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Hippocampal α5-GABA$_A$ Receptors Modulate Dopamine Neuron Activity in the Rat Ventral Tegmental Area

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ABSTRACT

BACKGROUND: Aberrant dopamine neuron activity is attributable to hyperactivity in hippocampal subfields driving a pathological increase in dopamine neuron activity, which is positively correlated with psychosis in humans. Evidence indicates that hippocampal hyperactivity is due to loss of intrinsic GABAergic (gamma-aminobutyric acidergic) inhibition. We have previously demonstrated that hippocampal GABAergic neurotransmission can be modulated by targeting α5-GABA$_A$ receptors, which are preferentially expressed in hippocampal regions. Positive and negative allosteric modulators of α5-GABA$_A$ receptors (α5-PAMs and α5-NAMs) elicit effects on hippocampal-dependent behaviors. We posited that the selective manipulation of hippocampal inhibition, using α5-PAMs or α5-NAMs, would modulate dopamine activity in control rats. Further, α5-PAMs would reverse aberrant dopamine neuron activity in a rodent model with schizophrenia-related pathophysiologies (methylazoxymethanol acetate [MAM] model).

METHODS: We performed in vivo extracellular recordings of ventral tegmental area dopamine neurons in anesthetized rats to compare the effects of two novel, selective α5-PAMs (GL-II-73, MP-III-022), a nonselective α-PAM (midazolam), and two selective α5-NAMs (L-655,708, TB 21007) in control and MAM-treated male Sprague Dawley rats (n = 5–9).

RESULTS: Systemic or intracranial administration of selective α5-GABA$_A$ receptor modulators regulated dopamine activity. Specifically, both α5-NAMs increased dopamine neuron activity in control rats, whereas GL-II-73, MP-III-022, and L-655,708 attenuated aberrant dopamine neuron activity in MAM-treated rats, an effect mediated by the ventral hippocampus.

CONCLUSIONS: This study demonstrated that α5-GABA$_A$ receptor modulation can regulate dopamine neuron activity under control or abnormal activity, providing additional evidence that α5-PAMs and α5-NAMs may have therapeutic applications in psychosis and other psychiatric diseases where aberrant hippocampal activity is present.

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The pathophysiology of schizophrenia is complex. It has long been hypothesized that positive symptoms (i.e., hallucinations and delusions) are mediated by hyperactivity of the mesolimbic dopamine system (1,2); however, no overt histopathology has been identified in dopamine neurons. Thus, aberrant dopamine system function is likely a consequence of disruptions in upstream brain regions. The hippocampus regulates the activity of dopamine neurons, its structure and function are altered in schizophrenia (3–7), and positive symptoms are correlated with heightened baseline hippocampal activity (3,7,8), together promoting the hippocampus as a putative region mediating aberrant dopamine activity in schizophrenia.

Rodent studies have repeatedly demonstrated that hippocampal hyperactivity drives aberrant dopamine system function via a multisynaptic pathway consisting of the nucleus accumbens, ventral pallidum, and ventral tegmental area (VTA) (9–13). Further, reversing aberrant hippocampal activity using pharmacological (14), neurosurgical, and cell-based approaches in rodent models is successful at restoring dopamine system function as well as behavioral correlates of positive, negative, and cognitive symptoms (11–13,15,16). Together, hippocampal hyperactivity may play a crucial role in the pathophysiology of schizophrenia, such that decreasing hippocampal activity may be a beneficial treatment.

Hippocampal hyperactivity is observed in individuals with schizophrenia as well as in rodent models and is thought to result from deficits in GABAergic (gamma-aminobutyric acidergic) inhibition (11,12,17,18). Postmortem studies from patients with schizophrenia report a loss of specific GABAergic interneuron subtypes in the hippocampus (19,20). These interneurons regulate hippocampal activity, such that decreases in interneuron function led to aberrant pyramidal cell firing (18,21,22) and uncoordinated activity (23,24). Prenatal exposure to methylazoxymethanol acetate (MAM) is frequently used in rodents to induce anatomical, physiological, and behavioral deficits that model those observed in schizophrenia (25,26). A

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selective loss of hippocampal interneuron subtypes and corresponding hippocampal hyperactivity are observed in the MAM model (11,12,24), which can be reversed by transplanting into the hippocampus parvalbumin and somatostatin interneurons, derived from embryonic stem cells or fetal interneuron precursor cells (12,16). Thus, augmenting hippocampal interneuron function may represent a novel therapeutic modality for the symptoms of schizophrenia.

GABAergic control of hippocampal pyramidal neurons occurs via $GABA_A$ receptors ($GABA_A$Rs) and $GABA_B$ receptors ($GABA_B$Rs). $GABA_A$Rs are pentameric, ionotropic chloride channels typically containing a combination of two α, two β, and one γ subunit (27). The subunit composition of $GABA_A$Rs lends to unique expression patterns, with those containing the α5 subunit being highly expressed in the hippocampus, expressed to a much lesser extent in the cortex, and very minimally expressed in subcortical regions (28,29). The enriched distribution of α5-$GABA_A$Rs in the hippocampus makes them an ideal target for the selective manipulation of hippocampal activity. Previous studies have demonstrated that knockdown of the α5-$GABA_A$Rs in the hippocampus can produce deficits in behaviors associated with positive symptoms of schizophrenia, including latent inhibition and prepulse inhibition of startle (30,31). Conversely, overexpression of the α5 subunit of the $GABA_A$ receptor in the ventral hippocampus (vHipp) not only restores dopamine system function in MAM-treated rats, but also alleviates deficits in cognitive flexibility (32). Further, systemic injection of a positive allosteric modulator selective for the α5 subunit of the $GABA_A$ receptor (α5-PAM) (SH-053-2′F-R-CH3) was effective at normalizing dopamine neuron activity and improved dopamine-dependent behaviors in MAM-treated rats (14,33). Although SH-053-2′F-R-CH3 appears to provide the therapeutic benefit of reducing psychotic-like symptoms (14), it impaired performance in a hippocampal-dependent cognitive task and failed to alleviate deficits in social interaction (33). Here, we tested two novel selective α5-PAMs, GL-II-73 and MP-III-022. GL-II-73 has recently been developed and displays pro-cognitive, anxiolytic, and antidepressant-like effects in stressed and old mice (34), suggesting that GL-II-73 differs from other α5-PAMs. MP-III-022 has been shown to exert dose-dependent effects on cognition and social memory (35). Thus, GL-II-73 and MP-III-022 may be beneficial in treating the negative and cognitive symptom domains of schizophrenia, although their effects on dopamine system function have yet to be determined.

Negative allosteric modulators of α5-$GABA_A$Rs (α5-NAMs) were originally developed to serve as cognitive enhancers; however, recent studies have demonstrated multiple uses for these compounds. Specifically, α5-NAMs can improve performance in hippocampal-dependent cognitive tasks (36–39) and exert potent antidepressant-like efficacy in a variety of behavioral assessments (40–42). Pathological decreases in dopamine neuron population activity likely contribute to depressive-like symptoms (43). This idea is supported by the observation that the rapid-acting antidepressant, ketamine, can acutely restore dopamine neuron activity and synaptic plasticity in the hippocampus in a rodent model of helplessness (44). Therefore, it stands that increasing hippocampal activity with an α5-NAM may normalize dopamine system function in models used to study depression.

We posited that α5-PAMs and α5-NAMs modulate dopamine neuron activity through their activity on α5-$GABA_A$Rs in the hippocampus (Figure 1). Here, we performed in vivo extracellular recordings of dopamine neurons in the VTA to investigate the effects of both systemic administration and direct intrahippocampal microinjection of two novel selective α5-PAMs (GL-II-73 and MP-III-022), a nonselective α-PAM (midazolam), and two selective α5-NAMs (L-655,708 and TB 21007) in saline-treated control rats and MAM-treated rats. Gaining a better understanding of how α5-$GABA_A$ receptor modulation can regulate dopamine neuron activity is warranted, as such compounds have multiple potential therapeutic applications, including in schizophrenia and other psychiatric diseases where aberrant hippocampal activity is present.

METHODS AND MATERIALS

All experiments were performed in accordance with the guidelines outlined in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and the Use Committee of UT Health San Antonio.

Animals

To generate rodents displaying circuit-level alterations relevant to psychosis, we administered MAM as previously described (25,26). In brief, timed pregnant female Sprague Dawley rats were obtained from Envigo on gestational day 16 and injected with MAM (22 mg/kg diluted in saline via intraperitoneal route) or saline (1 mL/kg via intraperitoneal route) on gestational day 17. Male pups were weaned on postnatal day 21 and housed with littermates in groups of two or three. All experiments were performed on multiple litters of MAM- and saline-treated rats during adulthood (>8 weeks old; approximately 250–400 g). Male Sprague Dawley rats (>12 weeks old; 350–450 g) were obtained from Envigo and used to perform studies with pharmacological hyperactivation of the vHipp and respective control rats. The doses of PAMs and NAMs used for systemic administration were 1 mg/kg for midazolam , 10 mg/kg for GL-II-73 and MP-III-022, 3 mg/kg for L-655,708, and 0.3 mg/kg for TB 21007 and were administered approximately 20 minutes before in vivo electrophysiology. Doses were chosen based on previously published literature using these compounds (34,40,45,46).

Intrahippocampal Microinjections

Rats were anesthetized with chloral hydrate via intraperitoneal injection before placement in a stereotaxic apparatus (Kopf). A core body temperature of 37 °C was maintained. This anesthetic (chloral hydrate) was used for examination of dopamine neuron physiology, as it does not significantly alter dopamine activity when compared to conscious rats (47). Supplemental anesthesia was administered as required to maintain suppression of the limb withdrawal reflex. Before dopamine neuron electrophysiology, untreated male Sprague Dawley rats were unilaterally injected into the vHipp (anteroposterior +5.3 mm and mediolateral ±5.3 mm from bregma; dorsoventral −7.0 mm ventral of the brain surface) with vehicle (0.5 μL Dulbecco's phosphate-buffered saline) or NMDA (0.75 μg/0.5 μL), followed by an α5 modulator (GL-II-73, MP-III-022,

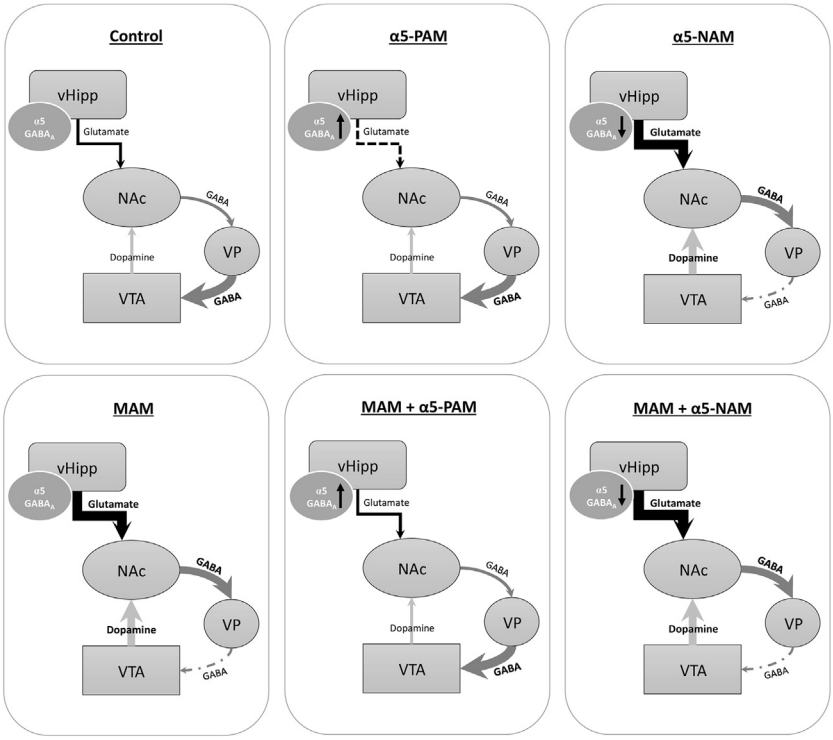

Figure 1. Under normal conditions, the VP exerts tonic inhibitory control of VTA dopamine neurons. Our hypothesis suggested that in the MAM model, increased hippocampal activity drives the NAc, which in turn inhibits the tonic activity of the VP. This results in a loss of GABAergic transmission from the VP to the VTA, which causes an increase in dopamine neuron population activity. NAMs of the α5 receptor cause an increase in hippocampal activity and mimic what is observed in models that display psychosis-related pathologies. In contrast, PAMs of the α5-GABA$_A$ receptor decrease aberrant vHipp activity, thus restoring dopamine system function in models with hippocampal hyperactivity and dopamine system dysfunction. GABAergic, gamma-aminobutyric acidergic; MAM, methylazoxymethanol acetate; NAc, nucleus accumbens; NAM, negative allosteric modulator; PAM, positive allosteric modulator; vHipp, ventral hippocampal; VP, ventral pallidum; VTA, ventral tegmental area.

midazolam, L-655,708, TB 21007; 0.75 μL of 100 ng/μL) or vehicle (0.5 μL; 50% dimethyl sulfoxide in distilled water or 1% Tween 80 [Sigma-Aldrich], 14% propylene glycol, and 85% distilled water) at a rate of approximately 0.5 μL/min. A subset of MAM- and saline-treated rats received an intra-vHipp injection of an α5 modulator or vehicle. Intra-vHipp injections specifically target the ventral CA1/subiculum region of the vHipp, as this region strongly regulates the mesolimbic dopamine system (48,49). Doses were based on pharmacokinetic data obtained with GL-II-73 (34). Dopamine neuron electrophysiology was performed starting approximately 20 minutes after microinjection. Hippocampal microinjections and dopamine recordings were performed bilaterally to minimize the number of experimental animals used.

Dopamine Neuron Electrophysiology

Extracellular glass microelectrodes (impedance 6–10 MΩ) were lowered into the VTA (anteroposterior −5.3 mm and mediolateral ±0.6 mm from bregma; dorsoventral −6.5 to −9.0 mm ventral of the brain surface). Six to 9 vertical passes were made throughout the cell body region of the VTA. Spontaneously active dopamine neurons were identified using the following previously established criteria (50): 1) action potential duration >2 ms and 2) frequency between 0.5 and 15 Hz. The following three parameters of dopamine activity were measured: 1) population activity (the number of spontaneously active dopamine neurons encountered per track); 2) basal firing rate; 3) the proportion of action potentials occurring in bursts (defined as the incidence of spikes with <80 ms between them; termination of the burst is defined by >180 ms between spikes). The same vehicle control rats were used in the PAM and NAM analyses.

Histological Verification

To verify electrode and cannula placement, rats were rapidly decapitated at completion of all experiments. Brains were extracted, fixed for at least 24 hours (4% formaldehyde in saline), and cryoprotected (10% w/v sucrose in phosphate-buffered saline) until saturated. Brains were coronally sectioned (25 μm) using a cryostat (Leica BioSystems). Sections containing electrode or cannula tracks were mounted onto gelatin-chrome alum-coated slides, stained with neutral red (0.1%) and thionin acetate (0.01%) and cover slipped with DPX Mountant (Sigma-Aldrich) for histochemical confirmation within the VTA (electrode) (Figure 2A) or vHipp (cannula) (Figure 2B) with reference to a stereotaxic atlas (51).

Statistical Analysis

Data are represented as the mean ± SEM with *n* values representing the number of animals per experimental group unless otherwise stated. Statistical analyses were performed using SigmaPlot (Systat Software Inc.). Electrophysiological data were analyzed by two-way analysis of variance (strain × treatment) followed by the Holm-Sidak post hoc test. Significance was determined at $p < .05$.

Materials

MAM was purchased from MRIGlobal. Proprietary compounds, MP-III-022 and GL-II-73, were generated by the University of Wisconsin in Milwaukee and supplied by the

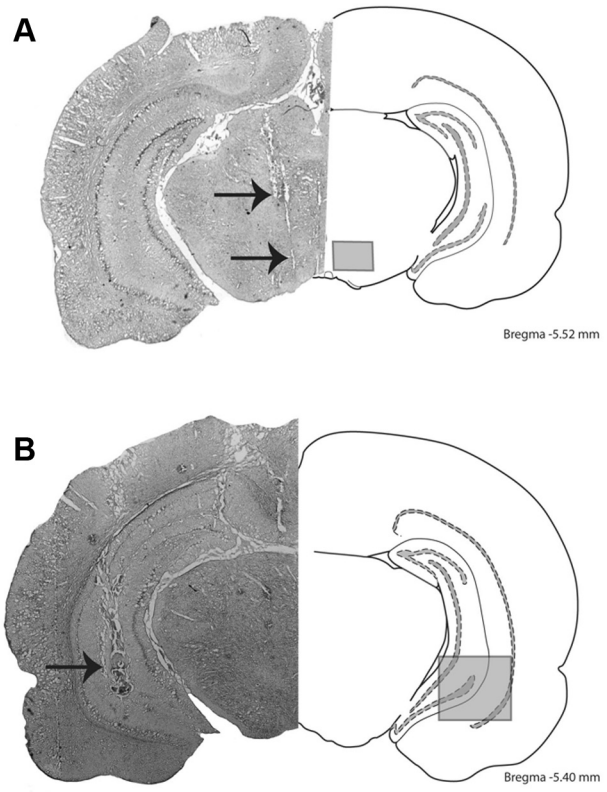

Figure 2. **(A)** Representative brain slice with an electrode track (arrows) in the ventral tegmental area (left) and corresponding schematic of the brain section (right). Gray boxes indicate the area where cannula and electrode tracks were observed. **(B)** Representative brain slice with a cannula track (arrow) in the ventral hippocampus (left) and corresponding schematic of the brain section (right).

Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health. L-655,708 and TB 21007 were purchased from Tocris. Midazolam hydrochloride was obtained from Akorn, Inc. Chloral hydrate, NMDA, propylene glycol, and dimethyl sulfoxide were obtained from Sigma-Aldrich.

RESULTS

Systemic Administration of α5-PAMs and α5-NAMs Exerts Differential Effects on Dopamine Neuron Population Activity in MAM-Treated Rats

Rodent models used to study schizophrenia-related pathologies consistently display aberrant dopamine system function (9,10,12,52). As expected, we observed a significant increase, about a doubling, in dopamine neuron population activity (strain: $F_{1,20} = 101.511$, $p < .001$; treatment: $F_{3,50} = 4.123$, $p = .012$; interaction: $F_{3,50} = 5.538$, $p = .003$) in MAM-treated vehicle rats ($n = 7$, 1.97 ± 0.14) compared with saline-treated vehicle rats ($n = 9$, 0.94 ± 0.05; $t = 7.53$, $p < .001$) (Figure 3A). In saline-treated rats, systemic administration with α5-PAMs GL-II-73 ($n = 6$, 1.05 ± 0.06) and MP-III-022 ($n = 6$, 1.06 ± 0.08) and with the nonselective α-PAM midazolam ($n = 6$, 1.00 ± 0.04) failed to alter population activity (Figure 3A), firing rate (Figure S1A), or bursting (Figure S1B). Aberrant population activity in MAM-treated rats was significantly decreased by systemic injection of MP-III-022 ($n = 6$, 1.29 ± 0.08; $t = 4.67$, $p < .001$); however, GL-II-73 ($n = 6$, 1.96 ± 0.18) and midazolam ($n = 6$, 1.87 ± 0.13) had no effect (Figure 3A). No differences were observed in average firing rate and burst firing pattern between any of the groups treated with α5-PAMs.

Conversely, the α5-NAM, L-655,708, increased population activity (strain: $F_{1,39} = 4.352$, $p = .045$; interaction: $F_{2,39} = 27.244$, $p < .001$) in saline-treated rats ($n = 6$, 1.71 ± 0.16; $t = 4.61$, $p < .001$) and restored normal dopamine system function in MAM-treated rats ($n = 6$, 1.00 ± 0.06; $t = 5.78$, $p < .001$) (Figure 3B). Systemic administration of the α5-NAM, TB 21007, had no effect in saline-treated rats ($n = 6$, 1.24 ± 0.15), but attenuated activity in MAM-treated rats ($n = 6$, 1.56 ± 0.13; $t = 2.48$, $p = .018$). α5-NAMs did not alter firing rate or bursting in saline-treated rats; however, burst firing was significantly increased in MAM-treated rats (Figure S1C, D).

Intra-vHipp Microinjections of α5-PAMs and α5-NAMs Exert Effects Downstream on Dopamine Neuron Population in Saline- and MAM-Treated Rats

Saline-treated rats receiving intra-vHipp vehicle displayed a population activity of 1.01 ± 0.05 ($n = 6$), whereas MAM-treated rats receiving vehicle had a significantly higher population activity of 1.98 ± 0.12 ($n = 6$; $t = 7.76$, $p < .001$; strain: $F_{1,42} = 48.211$, $p < .001$; treatment: $F_{3,42} = 6.379$, $p = .001$; interaction: $F_{3,42} = 10.446$, $p < .001$) (Figure 4A). Direct intra-vHipp microinjections of α5-PAMs had no effect in saline-treated rats (GL-II-73: $n = 5$, 1.14 ± 0.06; MP-III-022: $n = 6$, 1.06 ± 0.06; midazolam: $n = 5$, 1.02 ± 0.13), consistent with systemic administration; however, GL-II-73 completely reversed the elevated population activity in MAM-treated rats (GL-II-73: $n = 5$, 1.10 ± 0.12; $t = 6.681$; $p < .001$) (Figure 4A). To a smaller extent, MP-III-022 and midazolam also significantly attenuated the population activity in MAM-treated rats (MP-III-022: $n = 5$, 1.64 ± 0.12; $t = 2.597$, $p = .04$; midazolam: $n = 5$, 1.37 ± 0.13; $t = 4.664$, $p < .001$). Only relatively small effects were observed on firing rate and bursting (Figure S2A, C).

Intra-vHipp microinjection of α5-NAMs (treatment: $F_{1,31} = 3.424$, $p = .048$; interaction: $F_{5,74} = 31.362$; $p < .001$) (Figure 4B) elicited a significant increase in population activity in saline-treated rats (L-655,708: $n = 5$, 1.66 ± 0.14; $t = 4.514$, $p < .001$; TB 21007: $n = 5$, 1.61 ± 0.09; $t = 4.193$, $p < .001$). Both α5-NAMs produced a significant reduction, albeit to a different extent, in population activity of MAM-treated rats (L-655,708: $n = 5$, 1.03 ± 0.12; $t = 6.553$, $p < .001$; TB 21007: $n = 5$, 1.64 ± 0.09; $t = 2.368$, $p = .026$). Only relatively small effects were observed on firing rate and bursting (Figure S2B, D).

Intra-vHipp Administration of α5-PAMs and α5-NAMs Alters Dopamine System Function After Pharmacological Activation of vHipp

Using a model of pharmacological hyperactivation of the vHipp, via infusion of NMDA, we observed a significant

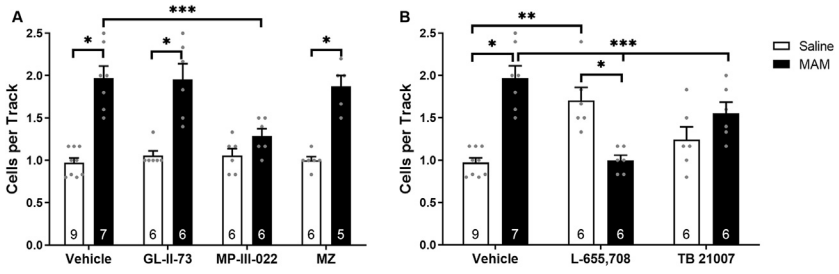

Figure 3. Dopamine neuron population activity can be modulated by systemic administration of positive and negative allosteric modulators of α5-$GABA_A$ receptors. **(A)** Population activity (average number of spontaneously active dopamine neurons per electrode track) is significantly higher in MAM-treated rats, which is reversed by systemic administration of the selective α5-$GABA_A$ positive allosteric modulator, MP-III-022. **(B)** Systemic administration of the α5-$GABA_A$ negative allosteric modulator, L-655,708, restored normal dopamine system function in MAM-treated rats and increased dopamine activity in saline-treated rats. In contrast, the α5-$GABA_A$ negative allosteric modulator, TB 21007, had no effect in saline-treated rats and only attenuated population activity in MAM-treated rats. *$p < .05$ denotes significance from respective saline-treated control; ***$p < .05$ denotes significance from MAM-treated vehicle group; **$p < .05$ denotes significance from saline-treated vehicle group. $GABA_A$, gamma-aminobutyric acid A; MAM, methylazoxymethanol acetate; MZ, midazolam.

increase in dopamine neuron activity (n = 6, 2.03 ± 0.18; t = 5.55, $p < .001$) (Figure 5A) compared with vehicle (n = 6, 1.04 ± 0.10; strain: $F_{1,47}$ = 3.869, p = .056; treatment: $F_{3,47}$ = 4.967, p = .005; interaction: $F_{3,47}$ = 18.594, $p < .001$). GL-II-73 caused a significant increase in population activity in vehicle rats (n = 6, 1.78 ± 0.12; t = 4.12, $p < .001$); that was not observed with MP-III-022 (n = 6, 1.08 ± 0.07) or midazolam (n = 6, 0.94 ± 0.06). Further, both GL-II-73 (n = 6, 0.97 ± 0.03; t = 5.94, $p < .001$) and MP-III-022 (n = 6, 1.08 ± 0.08; t = 5.35, $p < .001$) completely reversed the increase in population activity observed following NMDA activation of the vHipp, whereas midazolam had a modest effect (n = 6, 1.47 ± 0.24; t = 3.17, p = .012).

No significant differences were observed following either α5-NAM in vehicle rats (L-655,708: n = 6, 1.38 ± 0.11; TB 21007: n = 6, 1.51 ± 0.14; strain: $F_{1,35}$ = 5.733, p = .023; treatment: $F_{2,35}$ = 4.247, p = .024; interaction $F_{2,35}$ = 8.474, p = .001) (Figure 5B). L-655,708 caused a significant decrease in population activity (n = 6, 1.03 ± 0.09; t = 4.357, $p < .001$) following NMDA-induced activation of the vHipp, but no change was observed after administration of TB 21007 (n = 6, 1.82 ± 0.28) (Figure 5B). No differences in firing rate or burst firing were observed (Figure S3).

DISCUSSION

There is increasing evidence that aberrant dopamine system function observed in individuals with schizophrenia may be secondary to hippocampal hyperactivity (8). Also, preclinical studies demonstrate the ability of the vHipp to modulate the activity of dopamine neurons in rodent models (9,12,53). Therefore, exploring targets within the hippocampus may represent innovative and efficacious approaches for the treatment of schizophrenia and provide an effective treatment without the side effects observed with conventional antipsychotics (54). Here, we provide evidence that selective pharmacological modulation of vHipp activity, using novel selective α5-$GABA_A$ receptor modulators, can reduce aberrant dopamine neuron activity observed in a model used to study schizophrenia-related pathologies.

Previous studies have demonstrated schizophrenia-like deficits following knockdown of α5-$GABA_A$Rs, specifically in the hippocampus (30,31), whereas overexpression of the $GABA_A$ receptor α5 subunit can reverse deficits in dopamine system function commonly observed in rodent models used to study schizophrenia-related pathologies (32). Previous studies showed that systemic injection of the selective α5-PAM, SH-053-2′F-R-CH3, normalized dopamine system function and improved dopamine-dependent behaviors; however, it was ineffective at resolving behaviors associated with negative symptoms and cognitive deficits (14). In contrast, the selective α5-PAM used in the present study, GL-II-73, has been established as an anxiolytic, antidepressant, and pro-cognitive compound, thus demonstrating therapeutic potential for the treatment of negative symptoms and cognitive decline associated with schizophrenia (34). As shown in this study, when administered directly into the vHipp, GL-II-73 can reverse aberrant VTA dopamine neuron activity implicated in schizophrenia (1,2).

MAM-treated rats exhibit anatomical, physiological, and behavioral deficits consistent with those observed in

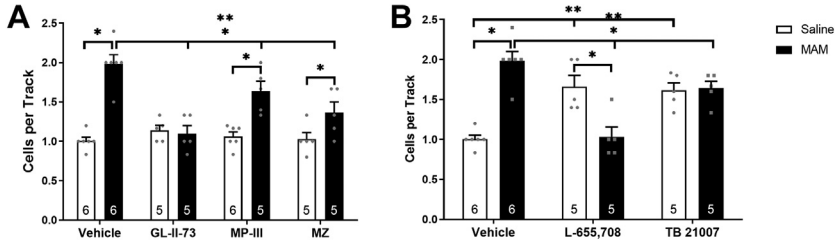

Figure 4. Direct intra–ventral hippocampal administration of positive and negative allosteric modulators of α5-$GABA_A$ receptors can attenuate dopamine neuron population activity in MAM-treated rats. **(A)** The α5-$GABA_A$ positive allosteric modulator, GL-II-73, completely restored dopamine system function, while MP-III-022 and MZ only attenuated activity in MAM-treated rats, with no effects in saline-treated rats. **(B)** Saline-treated rats displayed a significant increase in population activity following intra–ventral hippocampal administration of both α5-$GABA_A$ negative allosteric modulators, L-655,708 and TB 21007. Further, dopamine system function was restored in MAM-treated rats following L-655,708, and population activity was attenuated following TB 21007. *$p < .05$ denotes significance from respective saline-treated control; ***$p < .05$ denotes significance from MAM-treated vehicle group; **$p < .05$ denotes significance from saline-treated vehicle group. $GABA_A$, gamma-aminobutyric acid A; MAM, methylazoxymethanol acetate; MZ, midazolam.

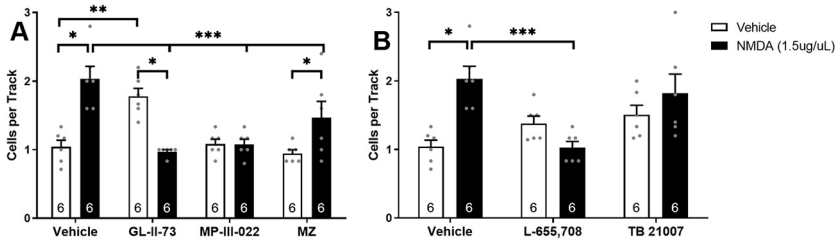

Figure 5. **(A)** Rats with NMDA-induced hippocampal hyperactivity displayed a significant increase in dopamine neuron population activity, which was reversed by intra–ventral hippocampal administration of the specific α5-$GABA_A$ positive allosteric modulators, GL-II-73 and MP-III-022, and attenuated by MZ. Intra–ventral hippocampal GL-II-73 administration caused a significant increase in population activity in vehicle-treated control rats. **(B)** The α5-$GABA_A$ negative allosteric modulator, L-655,708, restored dopamine system function in rats with NMDA-induced hippocampal hyperactivity. $^*p < .05$ denotes significance from respective saline-treated control; $^{***}p < .05$ denotes significance from MAM-treated vehicle group; $^{**}p < .05$ denotes significance from saline-treated vehicle group. $GABA_A$, gamma-aminobutyric acid A; MAM, methylazoxymethanol acetate; MZ, midazolam.

schizophrenia. In these rats, systemic administration of the selective α5-PAM, GL-II-73, did not change dopamine neuron population activity, although MP-III-022 successfully restored dopamine system function. By contrast, after intra-vHipp administration, both GL-II-73 and MP-III-022 were able to modulate dopamine neuron population activity. The difference in effect of GL-II-73 following these two routes of administration is likely associated with its pharmacokinetic profile. GL-II-73 undergoes significant first-pass metabolism in rats (data not shown), resulting in insufficient brain concentrations to produce a detectable effect on population activity. This is specific for rats, as previously published literature demonstrated effects of GL-II-73 in mice when administered systemically (34). Intra-vHipp administration of both GL-II-73 and MP-III-022 reduced the elevated dopamine neuron population activity in MAM-treated rats; however, only GL-II-73 restored dopamine neuron population activity back to control levels, whereas MP-III-022 attenuated it. We suspect the difference in the magnitude of the effect between the α5-selective $GABA_A$ receptor PAMs stems from their differential affinities for the α5 subunit. MP-III-022 has a greater binding affinity and functional potentiation efficacy at α5-$GABA_A$ receptor compared with GL-II-73 but displays an inverted U–shaped dose response relationship (35), which could underlie the results detailed here. In addition, midazolam, a nonspecific α-PAM, was able to attenuate elevated population activity only when given directly into the vHipp and had no effect when administered systemically. Given the widespread effects of benzodiazepines, it is possible that activation of non-α5 receptors opposes the beneficial effects of activation of α5-$GABA_A$Rs and could reflect differences between synaptic and extrasynaptic $GABA_A$Rs (55,56). Indeed, the dichotomy between extrasynaptic and synaptic $GABA_A$Rs has been highlighted in previous studies, in which overexpression of extrasynaptic (α5) receptors in the vHipp restored dopamine system function in MAM-treated rats, whereas overexpression of synaptic (α1) receptors did not (32). Interestingly, α5-$GABA_A$Rs themselves have been found in the synapse (55,56), and recent work has demonstrated that the localization of α5-$GABA_A$Rs is dynamic, such that changes in neuronal activity can cause receptors to shift between the synaptic and extrasynaptic spaces, altering their contribution to tonic and phasic inhibition (57).

Interestingly, α5-NAMs have been shown to produce similar behavioral effects (i.e., pro-cognitive and antidepressant-like) to those observed with α5-PAMs under different experimental conditions (34,40,41,58). Specifically, α5-PAMs produce antidepressant-like effects acutely, whereas the effect of α5-NAMs appear at 24 hours following administration. The reason for this is that α5 modulators restore the excitatory signal-to-noise ratio, albeit by different mechanisms. The α5-PAMs are thought to decrease noise, while the disinhibition produced by α5-NAMs leads to a glutamatergic surge and an augmented signal. Both can support appropriate processing of information, leading to improvements in behavior and reduced symptoms (59). It has been suggested that a delicate balance of excitation and inhibition is necessary for optimal signal transduction and that alterations to α5-$GABA_A$ receptor signaling in either direction may negatively impact cognition (59). Therefore, depending on the pathology, either an NAM or a PAM may be the most appropriate therapeutic intervention to influence dopamine neuron activity. Indeed, in conditions of hippocampal hyperfunction such as schizophrenia (8,9), a PAM may be warranted, whereas under conditions of dopamine hypofunction [e.g., depression (60,61)], an NAM may be a more effective therapeutic to exert vHipp control over dopamine neuron activity in the VTA. However, depression is also associated with reduced cortical inhibition, in which case α5-PAMs seem to provide more beneficial effects than α5-NAMs, in particular on cognition (59), so the contributions of both PAMs and NAMs in complex disease remain to be clarified. As α5-NAMs can increase activity in the hippocampus (40), and activation of the hippocampus is associated with increased dopamine neuron population activity (9–13), we reasoned that α5-NAMs should increase dopamine neuron population activity in control rats. Indeed, when given either systemically or intra-vHipp, L-655,708 increased dopamine neuron population activity in saline-treated control rats, providing further evidence that activation of the vHipp can drive the increases in dopamine neuron population activity. Further, L-655,708, but not TB 21007, decreased population activity in MAM-treated rats. It is unclear why L-655,708 would reverse aberrant dopamine neuron activity in MAM-treated rats, while increasing it in saline-treated rats.

Given the potential for other disruptions associated with prenatal MAM treatment [for review, see (26)], we employed a simplified model where vHipp activity was enhanced pharmacologically by direct microinjection of NMDA into the vHipp. Consistent with previous reports (48), intra-vHipp NMDA administration caused a significant increase in population activity, similar to observations in MAM-treated rats (9). As expected, intra-vHipp administration of α5-NAMs increased dopamine neuron population activity in control

rats receiving intra-vHipp vehicle, albeit to a lesser extent than seen in the control rats for the MAM experiments. This is likely associated with the multiple injections into the vHipp, i.e., both NMDA and the α5-NAMs. In NMDA-treated rats, L-655,708 decreased population activity. No significant effects were present with TB 21007 administration. This mirrored our results in MAM-treated rats, suggesting that in conditions of vHipp hyperactivity, L-655,708, but not TB 21007, is able to restore dopamine system function. The ability of L-655,708 to reverse aberrant dopamine neuron activity was unexpected, but may be due to inherent differences between L-655,708 and TB 21007. L-655,708 has much higher selectivity for α5-$GABA_A$ receptor over other $GABA_A$Rs in terms of affinity compared with TB 21007 (50–100 times and 10 times, respectively). Despite this difference in affinity, TB 21007 is still functionally selective for α5-$GABA_A$Rs based on efficacy (46). L-655,708 and TB 21007 have also been shown to differentially prefer certain α5-$GABA_A$R isoforms based on β subunit composition (62). It is possible that in the context of high hippocampal activity, as seen in MAM-treated or intra-vHipp NMDA-treated rats, these otherwise subtle differences are accentuated, leading to the unanticipated effects of L-655,708 in these groups. The important point then is that as a class of drugs, individual α5-NAMs may not all act the same way.

The potential beneficial effects of α5-PAMs seen in MAM-treated rats were also examined in the NMDA-induced model of hippocampal hyperactivity. These data replicated data observed in MAM-treated rats where intra-vHipp GL-II-73 and MP-III-022 completely reversed aberrant dopamine neuron population activity and midazolam partially restored dopamine system function. These results reinforce the idea that selectively augmenting α5-$GABA_A$ receptor activity in the vHipp can reverse aberrant dopamine neuron activity associated with hippocampal activation. Surprisingly, GL-II-73 caused a significant increase in population activity in vehicle-treated rats. While this effect was robust, we believe it to be spurious, as intra-vHipp injection of GL-II-73 did not increase population activity in control rats in our earlier experiment.

In summary, we have demonstrated that pharmacological manipulation of activity in the vHipp with α5-PAMs and α5-NAMs differentially modulates dopamine neuron population activity in the VTA. Specifically, under conditions of hippocampal hyperactivity, aberrant dopamine system function can be reversed by the administration of α5-PAMs. By demonstrating that multiple α5-PAMs can reverse aberrant dopamine neuron activity, this study provides evidence for potential antipsychotic properties of this class of compounds. Furthermore, it adds schizophrenia as well as other conditions in which hippocampal hyperactivity is present to the growing list of psychiatric disorders for which these compounds may have therapeutic utility. Moreover, α5-PAMs offer added benefit over traditional anxiolytic medications (nonspecific $GABA_A$R PAMs), as their preference for α5 minimizes the undesired side effect of sedation, typically associated with the α1 subunit (63). Specifically, the α5-PAM GL-II-73 can reverse pathological increases in dopamine neuron population activity in the MAM model as well as stress-associated cognitive deficits, anxiety, and some depressive-like behaviors (34), suggesting that this compound may address affective and cognitive deficits observed in schizophrenia in addition to dopamine dysfunction. Indeed, future studies will test the utility of α5-PAMs in other rodent models that display psychosis-related pathologies. Furthermore, we have demonstrated that α5-NAMs can significantly increase baseline dopamine neuron activity in control rats suggesting that these might be a therapeutic approach for conditions associated with dopamine hypofunction. Taken together, we have demonstrated robust hippocampal modulation of dopamine system function by α5-$GABA_A$ receptor allosteric modulators, which may provide a beneficial approach for the treatment of several psychiatric disorders.

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ES, JMC, and TDP are coinventors or listed on U.S. patent applications that cover GABAergic ligands and their use in brain disorders. ES is cofounder of DAMONA Pharmaceuticals, a biopharmaceutical company dedicated to treatment of cognitive deficits in brain disorders. DJL and AF are coinventors of a patent application covering novel analgesics, and DJL has an active collaboration with Sosei-Heptares; these are both unrelated to the work described in the current article. All other authors report no biomedical financial interests or potential conflicts of interest.

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