Activation of the mGlu1 metabotropic glutamate receptor has antipsychotic-like effects and is required for efficacy of M4 muscarinic receptor allosteric modulators

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Received: 29 December 2017 / Revised: 1 June 2018 / Accepted: 28 June 2018 © Springer Nature Limited 2018

Abstract
Recent clinical and preclinical studies suggest that selective activators of the M4 muscarinic acetylcholine receptor have potential as a novel treatment for schizophrenia. M4 activation inhibits striatal dopamine release by mobilizing endocannabinoids, providing a mechanism for local effects on dopamine signaling in the striatum but not in extrastriatal areas. G protein-coupled receptors (GPCRs) typically induce endocannabinoid release through activation of Goq/11-type G proteins whereas M4 transduction occurs through Goi/o-type G proteins. We now report that the ability of M4 to inhibit dopamine release and induce antipsychotic-like effects in animal models is dependent on co-activation of the Goq/11-coupled mGlu1 subtype of metabotropic glutamate (mGlu) receptor. This is especially interesting in light of recent findings that multiple loss of function single nucleotide polymorphisms (SNPs) in the human gene encoding mGlu1 (GRM1) are associated with schizophrenia, and points to GRM1/mGlu1 as a gene within the “druggable genome” that could be targeted for the treatment of schizophrenia. Herein, we report that potentiation of mGlu1 signaling following thalamo-striatal stimulation is sufficient to inhibit striatal dopamine release, and that a novel mGlu1 positive allosteric modulator (PAM) exerts robust antipsychotic-like effects through an endocannabinoid-dependent mechanism. However, unlike M4, mGlu1 does not directly inhibit dopamine D1 receptor signaling and does not reduce motivational responding. Taken together, these findings highlight a novel mechanism of cross talk between mGlu1 and M4 and demonstrate that highly selective mGlu1 PAMs may provide a novel strategy for the treatment of positive symptoms associated with schizophrenia.

Introduction
Currently approved antipsychotics are efficacious in treating positive symptoms of schizophrenia in many patients, however they offer little to no benefit for negative or cognitive symptoms, and are associated with a number of adverse effects [1, 2]. Thus, there is a critical need to develop fundamentally new approaches for treating schizophrenia that provide improved efficacy and induce fewer adverse effects than current medications.

In recent years, intense translational efforts suggest that highly selective positive allosteric modulators (PAMs) of the M4 muscarinic acetylcholine receptor (mAChR) may provide a novel approach for the treatment of schizophrenia. Most notably, a four-week, double-blind, placebo-controlled outcome trial revealed that an M1/M4 subtype-preferring mAChR agonist, xanomeline, produced statistically significant improvements in total Positive and
Negative Syndrome Scale (PANSS) in schizophrenia patients, as well as trends toward improvements in the PANSS positive and negative subscales, and specific domains of cognitive function [3]. Furthermore, xanomeline displays efficacy in animal models that predict clinical efficacy across these three symptom domains [4–6]. Interestingly, the antipsychotic-like effects of xanomeline are absent in M₄ knockout mice [7] and mimicked by administration of highly selective M₄ PAMs [8–11], suggesting that some of the antipsychotic-like effects of xanomeline are mediated by M₄ activation. Finally, genetic studies reveal that single nucleotide polymorphisms (SNPs) of CHRM4, the gene encoding M₄, were associated with an increased risk of developing schizophrenia [12].

Hyperactivity in subcortical dopamine (DA) signaling contributes to the manifestation of positive symptoms in schizophrenia [13, 14] and imaging studies suggest that schizophrenia patients’ display selective increases in DA release in the dorsal striatum, with decreases in extrastriatal DA release [15]. Interestingly, detailed preclinical, cellular, genetic, and optogenetic studies reveal that M₄ PAMs inhibit DA release in the dorsolateral striatum (DLS) by specific actions on M₄ in a subpopulation of spiny projection neurons (SPNs) that express D₁-DA receptors (D₁-SPNs) [11]. Activation of M₄ on D₁-SPNs induces an inhibition of DA release that is dependent upon both synthesis of the endocannabinoid (eCB) 2-α-arachidonoylglycerol (2-AG) and activation of CB₂ receptors located on neighboring DA terminals [11]. The mobilization of 2-AG by M₄ provides a novel mechanism that allows local inhibition of DA signaling in striatal regions that are most critical for positive symptoms of schizophrenia, without inhibiting DA in other regions where DA signaling is already impaired. However, despite this promising profile, activation of M₄ also directly inhibits D₁ signaling in D₁-SPNs through activation of Gαq/11- type G proteins, which inhibits D₁ receptor-mediated activation of adenylyl cyclase [16]. Therefore, M₄ activation could excessively inhibit D₁ relative to D₂ DA receptor signaling, rather than maintaining balanced inhibition of both D₁ and D₂-dependent signaling pathways.

The finding that M₄ activation inhibits DA release through stimulation of 2-AG was somewhat surprising in light of studies showing that G protein-coupled receptors (GPCRs) typically induce eCB release by activation of Gαq/11 and induction of intracellular calcium (Ca²⁺) [17, 18]. M₄ signals through Gαq/11 and does not couple to Gαq/11 or induce Ca²⁺ mobilization in SPNs [11]. This raises the possibility that M₄-induced release of 2-AG and inhibition of DA release may require co-activation of another GPCR that activates Gαq/11 and facilities Ca²⁺ mobilization. If so, this Gαq/11-coupled GPCR could provide a novel target that may be more proximal to eCB synthesis and inhibition of DA release, and may inhibit DA release without altering the balance between D₁ and D₂ signaling pathways.

Based on previous studies, the group I metabotropic glutamate (mGlu) receptors (mGlu₁ and Glu₅), are prime candidates as Gαq/11-coupled GPCRs that may interact with M₄ and inhibit DA release through eCB signaling. Group I mGlu receptors are heavily expressed in striatal SPNs [19, 20], where they couple to Gαq/11 and their signal transduction pathway induces Ca²⁺ mobilization [21–23] and activates eCB signaling [24]. Furthermore, multiple loss of function SNPs in the human gene encoding mGlu₁ (GRM1) are associated with schizophrenia [25, 26], suggesting that mGlu₁ may play a key role in modulating schizophrenia-related circuitry. We now report a series of studies using ex vivo and in vivo cyclic voltammetry, along with genetic and optogenetic approaches, to show that activation of mGlu₁ is critical for M₄-induced reductions in DA release and antipsychotic-like effects in animal models. Furthermore, synaptic or agonist-induced activation of mGlu₁ is sufficient to inhibit DA release and a highly selective mGlu₁ PAM induces robust antipsychotic-like effects, which are dependent on eCB signaling. Interestingly, unlike M₄, mGlu₁ activation does not directly inhibit DA D₁ signaling and does not reduce motivational responding. Collectively, these findings provide strong evidence that mGlu₁ PAMs have robust antipsychotic efficacy and highlight the mGlu₁ receptor as an exciting novel target for the treatment of positive symptoms associated with schizophrenia.

Materials and methods

Animals

Adult, drug-naïve, C57BL/6J mice (Jackson Laboratory; 6–10 weeks old) and CB₂ knockout (KO) mice (Jackson Laboratory; 005786; 8–10 weeks old) were kept on a 12 h light/dark cycle and were tested during the light phase (lights on at 7:00 h). All experiments were approved by the Institutional Animal Care and Use Committee, Vanderbilt University. Detailed materials and methods are provided as Supplementary Information. All efforts were made to minimize the number of animals while maintaining statistical rigor.

Ex vivo fast scan cyclic voltammetry

Striatal DA release was measured as described previously [11] (See Supplemental Methods).
Activation of the mGlu1 metabotropic glutamate receptor has antipsychotic-like effects. Figure 1 shows the time courses of 30 μM Oxo-M-induced inhibition of electrically-evoked striatal DA release alone or in the presence of either 3 μM of the mGlu5 negative allosteric modulator (NAM), MTEP, or the mGlu1 NAM VU0469650 (VU’650). All time-course data are depicted as mean ± SEM.

- Figure 1a: Time courses of 30 μM Oxo-M-induced inhibition of electrically-evoked striatal DA release alone or in the presence of 3 μM MTEP or VU’650.
- Figure 1b: Boxplot summaries depicting the percent inhibition of striatal DA release observed under different conditions at peak time points (n = 6–8; *significant from 30 μM Oxo-M; p < 0.05; one-way ANOVA with Dunnett’s multiple comparison posthoc test).
- Figure 1c: Total locomotor counts are depicted as the mean ± SEM.
- Figure 1d: Averaged data showing percent prepulse inhibition (PPI) observed in WT mice following administration of vehicle (VEH; 10% Tween 80), 4 mg/kg amphetamine (AMP), 10 mg/kg VU0467154 (VU’154), and 60 mg/kg VU0469650 (VU’650; n = 15–20; *p < 0.05, significant difference from AMP/VEH condition; **p < 0.01, significant difference from AMP/VEH condition; one-way ANOVA with Dunnett’s multiple comparison posthoc test). PPI data are depicted as the mean ± SEM.
Whole-cell patch clamp

Slice preparation and recordings were conducted using procedures previously described [16] (See Supplemental Methods).

In vivo fast scan cyclic voltammetry

Mice were anesthetized and immobilized in a stereotaxic apparatus as described previously [27] (See Supplemental Methods). In sum, a twisted, bipolar, stimulating electrode was implanted into the medial forebrain bundle (MFB; mm from bregma, AP: -1.1, ML: ± 1.4; DV: 1.3) and a carbon fiber working electrode into the dorsal striatum (DLS; mm from bregma, AP: + 1.3; ML: ± 2.3, DV: -2.7). Biphasic current pulse (± 450 μA, 60 Hz, 4 ms pulse width) were applied for 2 s to the DA axons in the MFB to evoke DA release in the DLS following administration of vehicle (10% Tween 80, intraperitoneal injection (i.p.)) or the mGlu4 PAM VU6004909 (60 mg/kg, i.p.). Sufficient time (i.e., five minutes) was allowed between stimulations for evoked responses to recover.

Microdialysis

Guide cannulae were implanted into the medial prefrontal cortex (mPFC) and were collected as described previously [10] (see Supplemental Methods). DA in dialysate samples were analyzed by the Vanderbilt University Neurochemistry Core using liquid chromatography (LC)-mass spectrometry. Only animals with accurate probe placement that showed three consecutive stable baseline values (within ±20%) were included in the statistical analysis. Prior to analysis, samples (5 μL) were derivatized with benzoyl chloride [28]. LC was performed on a 2.0 × 50 mm, 1.7 μM particle Acquity BEH C18 column (Waters Corporation, Milford, MA, USA) using a Waters Acquity UPLC. Mobile phase A was 15% aqueous formic acid and mobile phase B was acetonitrile. Samples were separated by a gradient of 98–5% of mobile phase A over 11 min at a flow rate of 0.6 mL/min prior to delivery to a SCIEX 6500+ QTrap mass spectrometer (AB Sciex LLC, Framingham, MA, USA). Chromatograms were analyzed using MultiQuant 3.0.2 Software from SCIEX.

Behavioral tests

Tests for locomotor activity [29], prepulse inhibition (PPI) of the acoustic startle reflex [30], motivated behavior [31], rotarod and TreadScan were conducted, as previously described and outlined in supplemental materials. Randomizations were performed for counter-balanced behavioral assays by alternating mice based on mouse number. Rotarod and TreadScan measures were measured and recorded by a blinded investigator. All other behavioral data was collected by MedAssociates software.

Statistics

When appropriate, data are represented as the mean ± SEM. Statistical analyses were performed using two-tailed Student’s t-test, one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison post hoc test and two-way ANOVA followed by Bonferroni’s post hoc test, as described in the figure legends. Samples sizes are indicated in figure legends. A value of p < 0.05 was considered as statistically significant. All comparisons met the assumptions of the test used, including similar variance between groups being compared. Post hoc power analyses ensured a sufficient number of slices and mice were used.

Results

mGlu1 activation is required for M4-induced reductions in striatal DA release and antipsychotic-like effects

Due to the localization of group I mGlu receptors in D1-SPNs [19, 20, 32] and their well-defined role in modulating SPNs via eCB production [33, 34], we tested the hypothesis that M4-induced reductions in DA release require co-activation of group I mGlu receptors. The effect of the muscarinic acetylcholine receptor (mAChR) agonist oxotremorine-M (Oxo-M) on electrically-evoked DA release in striatal slices was assessed using fast-scan cyclic voltammetry (FSCV) in the absence or presence of selective negative allosteric modulators (NAMs) of individual group I mGlu receptor subtypes. All studies were performed in the presence of the nicotinic acetylcholine receptor antagonist dihydro-β-erythroidine hydrobromide (DHβE, 1 μM). Consistent with previous studies [11], 30 μM Oxo-M induced a sustained M4-mediated inhibition of DA release (Fig. 1a, d). This effect of Oxo-M on DA release was not blocked by the selective mGlu5 NAM, MTEP (3 μM) (Fig. 1b, d), but was significantly attenuated by application of an mGlu1 NAM VU0469650 (3 μM) (46.7 ± 9.02 in the absence and 21.37% ± 3.14 in the presence of VU0469650; one-way ANOVA, p < 0.05; Fig. 1c, d), suggesting that the M4-mediated reduction in DA release is dependent upon mGlu1 activation. Consistent with this, VU0469650 also blocked the inhibition of DA release observed following application of the M4 PAM VU0467154 (rat M4 EC50 = 17.7 nM, inactive at rat M1,2,3,5) [9] with a submaximal concentration of Oxo-M (10 μM) (52% ± 6.07 in the absence and 9.13% ±
2.72 in the presence of VU0469650; students t-test, \( p < 0.01 \); Supplementary Fig. 1A, B).

M₄ PAMs have robust efficacy in multiple animal models of antipsychotic activity, including an ability to attenuate amphetamine-induced hyperlocomotion (AHL) and disruption of pre-pulse inhibition (PPI) [8–10], and these effects are thought to be mediated, at least in part, by inhibition of DA release. Thus, we examined the ability of
mGlu₁ blockade to blunt the efficacy of M₄ PAMs on AHL and PPI. Consistent with previous findings [9–11], VU0467154 (1 mg/kg, intraperitoneal (i.p.)), reversed AHL (Fig. 1e; one-way ANOVA, p < 0.001) and VU0467154 (10 mg/kg) attenuated amphetamine-induced deficits in PPI (Fig. 1f; one-way ANOVA, p < 0.01). Interestingly, the effects of the M₄ PAM on AHL (one-way ANOVA, p < 0.05; Fig. 1e) and PPI (one-way ANOVA, p < 0.01; Fig. 1f) were attenuated by prior administration of the mGlu₁ NAM, VU0469650 (60 mg/kg). In both AHL and PPI, all treatment conditions had similar baseline activity counts (data not shown) and acoustic startle responses were not altered by dosing with amphetamine, VU0467154 or VU0469650 alone or in combination (data not shown). Finally, the effects of M₁/M₄ preferring agonist xenonoline on AHL were also partially attenuated by prior administration of the mGlu₁ NAM, VU0469650 (60 mg/kg) (one-way ANOVA, p < 0.05; Supplemental Fig. 1C, D). Taken together, these findings suggest that mGlu₁ is a critical modulator of the antipsychotic-like effects of M₄ activation.

Activation of mGlu₁ reduces striatal DA release via activation of CB₂ cannabinoid receptors

Previous studies suggest that a non-selective agonist of group I mGlu receptors can inhibit striatal DA release [35]. This raises the possibility that mGlu₁ may not only be required for M₄ PAMs to inhibit DA release, but may be capable of inducing this response in the absence of M₄ agonists. We now report that application of 30 µM DHPG, a group I mGlu receptor agonist, induces a robust inhibition of DA release compared to baseline (25.415% ± 5.856 and −2.576% ± 1.106, respectively; students paired t-test, p < 0.001; Fig. 2a, b) and this response was completely blocked by the mGlu₁ NAM VU0469650 (3 µM) but not by the mGlu₁ NAM MTEP (3 µM) (Fig. 2c, d; one-way ANOVA, p < 0.01). These results suggest that DHPG-induced reductions in striatal DA release are mediated by mGlu₁ activation. To further evaluate the potential role of mGlu₁ on DA release, we determined the effects of two selective mGlu₁ PAMs, Ro-07-11401 [36] and VU6004909 (rat mGlu₁ EC₅₀ = 31 nM, inactive at mGlu₂,₃,₅,₇,₈; EC₅₀ > 10 µM at mGlu₁) [37], on a subthreshold concentration of DHPG (10 µM). In contrast to 30 µM DHPG, application of 10 µM DHPG alone did not produce a significant inhibition in striatal DA release (students t-test, p > 0.05; Fig. 2a, b). However, this concentration of DHPG induced a robust inhibition of DA release when co-applied with either 3 µM Ro-07-11401 (12.180 ± 3.229; students t-test, p < 0.01; Supplemental Fig. 2A, B) or 3 µM VU6004909 (22.748 ± 7.519; students t-test, p < 0.01; Fig. 2e, f). Collectively, these results suggest that activation of mGlu₁ inhibits stimulus-induced DA release in the striatum.

Activation of mGlu₁ generates diacylglycerol, which is converted to 2-AG by the Ca²⁺-dependent enzyme diacylglycerol lipase (DAGL) [38, 39], raising the possibility that mGlu₁-mediated reductions in DA release are similar to M₄-mediated effects in that they are mediated by eCB mobilization and activation of CB₂ receptors [11]. Consistent with this, inhibition of CB₂ receptors via the CB₂ antagonist AM630 (3 µM) significantly blocked DHPG-induced inhibition of DA release (one-way ANOVA, p < 0.001, Fig. 2g; Supplemental Fig. 2C). Furthermore, the effects of DHPG were absent in slices from CB₂ knockout (KO) mice (Fig. 2h; Supplemental Fig. 2D), confirming a critical role for the CB₂ receptor in mediating this response. In contrast to the CB₂ receptor antagonist, bath application of 3 µM AM251, a CB₁ antagonist, did not significantly attenuate 30 µM DHPG-induced reductions in striatal DA release (one-way ANOVA, p > 0.05, Fig. 2g; Supplemental Fig. 2C).

mGlu₁ activation following stimulation of thalamostriatal afferents attenuates striatal DA release

The striatum receives extensive glutamatergic innervation from the cortex [40] and several thalamic nuclei [41]. Both afferent inputs have been reported to modulate striatal DA release [35, 42, 43], however, it is critical to determine whether activation of mGlu₁ by these glutamatergic inputs inhibits DA release, and whether this response is specific to either corticostriatal or thalamo-striatal synapses. To test the hypothesis that endogenous glutamate acts on mGlu₁ to inhibit DA release, we selectively activated corticostriatal or thalamostriatal afferents...
thalamostriatal axons in the striatum by virally expressing ChR2-eYFP in the motor cortex (M1) or the paraventricular nucleus of the thalamus to the dorsal lateral striatum (DLS). In coronal sections containing ChR2-eYFP expression, we recorded electrically evoked DA in the presence of 1 µM DHβE prior to, during, and following optical stimulation (3 Hz, 12.5 min). Stimulation of thalamostriatal afferents produced a 15% inhibition in striatal DA release (Fig. 3b, c), which returned to baseline after terminating optical stimulation.

The mGlu1 NAM VU0469650 (3 µM) attenuated thalamostriatal induced reductions in DA release (students t-test; $p < 0.01$; Fig. 3b, c), whereas antagonists of ionotropic glutamate (iGluRs) and mGlu5 receptors did not inhibit this response (Supplemental Fig. 3A, B). Interestingly, selective activation of corticostriatal afferents produced a 27% sustained inhibition in striatal DA release, but this response was not inhibited by the mGlu1 NAM VU0469650 (Fig. 3e, f). The reduction in striatal DA release observed following

Fig. 3 Activation of mGlu1 following Stimulation of Thalamostriatal Afferents modulates Striatal DA Release. a Schematic of viral injection strategy to target glutamatergic afferents from the paraventricular nucleus (PVT) of the thalamus to the dorsal lateral striatum (DLS). b Time course depicting the effect of 3 Hz optical stimulation of thalamic inputs (black) on electrically-evoked striatal DA release, an effect that is blocked following application of the mGlu1 NAM VU0469650 (VU*650; red). c Boxplot summary depicting the percent inhibition of DA release at the peak time point following stimulation of thalamostriatal afferents ($n = 12–16$, **$p < 0.01$, significant difference from peak inhibition of optical stimulation; students t-test). d Schematic of viral injection strategy to target glutamatergic afferents from the motor cortex (M1) to the dorsal lateral striatum (DLS). e Time course depicting the effect of 3 Hz optical stimulation of cortical inputs to the striatum (black) on electrically-evoked striatal DA release, an effect that is not blocked following application of the mGlu1 NAM VU0469650 (VU*650; red). f Boxplot summary depicting the percent inhibition of DA release at the peak time point following stimulation corticostriatal afferents ($n = 12–16$; not significantly different, students t-test $p > 0.05$)
corticostriatal stimulation was eliminated by incubation with a cocktail of antagonists for other glutamate receptors (Supplemental Fig. 3C, D). Taken together, these findings suggest that mGlu1 inhibits striatal DA release in an input-specific manner and regulates DA release by glutamate from thalamostriatal but not corticostriatal afferents.

The mGlu1 PAM, VU6004909, reduces dorsolateral striatal DA release in vivo and displays antipsychotic efficacy

The finding that mGlu1 activation inhibits striatal DA release raises the possibility that selective mGlu1 PAMs could reduce DA release in vivo and have antipsychotic-like effects. To determine whether a selective mGlu1 PAM [37] reduces striatal DA release, we examined the effects of systemic administration of VU6004909 using FSCV in isoflurane anesthetized mice. DA release was monitored in the dorsolateral striatum (DLS) following electrical stimulation of the medial forebrain bundle (MFB) in animals treated with either vehicle (10% Tween 80) or 60 mg/kg VU6004909 (Fig. 4a–c). Compared to vehicle, VU6004909 significantly reduced striatal DA release 40 min after administration (Fig. 4b), which corresponds to the T_max for this compound [37]. The averaged peak effect, observed 60 minutes after administration, was statistically significant from vehicle-control conditions (students t-test, p < 0.01; Fig. 4c). These results confirm our ex vivo voltammetry studies and indicate that administration of the mGlu1 PAM can reduce striatal DA release in vivo.

Next, we assessed the ability of VU6004909 to exert antipsychotic-like activity in rodent models that are dependent on increased DA transmission and are known to be responsive to antipsychotic agents and M₄ PAMs. Consistent with previous studies [11, 44], amphetamine (4 mg/kg, s.c.) induced a robust decrease in PPI (one-way ANOVA, p < 0.001), an effect that was dose-dependently reversed by pretreatment with 30 mg/kg (p < 0.05) or 60 mg/kg VU6004909 (one-way ANOVA, p < 0.01; Fig. 4d). The highest dose of VU6004909 (60 mg/kg, i.p.) alone had no effect on PPI compared to vehicle (1409.42 ± 102.21 and 1164.19 ± 81.16, respectively; Supplemental Fig. 4A).

Amphetamine (4 mg/kg, s.c.) also produced a significant increase in locomotor activity, and this effect was attenuated by administration of 60 mg/kg VU6004909 (students t-test, p < 0.01; Fig. 4e; Supplemental Fig. 4B). Acute treatment with VU6004909 (60 mg/kg) alone induced a slight, but statistically significant reduction in total spontaneous locomotor activity compared to vehicle-treated animals (students t-test, p < 0.05; Supplemental Fig. 5A, B) but was without effect on cerebellum-dependent motor tasks, such as the rotarod and TreadScan (Supplemental Fig. 5C, D–F, respectively). Interestingly, the ability of VU6004909 to attenuate amphetamine disruptions in PPI (one-way ANOVA, p < 0.05; Supplemental Fig. 6C) and AHL (one-way ANOVA, p < 0.05; Supplemental Fig. 6A, B) were absent following systemic administration of a CB₂ antagonist (AM630). Administration of AM630 alone did not affect basal levels of locomotion or disrupt the startle response (Supplemental Fig. 6A, D, respectively). Together, these findings demonstrate that VU6004909 exerts antipsychotic-like effects, and these effects are dependent on CB₂ activation.

mGlu₁ does not directly inhibit signaling at the DA D₁ receptor and does not impair motivation

In addition to inhibiting DA release, we recently reported that M₄ PAMs directly inhibit D₁-mediated increases in GABA-mediated synaptic responses in terminals of D₁-SPNs in the substantia nigra pars reticulata (SNr), through Gαᵣ₀-dependent inhibition of cAMP formation [16]. Based on these findings, M₄ could induce an excessive inhibition of D₁ relative to D₂ signaling. D₁ plays a crucial role in regulating motor function, cognition, and motivation [16, 45–48], therefore a mechanism that maintains balance of D₁/D₂ signaling may be preferable for the treatment of schizophrenia [49–51]. Since mGlu₁ primarily couples to Gαₒ₁₁ rather than Gαᵣ₀, we would not expect mGlu₁ PAMs to directly inhibit D₁ signaling in SNr SPN terminals. To test this hypothesis, we performed whole-cell patch clamp recordings from GABAergic cells of the SNr and assessed the effects of an mGlu₁ PAM on miniature inhibitory postsynaptic currents (mIPSCs). As previously reported [16], the D₁ agonist SKF82958 (10 μM) induced a leftward shift in mIPSC cumulative probability plots with a ~40% increase in mIPSC frequency compared to baseline (Supplemental Fig. 7A–C). In contrast to effects of M₄ PAMs [16], pretreatment with the mGlu₁ PAM VU6004909 (3 or 10 μM) did not attenuate the effect of SKF82958 on mIPSCs (Supplemental Fig. 7A–C), suggesting that mGlu₁ activation does not inhibit effects of D₁ agonists on mIPSC frequency. Furthermore, in contrast to M₄ PAMs [16], VU6004909 did not attenuate SKF82958 (1 mg/kg, i.p.) induced increases in locomotor activity (Supplemental Fig. 7D, E) (students t-test, p > 0.05). Taken together, these results suggest that mGlu₁ PAMs do not directly inhibit effects of direct-acting D₁-receptor agonists.

Since reductions in DA D₁ signaling have been implicated in reduced motivation [48, 52, 53], we determined the effects of the mGlu₁ PAM (VU6004909), M₄ PAM (VU0467154), and the typical antipsychotic haloperidol on motivational responding in a traditional progressive ratio (PR) schedule [31], where rodents need to nose poke for a 30% Strawberry Ensure solution (Fig. 5a). We assessed
motivation as total number of pokes and highest ratio achieved. Consistent with prior reports [54], administration of haloperidol (0.2 mg/kg, i.p.), a dose that possesses antipsychotic efficacy following amphetamine challenges (data not shown) significantly decreased total pokes and highest ratio achieved (repeated measures ANOVA, p < 0.001; Fig. 5b, c). While a low dose of the M₄ PAM VU0467154 (0.3 mg/kg) did not have effects on responding in this model, a higher dose (3.0 mg/kg), significantly reduced total pokes and highest ratio achieved compared to vehicle
repeated measures ANOVA, \( p < 0.001 \)). These reductions in motivation are not due to an appetite suppressant effect (repeated measures ANOVA, \( p > 0.05 \); Fig. 5d) or reduction in spontaneous locomotion (students \( t \)-test, \( p > 0.05 \); data not shown), but rather the work requirements of the task. Interestingly 30 and 60 mg/kg VU6004909, doses that attenuate deficits in AHL and PPI, did not affect motivation (Fig. 5b, c).

Fig. 5 Effects of mGlu1 Activation on Progressive Ratio Performance and Ventral Striatal DA Release. a Schematic of the progressive ratio (PR) assay, where mice are required to nose poke for 30% Ensure reward. Mean (±SEM) number of total nose pokes (b) and highest ratio achieved (c). d Total amount of ensure consumed in a free feeding session (in grams; **\( p < 0.001 \), significant difference from VEH conditions; repeated measures ANOVA with Dunnett’s multiple comparison posthoc test; \( n = 12–24 \)). e Time course of 30 \( \mu \)M DHPG in the presence of 5 \( \mu \)M MTEP, mGlu5 NAM, and 1 \( \mu \)M DhβE on ventral striatal DA release. f Averaged ventral striatal DA release at baseline (BL) and following application of MTEP and DhβE to isolate mGlu1 specific effects (\( n = 8 \); students \( t \)-test \( p > 0.05 \)).
Reductions in operant responding have been correlated with reduced levels of DA in the ventral striatum [55] and with the manifestation of negative symptoms, including motivational deficits [53, 56]; therefore, we assessed mGlu1 activation on ventral striatum DA release. To test this, we performed ex vivo FSCV and isolated mGlu1 via bath application of the nACh antagonist DhβE (1 μM) and the mGlu5 NAM MTEP (5 μM), and recorded changes in DA release in the nucleus accumbens (NAc) following application of 30 μM DHPG. Interestingly, in contrast to its effects in DLS, activation of mGlu1 does not produce a significant change in NAc DA release compared to baseline (Fig. 5e, f; paired students t-test, p > 0.05). Furthermore, since some negative symptoms and cognitive disturbances in schizophrenia patients are associated with aberrant frontal lobe function [57, 58] and reduced DA levels in the prefrontal cortex (PFC) [59], we examined DA levels following mGlu1 activation in the PFC through both ex vivo FSCV (Supplemental Fig. 8A) and microdialysis. Our FSCV data demonstrates that activation of mGlu1 produced a slight rise, although not statistically significant from baseline, in PFC DA release (Supplemental Fig. 8B; students t-test, p > 0.05). Moreover, data collected from microdialysis, with probes implanted in the infralimbic or prelimbic PFC (Supplemental Fig. 8C), revealed that similar to ex vivo DA recordings, systemic administration of the mGlu1 PAM VU6004909 did not produce a significant change compared to vehicle-control nor a significant sample × treatment interaction (two-way ANOVA, p > 0.05) in PFC DA levels (Supplemental Fig. 8D). However, there was a significant effect of time (two-way ANOVA, p < 0.05), suggesting that DA in both treatment groups increased relative to baseline levels. Taken together, these findings suggest that by it’s self, activation of mGlu1 does not affect extracellular DA in the PFC.

**Discussion**

Several clinical and preclinical studies suggest that dysfunction at glutamatergic synapses may play a critical role in the pathophysiological changes that underlie schizophrenia [60–63]. However, specific changes in glutamate signaling that contribute to symptoms in different subpopulations of schizophrenia patients are not well understood. Interestingly, recent genetic studies identified multiple nonsynonymous SNPs in the human gene encoding mGlu1 (GRM1) that are associated with schizophrenia [26, 64]. Furthermore, we reported that these mutations lead to deficits in mGlu1 signaling [25, 26], raising the possibility that disrupted signaling of mGlu1 could contribute to the symptoms of schizophrenia in some patients. However, the roles of mGlu1 in brain circuits that are disrupted in schizophrenia patients are not understood.

Here, we show that activation of mGlu1 through application of exogenous agonists or selective stimulation of thalamostriatal afferents induces a robust inhibition of DA release in the dorsolateral striatum and that selective mGlu1 PAMs exert antipsychotic-like effects in rodent models. These findings are consistent with previous studies showing that mGlu receptor agonists reduce striatal DA release [34], mGlu1 KO mice have altered locomotor responses to amphetamine [65] and with anatomical studies suggesting that mGlu1 is preferentially expressed at thalamostriatal synapses [66]. Our data are especially interesting in light of a large body of studies suggesting that striatal dopaminergic hyperactivity is associated with psychotic symptoms in schizophrenic patients [15, 67, 68] and that excessive striatal DA predicts treatment response to current antipsychotics [69]. Furthermore, we have previously shown that mGlu1 PAMs reverse deficits in mGlu1 signaling observed with schizophrenia-associated mutations [25]. Taken together, these data raise the possibility that selective mGlu1 PAMs may provide a novel approach to treatment of positive symptoms both in a broad schizophrenia patient population, as well as in schizophrenia patients with GRM1 mutations.

Interestingly, our studies also suggest that mGlu1 activation is required for M4 PAM-induced inhibition of DA release and antipsychotic-like effects. M4 PAMs have received increasing attention as a novel approach to treatment of schizophrenia, and clinical studies suggest that mAChR agonists have efficacy in schizophrenia patients [3]. Thus, the present findings provide a mechanistic link between mGlu1 PAMs, and two clinically validated targets (muscarinic agonists and DA receptor antagonists). However, in contrast to available antipsychotic agents, the present results and previous studies [11] suggest that mGlu1 and M4 PAMs reduce DA signaling through local release of an eCB from striatal SPNs and activation of CB2 receptors on neighboring DA terminals. Interestingly, tonic eCB signaling does not appear to play a key role in DA release; however, when eCBs are mobilized by an mGlu1 PAM a CB2 antagonist can block these effects. These local effects are interesting in the light of recent clinical imaging studies suggesting that the symptoms in schizophrenia patients are associated with selective increases in striatal DA signaling while extrastriatal regions display hypo-dopaminergic function [70, 71]. Thus, mGlu1 and M4 PAMs may provide a mechanism for selective inhibition of DA release in striatal regions that are important for antipsychotic efficacy, without further disruptions in extrastriatal DA signaling.

While these studies suggest that the effects of M4 PAMs on DA release require activation of mGlu1, we have also found that these targets have important differences. Most...
notably, M₄ PAMs also directly inhibit D₁ signaling in D₁-SPN terminals in the SNr and this effect likely regulates M₄-dependent actions on locomotor activity [16]. In contrast, we found that mGlu₁ activation does not inhibit D₁/cAMP-mediated increases in transmission at GABAergic synapses of striatal D₁-SPNs and that mGlu₁ PAMs do not suppress D₁ agonist-induced increases in locomotor activity. While the relative importance of these different actions of M₄ PAMs is not entirely clear, the finding that mGlu₁ PAMs do not inhibit D₁ signaling through actions that are independent of DA release could provide therapeutic benefits over traditional antipsychotics and M₄ PAMs. Most notably, current antipsychotics [56, 72], as well as D₁ antagonists [48] reduce motivation, as well as ventral striatal and PFC DA release. Consistent with this, we found that therapeutically relevant doses of a typical antipsychotic, haloperidol, or an M₄ PAM significantly reduce motivational responding in a progressive ratio operant paradigm. Interestingly, low doses of M₄ PAMs do not impair motivated behavior but do possess antipsychotic-efficacy, suggesting that it is possible to provide efficacy for the positive symptom domain without inducing or worsening negative symptoms. In contrast, efficacious doses of an mGlu₁ PAM did not impair motivated performance or alter DA release in the ventral striatum. It is possible that differential effects of these agents on D₁ signaling and distinct actions in other brain regions, such as the PFC or ventral striatum, contribute to their different effects on motivational responding. The mesolimbic DA system and interconnected forebrain regions are a critical component of brain circuitry that regulate behavioral activation and motivated behavior [73, 74] and it will be important in the future to fully evaluate the effects of mGlu₁ and M₄ PAMs as compared to currently available antipsychotics in regions that may be important for motivated behavior.

In addition to modulating motivated behavior, DA release in the striatum and PFC are heavily implicated in the manifestation of negative symptoms in schizophrenia. Accordingly, future studies are needed to explore the efficacy of mGlu₁ PAMs on the negative symptoms, such as preclinical models of NMDA hypofunction, which are thought to recapitulate all symptom clusters. Previous studies have shown that M₄ PAMs display robust efficacy following challenge with an NMDA antagonist, such as MK801 [9]. Therefore, it will be of interest to compare the effects of mGlu₁ PAMs in these preclinical models to those observed following M₄ administration.

In conclusion, we present a series of studies that build upon extensive preclinical mechanistic and drug discovery efforts, as well as human genetics, imaging, and clinical intervention studies, that raise the possibility, that mGlu₁ PAMs may provide a novel approach for the treatment of the positive symptoms of schizophrenia. Future studies are needed to evaluate the therapeutic potential of mGlu₁ PAMs on negative and cognitive symptom domains. It will be important to develop a more complete understanding of the actions of mGlu₁ PAMs in disease relevant models as well as the impact of specific GRM1 mutations on identified circuits that have been implicated in schizophrenia.

Acknowledgements We would like to thank Douglas Shaw and Ginger Milne for their invaluable assistance. This work was supported by funding from an NIH Institutional Training Grant (T32 MH065215-14) and Ruth L. Kirschstein National Research Service Award (F32MH113266) to SEY, a NARSAD Young Investigator Award to DJF, grants to PJC from National Institute of Mental Health (MH062646) and the National Institute of Neurological Disease and Stroke (NS031373), National Institute on Drug Abuse grants to JFC (DA022340 and DA042595) and a NARSAD to DPC (DA041827). Research conducted by the Vanderbilt Neurochemistry Core is supported by the EKS NICHD of the NIH (U54HD083211). Data generated by the Neurochemistry Core is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Author contributions: SEY and PJC conceived the studies and wrote the manuscript. SEY, DJF, DPC, MSM, CKJ, JFC, and MB designed experiments. SEY, DPC, MSM, JG, ALB, HPC, and MB conducted experiments and analyzed the data. CWL and PMBG provided pharmacological tools utilized in this study.

Competing interests CWL and PJC are inventors on patents that protect different classes of metabotropic glutamate allosteric modulators. CWL has been funded by the NIH, Johnson and Johnson, Bristol-Myers Squibb, AstraZeneca, Michael J. Fox Foundation, as well as Seaside Therapeutics. He has consulted for AbbVie and received compensation. PJC has been funded by NIH, AstraZeneca, Bristol-Myers Squibb, Michael J. Fox Foundation, Dystonia Medical Research Foundation, CHDI Foundation, and Thorne Memorial Foundation. Over the past three years he has served on the Scientific Advisory Boards for Michael J. Fox Foundation, Stanley Center for Psychiatric Research Broad Institute, Karuna Pharmaceuticals, Lieber Institute for Brain Development, Clinical Mechanism and Proof of Concept Consortium, and Neurobiology Foundation for Schizophrenia and Bipolar Disorder. SEY, DJF, DPC, MSM, JG, PMGB, HPC, MB, ALB, MEJ, CKJ, and MB declare no potential conflicts of interest.

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