits in sensorimotor gating and whether IS altered vHipp α5-GABA$_A$R expression. We found that intra-vHipp administration of GL-II-73, a novel α5-GABA$_A$R PAM, reversed the electrophysiological and behavioral effects of IS, whereas vHipp overexpression of α5-GABA$_A$Rs was ineffective. IS exposure had no effect on the expression of α5-GABA$_A$Rs in the vHipp. The results of this study provide evidence that pharmacological targeting of α5-GABA$_A$Rs may represent a novel therapeutic strategy for the treatment of PTSD and comorbid psychosis.

MATERIALS AND METHODS

All experiments were performed in accordance with the guidelines outlined in the USPH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and the Use Committees of UT Health San Antonio and US Department of Veterans Affairs.

Animals

Adult, male Sprague Dawley rats (350–400 g; Envigo, Indianapolis, IN, USA) were used for all experiments. Rats were group housed to avoid social isolation stress and were kept on a 12-hour-light/-dark cycle. Food and water were provided ad libitum.

Inescapable Foot Shock Stress

Adult rats were randomly assigned to control (no shock) or shock groups who received 2 consecutive days of inescapable foot shock stress. The 2-day IS paradigm consists of rats placed in a 30.5 × 25.4 × 30.5 cm^3 square conditioning chamber with a stainless-steel grid shock floor (Coulbourn Instruments, Whitehall, PA, USA). One session of IS consisted of 60 × 15-second, 0.8-mA foot shocks with an average inter-trial interval of 30 seconds with a 25% deviation (±7.5 seconds) and lasts approximately 40 minutes. Control rats were handled daily but not exposed to conditioning chambers. Rats were further assigned to specific treatment groups (n = 5–6 electrophysiology, n = 8–12 PPI). Electrophysiology and behavioral experiments were conducted 24–48 hours after the last day of IS.

Materials

The proprietary compound GL-II-73 was supplied by the Centre for Addiction and Mental Health, Campbell Family Mental Health Research Institute (Toronto, ON, Canada). GL-II-73 or vehicle (85% H_20, 14% propylene glycol, 1% Tween80) was administered directly into the vHipp (100 ng/uL; 0.75 μL; AP −5.3, ML ± 5.0, DV −6.0 mm from bregma) 20 minutes prior to electrophysiology or behavior. This dose and timing was selected based on previous characterization (Prevot et al., 2019) as well as our own data (Perez et al., 2022). Although systemic administration would be a more therapeutically relevant approach, we chose intra-vHipp administration because rats seem to rapidly metabolize this compound (Prevot et al., unpublished observations). Importantly, this effect appears to be exclusive to rats, because GL-II-73 can be administered systemically to mice and achieves sufficient brain concentrations to produce robust behavioral effects (Prevot et al., 2019). Chloral hydrate (C8383), propylene glycol (P4347), and Tween80 (P1754) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Intra-Hippocampal Viral Administration

To overexpress the α5 subunit of the GABA$_A$ receptor under the control of the CAMKII promoter, lentiviral vectors were used as previously described (Donegan et al., 2019). In brief, a single cohort of rats was anesthetized using Fluriso (2–5% isoflurane, USP with oxygen flow at 1 L/min) and placed on a stereotaxic apparatus. Bilateral cannula lowered into the vHipp (AP −5.3, ML ± 5.0, DV −6.0 mm from bregma) were used to inject the α5 lentiviral vector expressing enhanced green fluorescent protein (EGFP) as a reporter (pLV-CaMKII-rGabrα5-IRES-EGFP; 2.86 × 10^9 TU/mL) or the control vector that expressed the reporter (pLV-EGFP:T2A:puro-EF1A > mCherry; 1.26 × 10^9 TU/mL) into each hemisphere (0.75 μL; VectorBuilder, Chicago, IL, USA) using an injector that extended 1 mm past the guide cannula. Coordinates were selected based on an atlas (Paxinos and Watson, 1988). Animals were allowed to recover for >6 weeks before behavioral and electrophysiological experiments to allow maximal gene expression.

Verification of Electrode and Cannula Placement

To verify electrode and cannula placement, rats were killed via chloral hydrate overdose and rapidly decapitated at completion of all experiments. Brains were extracted, fixed for at least 24 hours (4% phosphate buffered formaldehyde), and cryoprotected (10% w/v sucrose in phosphate-buffered saline) until saturated. Brains were coronally sectioned (20–25 μm) using a cryostat (Leica, Buffalo Grove, IL, USA). Sections containing electrode or cannula tracks were mounted onto gelatin-coated slides, stained with neutral red (0.1%) and thionin acetate (0.01%), and cover-slipped with DPX Mountant for histochemical confirmation within the VTA or vHipp (Fig 1A, B). For overexpression studies, images were captured on an upright fluorescent microscope (Zeiss, AxioLab. A1; Oberkochen, Germany), and stable transgene expression was confirmed in a subset of animals by green fluorescent protein (GFP)+ fluorescence in vHipp pyramidal cells (Fig 1C).

In Vivo Extracellular Dopamine Recordings

Adult rats were anesthetized with 8% chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus for the duration of the experiment (no longer than 3 hours). A maximum of 2 rats were recorded each day. Extracellular glass microelectrodes (impedance approximately 6–10 MΩ) were lowered into the VTA (from bregma: AP −5.3, ML ±0.6, and DV −6.5 to −9.0 mm) using a hydraulic micro-positioner (Model 640, Kopf Instruments, Tujunga, CA, USA). Spontaneously active dopamine neurons were identified using open filter settings (low-frequency cutoff: 30 Hz; high-frequency cutoff: 30 kHz) according to previously established electrophysiological criteria (Ungless and Grace, 2012). Three parameters of dopamine activity were measured and analyzed: the number of dopamine neurons firing spontaneously per track (population activity) (Lodge and Grace, 2011), firing rate, and proportion of action potentials occurring in bursts (defined as the incidence of spikes with <80 ms between them; termination of the burst is defined as >180 ms between spikes). Whereas population activity can be altered via the hippocampus (Lodge and Grace, 2007, 2011; Perez and Lodge, 2018; Donegan et al., 2019), changes in burst firing and firing rate are primarily mediated by other VTA afferents (Floresco et al., 2003; Lodge and Grace,

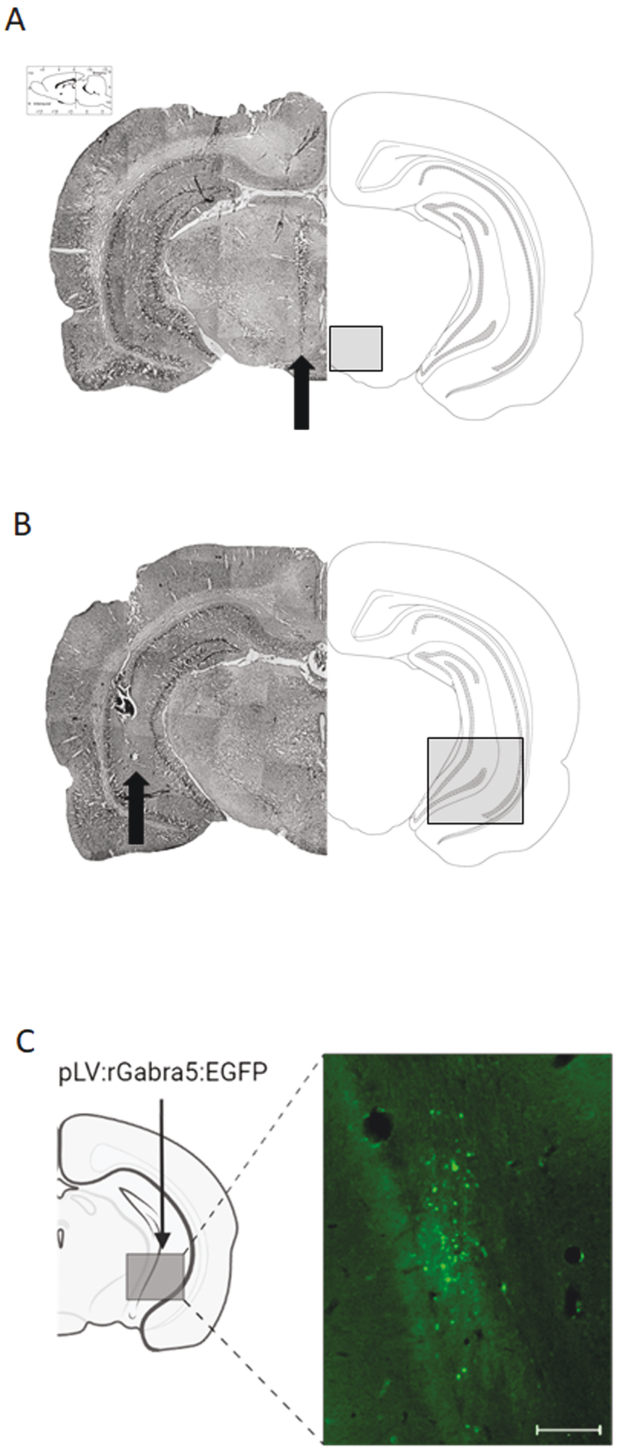

Figure 1. Verification of electrode and cannula placement and stable transgene expression. Representative brain slice with electrode placement in the ventral tegmental area (VTA) (A, left) and cannula placement for drug administration in the ventral hippocampus (vHipp) (B, left) indicated by the black arrow, with corresponding schematics of the brain sections (–5.40 mm posterior to bregma) with a box indicating the area in which tracks were found (right). Representative image containing a schematic of a brain slice (left –5.40 mm from bregma) with a box indicating the area in the vHipp near the tip of the cannula track where green fluorescent protein (GFP)-expressing pyramidal neurons were found (C, right, –5.40 mm from bregma). Scale bar denotes 50 μm.

2006a; Grace and Gomes, 2019). Analysis of dopamine neuron activity was performed using LabChart software (ADInstruments, Sydney, Australia).

Prepulse Inhibition of Startle

Adults rats were placed into a sound-attenuated chamber (SD Instruments) and allowed to acclimate to 65-dB background noise for 5 minutes. Rats were then exposed to 10 startle-only trials (40 ms, 120 dB, 15-second average inter-trial intervals). Next, rats were exposed to 24 trials where a pre-pulse (20 ms at 69, 73, and 81 dB) was presented 100 ms before the startle pulse. Each prepulse + startle pulse trial was presented 6 times in a pseudo-random order (15-second average inter-trial interval). The startle response was measured from 10 to 80 milliseconds after the onset of the startle pulse and recorded using SR-LAB Analysis Software (SD Instruments, San Diego, CA, USA); the entire test lasted approximately 15 minutes. Two cohorts of rats were used: 1 for drug experiments and 1 for virus experiments. All comparisons were made within cohorts (i.e., control groups were not combined).

Western Blot

Twenty-four hours after the last day of IS, brains (n = 9/group) were extracted and punches containing the vHipp were sonicated in lysis buffer and the proteins in the total lysate were separated in an sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel followed by blotting onto a 0.2-μm nitrocellulose membrane. Membranes were incubated with an antibody against α5-$GABA_A$Rs (UC Davis/NIH NeuroMab Facility, Davis, CA, USA 75–401; 1:1000 overnight at 4°C) and washed with Tris-buffered saline with 0.1% Tween 20 (TBST) prior to incubation with the secondary antibody (goat anti-mouse Sigma-Aldrich A9917; 1:30,000) for 1 hour at room temperature. Membranes were washed with TBST and incubated with a chemiluminescence kit (Pierce, Rockford, IL, USA) followed by exposure to X-ray film for detection. Antibodies were stripped using a commercially available stripping buffer (Thermo Scientific, Waltham, MA, USA), washed, blocked, and re-probed with an antibody against horseradish peroxidase (HRP)-conjugated β-actin (1:200 000 in 1% bovine serum albumin (BSA) in TBST, Sigma-Aldrich A3854). Densitometry analyses of immunoreactive bands for α5-$GABA_A$Rs or β-actin (loading control) were performed using the NIH Image J software from the scanned films. Densitometric arbitrary units obtained for α5-$GABA_A$Rs immunoreactive bands were normalized to β-actin and expressed as percent from no shock control.

Statistical Analysis

In all figures, data are shown as mean ± SEM, and n values representing either the number of rats or neurons are indicated in the figure legend. In all experiments, data were analyzed using SigmaPlot (Systat Software Inc., Chicago, IL, USA) either with a 2-way ANOVA (electrophysiology) or 3-way ANOVA (PPI) or t test (western blot). When significant main effects or interactions were detected, Holm–Sidak post-hoc tests were used. All tests were 2-tailed, and significance was determined at $P < .05$. Graphs were plotted with Prism software (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Intra-vHipp Administration of GL-II-73 Reversed Shock-Induced Increases in VTA Dopamine Neuron Population Activity

In rats, changes in mesolimbic dopamine activity can be directly examined using in vivo extracellular electrophysiology to quantify the number of spontaneously active dopamine neurons in the VTA (population activity). Following exposure

to 2 days of IS, a significant increase in dopamine neuron activity was observed. Specifically, a main effect of shock and drug were observed (Fig 2A; 2-way ANOVA; factors: shock × drug; $F_{(1,19)\ shock}=7.024$; $P=.016$; $F_{(1,19)\ drug}=7.258$; $P=.014$; $F_{(1,19)\ interaction}=4.149$; $P=.056$). Shocked animals treated with vehicle displayed a significantly higher number of spontaneously active dopaminergic cells per track compared with nonshocked, vehicle-treated animals (Holm–Sidak; $t=3.238$, $P=.026$, control/vehicle: 0.97 ± 0.07 cells per track; shock/vehicle: 1.68 ± 0.30 cells per track). This shock-induced increase in dopamine neuron activity was completely reversed by the intra-hippocampal administration of GL-II-73 (100 ng/μL; 0.75 μL) 20 minutes prior to electrophysiology (Holm–Sidak; $t=3.268$, $P=.024$, 0.96 ± 0.09 cells per track). There was no significant effect of GL-II-73 on population activity in nonshocked rats (Holm–Sidak; $t=0.476$, $P=.998$, 0.87 ± 0.10 cells per track). Two additional parameters of dopamine cell activity were examined: firing rate and percentage bursting. As expected, no significant differences were observed in average firing

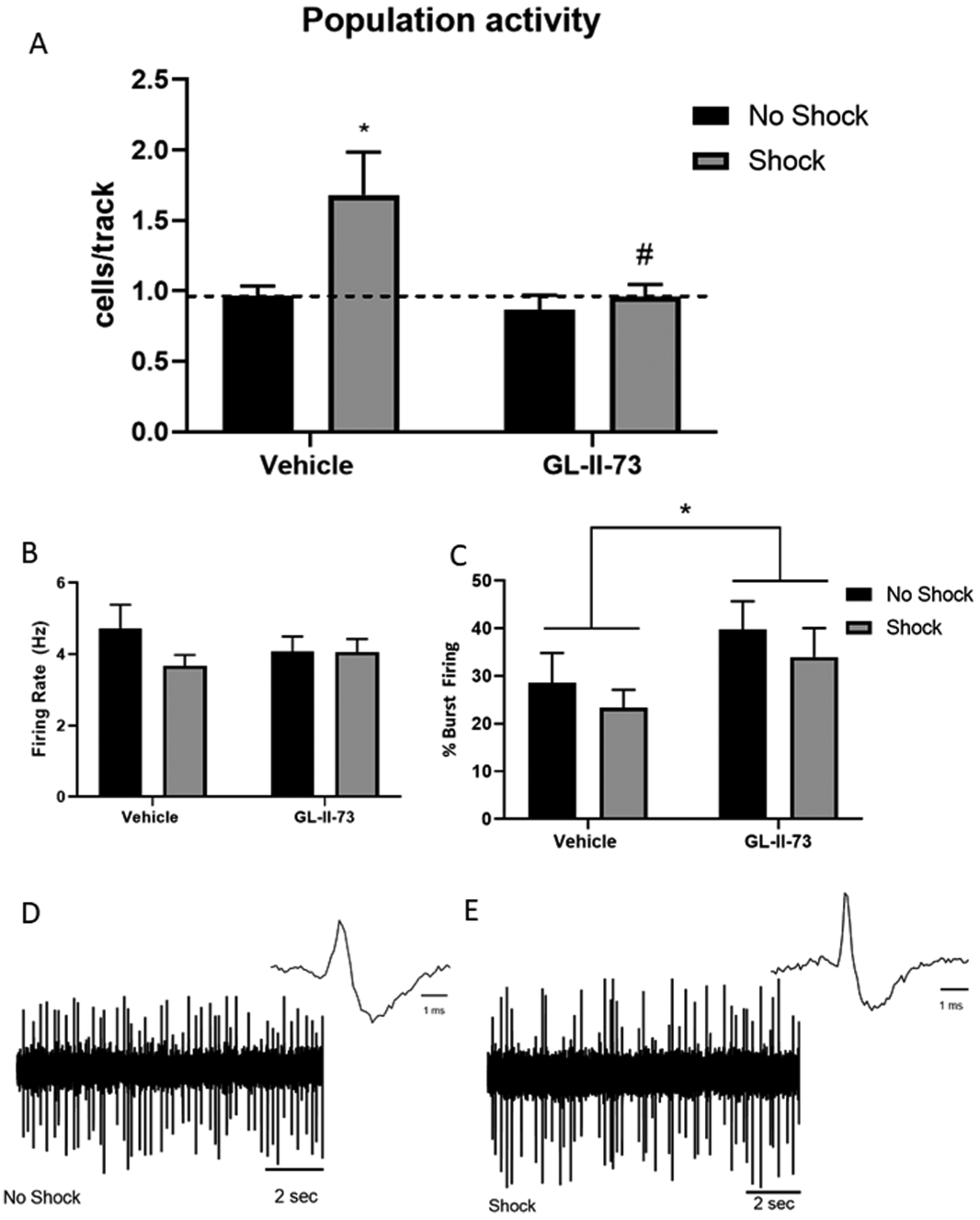

Figure 2. Direct intra-ventral hippocampus (vHipp) administration of the α5-selective positive allosteric modulator (PAM), GL-II-73, completely restored dopamine system function in shocked rats. In vivo extracellular electrophysiology was used to measure dopamine cell activity in the ventral tegmental area. (A) Inescapable shock exposure significantly increased the number of spontaneously active cells/track (population activity), which was reversed by intra-ventral hippocampus injection of GL-II-73 (100 ng/μL; 0.75 μL). (B) Firing rate was unaffected by shock or drug treatment; however, (C) there was a small effect of GL-II-73 on burst firing. Approximately 10-second example traces from control (D) or shocked (E) rats (bottom) and individual action potentials (top). *$P<.05$, significance from respective nonshocked control; #$P<.05$, significance from shock/vehicle group, n = 5–6/group.

rate (Fig 2B; 2-way ANOVA; factors: shock × drug; $F_{(1, 91)\ drug} = 0.075$, $P = .785$; $F_{(1, 91)\ shock} = 1.417$, $P = .237$; $F_{(1, 91)\ interaction} = 1.320$, $P = .254$). There was a main effect of GL-II-73 on percentage bursting (Fig 2C; 2-way ANOVA; factors: shock × drug; $F_{(1, 87)\ drug} = 4.000$, $P = .049$; $F_{(1, 87)\ shock} = 1.027$, $P = .314$; $F_{(1, 87)\ interaction} < 0.003$, $P = .958$); however, post-hoc analysis revealed no significant differences between individual groups (shock/vehicle: 23.36 ± 3.75%; shock/GL-II-73: 33.99 ± 6.09%; control/vehicle: 28.61 ± 6.24%; control/GL-II-73: 39.81 ± 5.86%). The lack of robust effects of GL-II-73 on burst firing and firing rate are expected because, unlike VTA population activity, which can be altered via the hippocampus (Lodge and Grace, 2007, 2011; Perez and Lodge, 2018; Donegan et al., 2019), changes in burst firing and firing rate are primarily mediated by other VTA afferents, such as the laterodorsal tegmental nucleus and pedunculopontine tegmental nucleus (Floresco et al., 2003; Lodge and Grace, 2006a; Grace and Gomes, 2019) (Fig. 2D,E).

GL-II-73 Reversed Shock-Induced Deficits in PPI

Consistent with previous reports (Elam et al., 2021), IS induced a deficit in PPI (Fig. 3; 3-way mixed ANOVA; factors: shock × drug × pre-pulse intensity; $F_{(1, 33)\ shock} = 1.431$; $P = .240$; $F_{(1, 33)\ drug} = 1.159$; $P = .2894$; $F_{(2, 66)\ interaction} = 5.543$; $P = .025$). Interestingly, this shock-induced deficit in sensorimotor gating was completely reversed at 73 dB by the intra-hippocampal administration of GL-II-73 (Holm–Sidak; $t = 3.546$, $P < .001$). There was no significant effect of GL-II-73 on PPI in nonshocked animals (Holm–Sidak; $t = 1.240$, $P = .218$).

Overexpression of α5 in the vHipp Did Not Alter Dopamine Population Activity

We confirmed stable transgene expression in the vHipp by the presence of GFP-labeled cells in the pyramidal cell layer of the vHipp, similar to previous characterizations of this approach (Donegan et al., 2019) (Fig. 1C). Consistent with our previous findings (Elam et al., 2021) and data from vehicle-treated rats (Fig. 2A), inescapable shock significantly increased VTA dopamine neuron population activity, but there was no effect of α5-subunit overexpression (Fig. 4A; 2-way ANOVA; factors: shock × virus; $F_{(1,23)\ shock=} 22.33$; $P < 0.0001$; $F_{(1,23)\ virus} = 2.092$; $P = .162$; $F_{(1,23)\ interaction} = 0.4849$; $P = .485$). Post hoc tests revealed a significant difference between control/nonshocked and control/shock groups (Holm–Sidak; $t = 3.018$, $P = .024$; control/nonshocked: 0.98 ± 0.05 cells per track; control/shock: 1.56 ± 0.19 cells per track) and between α5/nonshocked and α5/shock groups (Holm–Sidak: $t = 3.641$, $P = .007$; α5/nonshocked: 1.08 ± 0.11 cells per track; α5/shock: 1.87 ± 0.20 cells per track). There was no effect of α5-subunit overexpression on firing rate (Fig. 4B; 2-way ANOVA; factors: stress × virus; $F_{(1,203)\ shock} = 1.776$; $P = .185$; $F_{(1,203)\ virus} = 1.445$; $P = .231$; $F_{(1,203)\ interaction} = 0.011$; $P = .918$; control/GFP: 3.98 ± 0.32 Hz; stress/GFP: 3.65 ± 0.21 Hz; control/α5: 3.68 ± 0.32 Hz; stress/α5: 3.29 ± 0.23 Hz). There was a main effect of shock on percentage bursting (Fig. 4C; 2-way ANOVA; factors: stress × virus; $F_{(1,202)\ shock} = 5.388$; $P = .021$; $F_{(1,202)\ virus} = 0.982$; $P = .323$; $F_{(1,202)\ interaction} = 0.049$; $P = .824$), though post-hoc analysis revealed no significant differences between groups (control/GFP: 30.82 ± 3.38%; shock/GFP: 23.61 ± 2.93%; control/α5: 35.00 ± 4.68 %; shock/α5: 26.25 ± 3.00 %). These results demonstrate that viral overexpression of α5-$GABA_A$R is insufficient to reverse aberrant dopamine neuron activity following shock exposure.

α5 Overexpression Did Not Alter PPI

Consistent with our earlier results (Fig. 3), a main effect of shock was observed and there was a trend towards a main effect of the virus (Fig. 5; 3-way mixed ANOVA; factors: shock × virus × PPI; $F_{(1,32)\ shock} = 4.653$; $P = .039$; $F_{(1,32)\ virus} = 3.369$; $P = .070$; $F_{(1,32)\ interaction} = 0.021$; $P = .887$), replicating our laboratory's previous finding that IS induces deficits in PPI (Elam et al., 2021). However, post-hoc analysis revealed no relevant statistical differences between individual groups.

Inescapable Shock Did Not Alter α5-$GABA_A$R Expression Within the vHipp

Other rodent models used to study psychosis display deficits in hippocampal α5-$GABA_A$R expression (Kiemes et al., 2021). To determine whether inescapable shock altered total levels of α5-$GABA_A$Rs in the vHipp, we performed western-blot analysis in control and shocked rats and demonstrated that IS did not significantly alter α5-$GABA_A$Rs levels in the vHipp (Fig. 6: t test; $t = 0.469$, $P = .645$).

Discussion

The dopamine hypothesis of psychosis suggests that symptoms of psychosis are attributable to a pathological increase in mesolimbic dopamine transmission. Increasing evidence from our laboratory and others supports the hypothesis that dysregulation of the dopamine system observed in animal models of psychosis is driven by aberrant hippocampal output. Specifically, hippocampal hyperactivity can drive increases in VTA dopamine neuron population activity (Lodge and Grace, 2007, 2011; Perez and Lodge, 2013; Perez et al., 2013; Donegan et al., 2019). Therefore, we examined if reducing hippocampal activity in a rodent model of stress-related psychopathology used to study PTSD could normalize dopamine neuron activity as well as behavioral correlates of psychosis. In the current study, we utilized a 2-day IS procedure, which produces physiological and behavioral changes consistent with those of PTSD (i.e., hyperarousal, anxiety-like behavior, and sleep alterations) (Van Dijken et al., 1992; Pynoos et al., 1996; Pawlyk et al., 2005) to study psychosis-like alterations in physiology and behavior. Consistent with previous work from our laboratory and others, we found that foot shock exposure produced robust changes in dopamine neuron population activity and deficits in PPI (Valenti et al., 2011; Gomes and Grace, 2017; Gomes et al., 2020; Elam et al., 2021). Further, intra-vHipp administration of GL-II-73, a selective α5-$GABA_A$R PAM, reversed the effects of IS on dopamine neuron population activity and PPI, suggesting that α5-$GABA_A$Rs

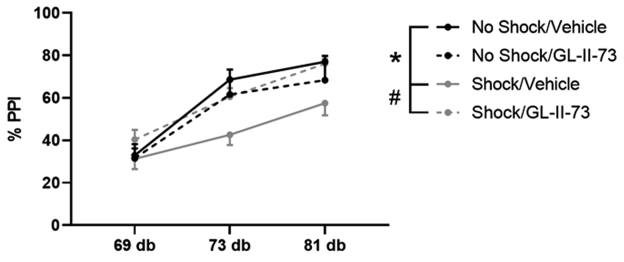

Figure 3. Direct intra-ventral hippocampus (vHipp) administration of GL-II-73 reversed shock-induced deficits in prepulse inhibition of startle. Two days of inescapable shock significantly decreased prepulse inhibition in rats that received vehicle, but not rats that received GL-II-73. *$P < 0.05$, difference between no shock/vehicle and shock/vehicle; #$P < 0.05$, difference between shock/vehicle and shock/GL-II-73, n = 8–12/group.

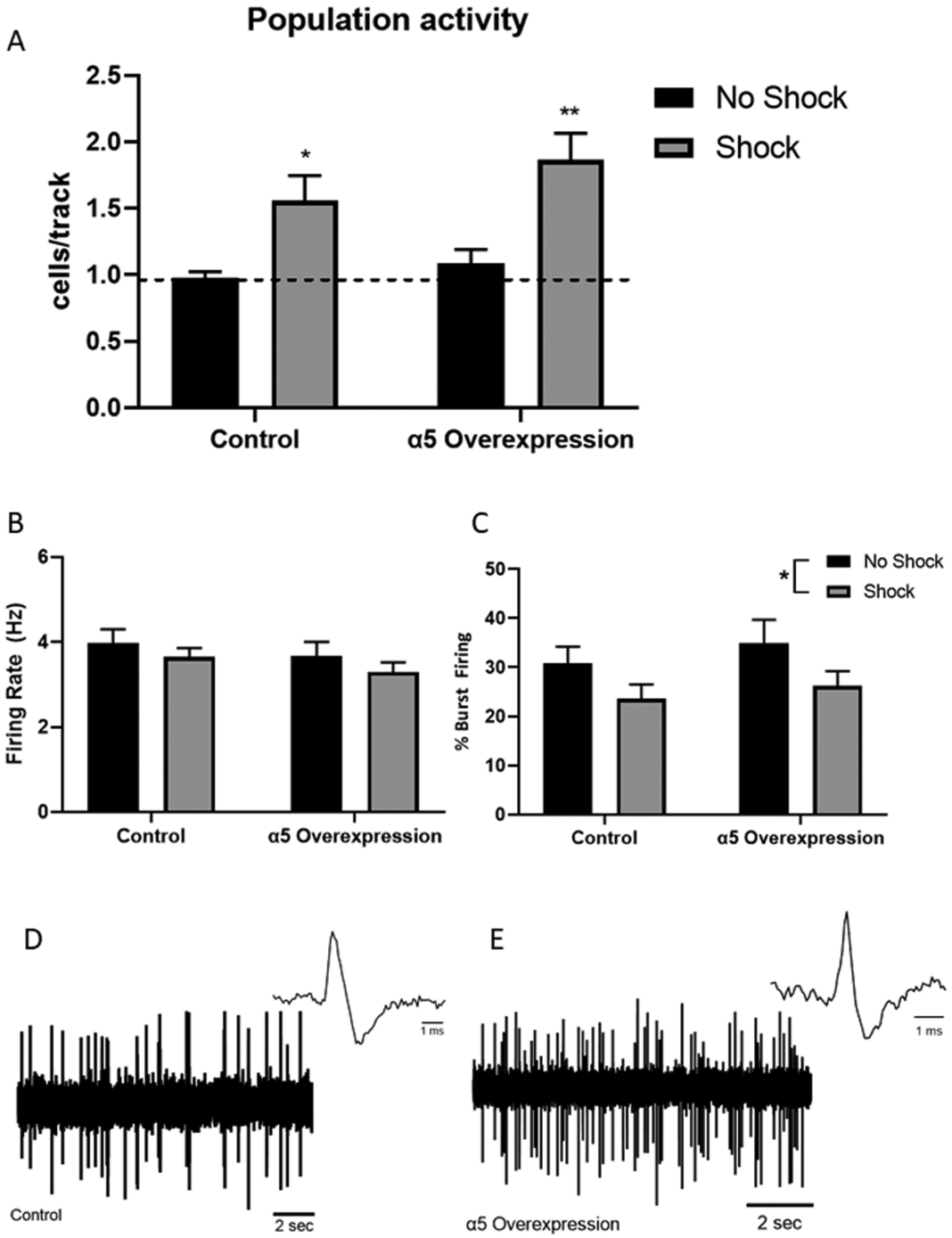

Figure 4. Ventral hippocampus (vHipp) overexpression of the α5 subunit of the $GABA_AR$ failed to restore dopamine system function in stressed rats. (A) Shock exposure significantly increased ventral tegmental area population activity in both control rats and rats with overexpression of the α5 subunit. (B) Firing rate was unaffected by shock or α5 overexpression. (C) A small decrease in burst firing was observed in shocked animals, but there was no effect of virus. Approximately 10-second example traces from control (D) or overexpression (E) rats (bottom) and individual action potentials (top). *$P < .05$; **$P < .01$ denotes significance from nonshocked control, n = 6–8/group.

may be a novel target for treating PTSD and comorbid psychosis. Although administration of GL-II-73 did produce a statistically significant change in the burst firing activity of VTA dopamine neurons, we do not believe this to be physiologically relevant because our previous work demonstrates that multiple α5-$GABA_AR$ PAMs (Perez et al., 2022), or other hippocampal manipulations (Perez et al., 2013; Perez and Lodge, 2013, 2018), do not consistently alter burst firing. Indeed, previous studies have established that burst firing is largely independent of hippocampal activity and mediated by other regions, such as the laterodorsal tegmental nucleus and pedunculopontine tegmental nucleus (Floresco et al., 2003; Lodge and Grace, 2006a; Grace and Gomes, 2019).

The hippocampus, specifically the vHipp, has received considerable attention as a target for treating symptoms of psychosis. Previous studies have demonstrated that the vHipp can regulate dopamine activity in the VTA through a multisynaptic pathway, including the nucleus accumbens and ventral pallidum

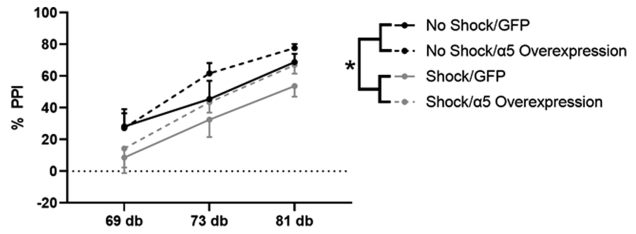

Figure 5. Ventral hippocampus (vHipp) overexpression of the α5 subunit of the $GABA_AR$ failed to reverse deficits in prepulse inhibition of startle. Two days of inescapable shock significantly decreased prepulse inhibition in both control and overexpression rats. There was no effect of α5 overexpression in control or shocked animals. *$P < .05$, main effect of shock, n = 8–10/group.

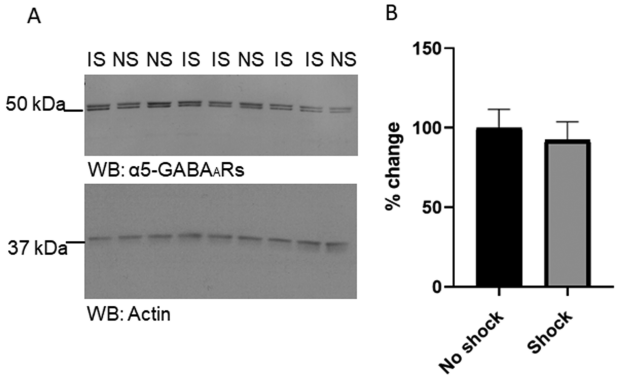

Figure 6. Inescapable shock did not change the α5-$GABA_AR$ levels in the ventral hippocampus (vHipp) . Representative image of western blot (WB) of vHipp α5-$GABA_A$Rs (top) and actin (bottom) immunoreactive bands (A). Quantification (B) revealed no differences between no shock (NS) and inescapable shock (IS) groups, $P = .64$, n = 9/group.

(Lodge and Grace, 2006b, 2007, 2011; Perez and Lodge, 2018). This indirect approach to modulating dopamine neuron activity via the vHipp may provide a more favorable side effect profile compared with currently available antipsychotic drugs, which directly target dopamine D2-like receptors and are associated with severe side effects (Lieberman et al., 2005). Furthermore, it differentially modulates activity in the VTA and substantia nigra, potentially limiting extrapyramidal effects (Bortz and Grace, 2018). In this study, we utilized pharmacological and overexpression approaches to examine whether increasing GABAergic tone to vHipp pyramidal cells can normalize stress-induced, psychosis-like alterations to physiology and behavior. We chose intra-vHipp administration of GL-II-73, a novel PAM selective for α5-$GABA_A$Rs (Prevot et al., 2019), rather than systemic administration for 2 reasons. First, although α5-$GABA_A$Rs are enriched in the hippocampus, they are not exclusively expressed in the hippocampus (Fritschy and Mohler, 1995; Sur et al., 1999), and this method allows for the effects of GL-II-73 to be attributed to α5-$GABA_AR$ in the vHipp specifically. The second reason is that we have discovered, when given to rats systemically, this compound is rapidly metabolized, resulting in insufficient brain concentrations to produce measurable psychoactive effects (Prevot et al., unpublished observations). Importantly, this effect appears to be exclusive to rats, because GL-II-73 can be administered systemically to mice and achieves sufficient brain concentrations to produce robust behavioral effects (Prevot et al., 2019). Here, we demonstrated that intra-vHipp administration of GL-II-73 can reverse aberrant dopamine neuron activity and deficits in PPI observed after IS. These results are consistent with previous work demonstrating that another α5-$GABA_A$ PAM, SH-053-2'F-R-CH3, can reverse aberrant dopamine neuron activity in a rodent model used to study schizophrenia (Gill et al., 2011). Of note, both positive and negative α5-$GABA_AR$ modulators are being developed for clinical applications, including as antidepressants, anxiolytics, and cognitive enhancers (Atack et al., 2006; Collinson et al., 2006; Carreno et al., 2017; Prevot et al., 2019, 2020). This is particularly exciting for the treatment of PTSD because comorbid depression and anxiety as well as cognitive deficits are commonly present in this disorder (Brady et al., 2000; Quinones et al., 2020). In combination with its robust effects on dopamine neuron activity and PPI, it appears that GL-II-73 may be beneficial for treating multiple comorbidities associated with PTSD as well as other psychiatric disorders (Prevot et al., 2019, 2020; Perez et al., 2022).

We previously reported that overexpression of the α5 subunit in the vHipp can correct aberrant dopamine neuron activity and psychosis-like behaviors in a rodent model of schizophrenia (Donegan et al., 2019). As a proof of concept, we tested whether α5 overexpression in the vHipp could reverse shock-induced, psychosis-like alterations in a model of PTSD. Interestingly, overexpression of α5 in the vHipp did not affect dopamine neuron population activity and although there was a trend towards an effect of α5 overexpression on PPI, this was not statistically significant ($P = .07$). This may be due to low transfection; however, we do not believe this to be the case because we observed robust GFP expression in the vHipp. Alternatively, the additional α5 subunits may not have successfully incorporated into functional receptors. Indeed, oligomerization of $GABA_ARs$ is highly inefficient; only approximately 25% of translated subunits incorporate into receptors (Jacob et al., 2008). Even if overexpression produced functional receptors, it has been shown that α5-$GABA_AR$ localization (extrasynaptic vs synaptic) is highly dynamic (Loebrich et al., 2006; Hausrat et al., 2015; Davenport et al., 2021), and overexpression may shunt α5-$GABA_AR$ receptors, the majority of which are found extrasynaptically, into the synapse. This relocation is relevant to the work done here because we previously published data that suggest that targeting extrasynaptic, but not synaptic, $GABA_ARs$ in the vHipp can restore aberrant dopamine system function (Donegan et al., 2019; Perez et al., 2022). Although we did not measure inhibitory currents in the vHipp in this study, we previously observed an increase in tonic inhibition following transfection with the same vector and protocol utilized here, suggesting that this overexpression approach yields functional, extrasynaptic α5-$GABA_ARs$ (Donegan et al., 2019). Thus, the lack of effect of α5 overexpression here is likely due to differences between models used and the divergent pathology underlying developmental and stress-based insults. PTSD is a complex disorder, and numerous brain regions are implicated in its pathology, including, but not limited to, the vHipp (Semple et al., 2000; Shin et al., 2006). Indeed, the role of the paraventricular nucleus of the thalamus (PVT) in PTSD and psychosis is an active area of study (Elam et al., 2021). However, we have also demonstrated that afferents from the vHipp and PVT converge on single neurons within the NAc to regulate dopamine population activity in the VTA (Perez and Lodge, 2018), and regardless of which region is activated, decreasing activity in either the vHipp or PVT can restore dopamine system function. Thus, although the vHipp may not be the primary site of pathology, it may still be a promising site of pharmacologic intervention. A more parsimonious explanation for these results is that receptor overexpression does not address the underlying deficits observed in PTSD. In the methylazoxymethanol acetate (MAM) model used to study psychosis, rats have been shown to have

reduced number of interneurons (Lodge et al., 2009) as well as decreased α5-GABA$_A$R density in the hippocampus (Kiemes et al., 2021), which may account for why α5 overexpression successfully treated dopamine system dysfunction in MAM rats (Donegan et al., 2019). However, aberrant hippocampal function in PTSD may not be due to decreased GABARs (Gilbertson et al., 2002; Villarreal et al., 2002; Bremner et al., 2003; Girgenti et al., 2021). Indeed, we demonstrated that there is no deficit in vHipp α5-GABA$_A$R expression following IS exposure (Fig. 6). Thus, it is not surprising that increasing the number of α5-GABA$_A$Rs was ineffective in this model, although it does raise the question as to why GL-II-73 was effective. Although there may not be a deficit in receptor expression, a low-GABA state that is present in multiple psychiatric conditions (Prévot and Sibille, 2020) may also be present in the IS model, and enhancing response to GABA with a α5-GABA$_A$R may address this pathology if, for example, concentrations of GABA are lower.

In summary, we have demonstrated that GL-II-73, a novel α5-GABA$_A$R PAM, can reverse stress-induced, psychosis-like alterations in a rodent model used to study PTSD. Although α5 overexpression did not successfully reverse deficits associated with our shock model, the results with GL-II-73 demonstrate that pharmacological targeting of α5-GABA$_A$Rs is a useful strategy for treating psychosis. This study adds to the growing literature implicating α5-GABA$_A$Rs as a common therapeutic target for the treatment of psychosis in multiple animal models and provides evidence that GL-II-73 may be a novel compound that can treat psychosis in addition to a range of psychiatric disorders.

Acknowledgments

The authors thank Angela Boley, PhD, for assistance with data acquisition. Some figures were created with BioRender.com.
This work was supported by Merit Awards from the United States Department of Veterans Affairs, Biomedical Laboratory Research (BX004693 and BX004646 to D.J.L.) and Development Service and National Institutes of Health grants (R01-MH090067 to D.J.L. and T32-NS082145 to A.M.M.)

Interest Statement

E.S., T.D.P., and J.C. are co-inventors or listed on US patent applications that cover GABAergic ligands, including GL-II-73, and their use in brain disorders. E.S. is co-founder of DAMONA Pharmaceuticals, a biopharmaceutical company dedicated to treat cognitive deficits in brain disorders.

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