Partial mGlu₅ Negative Allosteric Modulators Attenuate Cocaine-Mediated Behaviors and Lack Psychotomimetic-Like Effects

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Cocaine abuse remains a public health concern for which pharmacotherapies are largely ineffective. Comorbidities between cocaine abuse, depression, and anxiety support the development of novel treatments targeting multiple symptom clusters. Selective negative allosteric modulators (NAMs) targeting the metabotropic glutamate receptor 5 (mGlu₅) subtype are currently in clinical trials for the treatment of multiple neuropsychiatric disorders and have shown promise in preclinical models of substance abuse. However, complete blockade or inverse agonist activity by some full mGlu₅ NAM chemotypes demonstrated adverse effects, including psychosis in humans and psychotomimetic-like effects in animals, suggesting a narrow therapeutic window. Development of partial mGlu₅ NAMs, characterized by their submaximal but saturable levels of blockade, may represent a novel approach to broaden the therapeutic window. To understand potential therapeutic vs adverse effects in preclinical behavioral assays, we examined the partial mGlu₅ NAMs, M-5MPEP and Br-5MPEPy, in comparison with the full mGlu₅ NAM MTEP across models of addiction and psychotomimetic-like activity. M-5MPEP, Br-5MPEPy, and MTEP dose-dependently decreased cocaine self-administration and attenuated the discriminative stimulus effects of cocaine. M-5MPEP and Br-5MPEPy also demonstrated antidepressant- and anxiolytic-like activity. Dose-dependent effects of partial and full mGlu₅ NAMs in these assays corresponded with increasing *in vivo* mGlu₅ occupancy, demonstrating an orderly occupancy-to-efficacy relationship. PCP-induced hyperlocomotion was potentiated by MTEP, but not by M-5MPEP and Br-5MPEPy. Further, MTEP, but not M-5MPEP, potentiated the discriminative-stimulus effects of PCP. The present data suggest that partial mGlu₅ NAM activity is sufficient to produce therapeutic effects similar to full mGlu₅ NAMs, but with a broader therapeutic index.

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INTRODUCTION

Cocaine use continues to be a public health concern, with an estimated 1.6 million cocaine users in the United States alone in 2012 (Administration SAMHSA, 2012). Comorbidity with other psychiatric conditions, including depression and anxiety disorders, is commonly seen in substance use disorders, including cocaine abuse (Volkow, 2004; Vorspan *et al*, 2015). Pharmacotherapies developed for

treating cocaine abuse have been largely unsuccessful (Shorter *et al*, 2015) and some purported treatment mechanisms elicit or exacerbate unwanted symptoms (eg, depression, anxiety, increased impulsivity, cognitive impairments, or psychosis) (Homayoun *et al*, 2004; Kishi *et al*, 2013; Moore *et al*, 2014). Developing pharmacotherapies targeting cocaine addiction and associated comorbidities while lacking adverse effects may provide greater therapeutic efficacy as well as medication compliance, increased abstinence rates and improved overall treatment success.

One novel treatment mechanism for cocaine addiction involves modulation of glutamatergic neurotransmission through antagonism of the metabotropic glutamate (mGlu) receptor subtype, mGlu₅ (Bird and Lawrence, 2009). Glutamate binds to and signals through both ionotropic and mGlu receptors. mGlu₅ is one of eight mGlu receptor subtypes identified and couples to G_{q/11} proteins, resulting in intracellular calcium mobilization and the activation of phosphoinositide hydrolysis (Niswender and Conn, 2010).

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Selective inhibitors of mGlu₅, known as negative allosteric modulators (NAMs), have been developed that do not interact with the highly conserved orthosteric binding site of glutamate, but instead bind to an allosteric site in the seven transmembrane-spanning domain of mGlu₅ and inhibit coupling of the receptor to GTP-binding proteins (Conn *et al*, 2009). These selective mGlu₅ NAMs have shown exciting efficacy across preclinical models (Emmitte, 2011; Christopoulos, 2014; Nickols and Conn, 2014) and in clinical development for the treatment of multiple CNS disorders (Berg *et al*, 2011; Jacquemont *et al*, 2011; Nickols and Conn, 2014; Lindemann *et al*, 2015).

Preclinical studies have shown that the first generation of selective full mGlu₅ NAMs, MPEP and MTEP (Gasparini *et al*, 1999; Cosford *et al*, 2003), reduced cocaine self-administration, attenuated drug and cue-mediated reinstatement of cocaine-seeking behavior, and/or blocked cocaine-induced conditioned place preference (CPP) in rodents and nonhuman primates (McGeehan and Olive, 2003; Herzig and Schmidt, 2004; Kenny *et al*, 2005; Lee *et al*, 2005; Paterson and Markou, 2005; Backstrom and Hyttia, 2006; Kumaresan *et al*, 2009; Brown *et al*, 2012). MPEP and MTEP also produced robust anxiolytic- and antidepressant-like activity, suggesting that mGlu₅ NAMs may be effective in treating the comorbid symptoms observed in cocaine addicts (Busse *et al*, 2004; Ballard *et al*, 2005). Unfortunately, clinical and preclinical studies using full mGlu₅ NAMs, with little chemotype diversity, exhibited a narrow therapeutic index between doses that produced efficacy and those that induced dose-limiting adverse effects (Kinney *et al*, 2003; Homayoun *et al*, 2004; Gravius *et al*, 2005). In particular, the mGlu₅ NAM fenobam (McN-3377) demonstrated anxiolytic-like effects in rodents and humans yet also induced psychotomimetic-like effects in humans (Friedmann *et al*, 1980; Pecknold *et al*, 1982; Porter *et al*, 2005).

Although the underlying cause of these adverse effects remains unknown, one possible mechanism is through downstream inhibition of ionotropic *N*-methyl-D-aspartate receptor subtype of glutamate receptors (NMDARs), to which mGlu₅ is physically and functionally coupled (Mannaioni *et al*, 2001; Pisani *et al*, 2001; Marino and Conn, 2002; Fujii *et al*, 2003; Gravius *et al*, 2006). Blockade of NMDARs induces psychotomimetic effects in humans and psychotomimetic-like effects in rodents (Kinney *et al*, 2003; Homayoun *et al*, 2004; Coyle, 2012). Full mGlu₅ NAMs can potentiate the behavioral effects of NMDAR antagonists, including PCP (Henry *et al*, 2002; Kinney *et al*, 2003; Pietraszek *et al*, 2004). Another possible explanation is that the psychotomimetic effects of full mGlu₅ NAMs are associated with inverse agonist activity (Gasparini *et al*, 1999; Porter *et al*, 2005). Although multiple reports demonstrate potential for novel mGlu₅ NAMs to reduce cocaine self-administration as well as antidepressant- and anxiolytic-like activity (Keck *et al*, 2012, 2014; Amato *et al*, 2013; Felts *et al*, 2013; Hughes *et al*, 2013; Rook *et al*, 2015), a remaining challenge includes developing compounds with a substantial therapeutic window.

One approach that may increase the therapeutic window for mGlu₅ NAMs involves the development of compounds that demonstrate submaximal, but saturable, NAM activity at mGlu₅, aka partial NAMs. By definition, a partial mGlu₅ NAM blocks less than 100% of the effects assessed *in vitro*

when compared with a full mGlu₅ NAM like MTEP; this submaximal blockade occurs at concentrations that fully occupy the allosteric site on mGlu₅ (Rodriguez *et al*, 2005). We previously reported the development and characterization of two highly selective partial mGlu₅ NAMs M-5MPEP and Br-5MPEPy that, in contrast to MPEP and MTEP, produced only a 50% inhibition of the maximal glutamate response *in vitro* within a concentration range that fully displaces binding of the radioligand [³H]methoxyPEPy to the MPEP allosteric binding site of mGlu₅ (Rodriguez *et al*, 2005). In the present studies, we compare the behavioral effects of the partial mGlu₅ NAMs M-5MPEP and Br-5MPEPy with the classical full mGlu₅ NAM MTEP in preclinical models of cocaine abuse, anxiolytic- and antidepressant-like activity and psychotomimetic-like effects. Both partial mGlu₅ NAMs attenuated cocaine-mediated behaviors and produced antidepressant- and anxiolytic-like effects comparable to effects with the full mGlu₅ NAM MTEP. In contrast to full mGlu₅ NAMs, the partial mGlu₅ NAMs did not exacerbate PCP-induced effects, cause sedation, or demonstrate inverse agonist activity *in vitro*. The present data suggest that partial mGlu₅ NAM activity is sufficient to produce similar effects as full mGlu₅ NAMs across multiple preclinical models of addiction and comorbid conditions. Importantly, the partial mGlu₅ NAMs exhibit less adverse effect potential, supporting a role for partial mGlu₅ NAM activity for the treatment of neuropsychiatric disorders, including cocaine abuse.

MATERIALS AND METHODS

Animals

Group-housed adult male Sprague-Dawley rats (two to three per cage) or CD-1 mice (four to five per cage; Harlan Laboratories, Indianapolis, IN) were maintained on a 12-h light/12-h dark cycle with standard rodent chow and water available *ad libitum* except for animals in self-administration and drug discrimination studies (see below). All studies were approved by the Institutional Animal Care and Use Committee of Vanderbilt University and followed the *Guide for the Care and Use of Laboratory Animals*.

Drugs and Reagents

MTEP, M-5MPEP, and Br-5MPEPy (synthesized in house) were dissolved in 10% Tween 80 in sterile water and administered intraperitoneally (i.p.) in a dosing volume of 1–2 ml/kg (rats) or 10 ml/kg (mice). Cocaine hydrochloride and phencyclidine hydrochloride (PCP; Sigma, St. Louis, MO) were dissolved in sterile saline and water, respectively, and administered i.p. and subcutaneously (s.c.), respectively. [³H] myo-inositol was obtained from Perkin-Elmer (Boston, MA). L-glutamic acid was obtained from Tocris. [³H]methoxyPEPy (76.3 Ci/mmol) was custom synthesized by PerkinElmer Life and Analytical Sciences (Waltham, MA). All other reagents were obtained from Sigma-Aldrich (St Louis, MO).

Plasma and Brain Exposure

Rats were injected with M-5MPEP or Br-5MPEPy (10 mg/kg) and 0.25, 0.5, 1, 3, and 6 h later, blood and brain were

collected. In addition, blood and brain were harvested 0.5 h after administration of 56.6 mg/kg of each compound. Samples were analyzed using LC/MS/MS (Bubser *et al*, 2014; See Supplementary Methods for details).

Occupancy

In vivo occupancy studies were conducted to provide a direct comparison between central mGlu₅ occupancy and behavioral effects utilizing the method of Anderson *et al* (2003). Briefly, animals were dosed with M-5MPEP (1.8–56.6 mg/kg), Br-5MPEPy (3–56.6 mg/kg) or MTEP (0.3–50 mg/kg). Twenty-nine minutes later, and 1 min before sacrifice, [³H]methoxy-PEPy (30 μ Ci/kg; 1 ml/kg in saline) was administered via tail vein injection. Animals were euthanized, bilateral hippocampal tissue was rapidly dissected and radioactivity (counts per minute; CPM) was measured (See Supplementary Methods for details). Percent occupancy was calculated by subtracting nonspecific CPM and normalizing to the maximal dose of the full mGlu₅ NAM MTEP (50 mg/kg). A nonlinear regression analysis was used to generate values corresponding with 50% receptor occupancy (RO₅₀; GraphPad Prism).

Cocaine Self-Administration

Self-administration procedures have been described previously (Amato *et al*, 2013; See Supplementary Methods). Individually housed rats were maintained at 85–90% of their free-feeding weights and trained to respond on a lever under a fixed ratio 10 (FR-10) schedule of reinforcement, with each completed ratio resulting in delivery of 0.5 mg/kg/infusion cocaine. This cocaine dose maintains high rates of responding under similar FR schedules and is located at or near the peak of the cocaine dose–response curve (Thomsen and Caine, 2005; Hiranita *et al*, 2010). Sessions lasted 2 h or 60 reinforcers, whichever occurred first.

To determine whether partial mGlu₅ NAMs could attenuate the reinforcing effects of cocaine to a similar degree as full mGlu₅ NAMs, we administered ascending doses of M-5MPEP, Br-5MPEPy, and MTEP to rats before cocaine self-administration sessions. Following stable responding, M-5MPEP, Br-5MPEPy (both 10–56 mg/kg, i.p.), and MTEP (1–10 mg/kg, i.p.), were injected 30 min before initiation of self-administration sessions. All the doses for a given drug were administered in a mixed order design, determined twice, and the number of cocaine infusions and response rate (total responses/minute, excluding timeouts) were recorded. Statistical comparisons using the mean group data were made by one-way ANOVA (SigmaStat, SYSTAT Software, Point Richmond, CA) followed by Holm-Sidak *post hoc* tests to compare drug with control (vehicle) sessions; significance was set at $p < 0.05$.

Drug Discrimination

Drug-discrimination studies were conducted as described by Gurkovskya and Winsauer (2009). Rats were trained in identical chambers as above, to respond on a lever under a FR-20 schedule of food reinforcement such that responding on one lever (eg, left) resulted in reinforcement following non-contingent drug injection (group 1: 10 mg/kg cocaine;

group 2: 1.8 mg/kg PCP) and responding on the alternative lever resulted in reinforcement following vehicle administration. During test sessions, responses on both levers produced reinforcement. During training and test sessions, responses emitted on one lever reset the FR counter on the alternative lever. Training drug (cocaine, PCP) or vehicle were administered 10 min before each session. Sessions lasted for 30 min or until rats completed 120 trials. To determine whether partial or full mGlu₅ NAMs would substitute for cocaine or PCP, M-5MPEP, Br-5MPEPy, and MTEP were administered 20 min before the start of the discrimination session and responding on both levers was reinforced. To determine whether partial or full mGlu₅ NAMs could alter the discriminative stimulus effects of cocaine or PCP, we evaluated a single dose corresponding with >50% occupancy of the more potent partial mGlu₅ NAM M-5MPEP (32 mg/kg) or the full mGlu₅ NAM MTEP (1 mg/kg) when administered 10 min before cocaine (1.8–18 mg/kg) or PCP (0.32–3.2 mg/kg). Substitution for or potentiation of PCP-induced effects has been proposed as a measure for assessing psychotomimetic-like effects in preclinical studies (Swedberg *et al*, 2014).

A two-way repeated measures ANOVA was used to analyze percent drug-lever responding and response rate for training drugs and drug combinations. For drug discrimination studies, complete and partial substitution for either training drug by a dose of test compound were operationally defined, respectively, as $\geq 80\%$ and ≥ 40 –79% mean responses on the drug-appropriate lever (Young, 2009). Data were excluded from analyses if the response rate was less than five responses per minute. ED₅₀ values were determined by linear regression using two or more data points reflecting the slopes of the ascending portions of the curve for each drug. Rats that did not reach 50% drug lever (%DL) responding were excluded from ED₅₀ analysis. The curves for %DL responding were considered to be significantly different if the ED₅₀ values for the drug combinations fell outside of the 95% confidence intervals obtained for PCP or cocaine discrimination alone.

Conditioned Place Preference

Methods described by Amato *et al* (2013) were used to perform conditioned place preference (CPP) studies to evaluate whether partial or full mGlu₅ NAMs would induce a CPP, indicative of an association between an interoceptive cue with an environmental context (See Supplementary Methods). On day 1, rats were placed into a three-chamber box (Williams Machine, Greenbrier, TN) and allowed to move freely between compartments. The outer chambers had distinct visual and tactile cues and were separated by a clear middle chamber. Time spent in each compartment was recorded. On days 2–5, rats were pre-treated (alternating days) either with vehicle or compound to determine whether cocaine (10 mg/kg) or mGlu₅ NAMs (M-5MPEP, 10–56 mg/kg or MTEP, 1.8–5.6 mg/kg) engendered CPP. Cocaine was administered immediately before and mGlu₅ NAMs 20 min before daily conditioning sessions during which rats were confined to a single compartment for 30 min. To determine whether mGlu₅ NAMs block induction of a cocaine CPP, doses of mGlu₅ NAMs corresponding with $\sim 80\%$ mGlu₅ occupancy (MTEP (3.2 mg/kg) and M-5MPEP (56 mg/kg))

were administered 20 min before 10 mg/kg cocaine on drug training days. On the test day (day 6), no compound was administered and rats were allowed to explore all the three chambers. The time spent in the non-preferred compartment on Day 1 (pre-test) was determined and compared with the time spent in the same chamber on Day 6 (test) using a repeated-measures two-way ANOVA followed by Holm–Sidak *post hoc* tests. Significance was set at $p < 0.05$.

Spontaneous Locomotor Activity and PCP-induced Hyperlocomotion

To determine whether reductions in cocaine self-administration and other, subsequent assays were related to nonspecific motor impairments or sedation, we examined the effects of the partial and full mGlu₅ NAMs on spontaneous locomotor activity in a novel environment. Open-field activity was tested using SmartFrame open-field chambers (Kinder Scientific, San Diego, CA) as described previously (Jones *et al*, 2008; Byun *et al*, 2014). To examine potential sedative effects of compounds, rats were injected with vehicle, M-5MPEP, or Br-5MPEPy (18–56.6 mg/kg), or MTEP (5 mg/kg) and 30 min later placed in the test chambers for 60 min. To determine the effects of compounds on PCP-induced hyperlocomotion, rats were habituated in the open field for 30 min, followed by the administration of vehicle, or doses corresponding to ~80% mGlu₅ occupancy of M-5MPEP or Br-5MPEPy (56.6 mg/kg), or MTEP (5 mg/kg). After an additional 30 min, vehicle or PCP (2.5 mg/kg) was injected and locomotor activity was recorded for another 120 min. The time course of drug-induced changes in ambulation was expressed as number of beam breaks/5 min over the 60-min (spontaneous locomotion) or 180-min session (PCP hyperlocomotion). Total locomotor activity was calculated as the sum of beam breaks per 30-min (spontaneous locomotion) or 60-min block (PCP hyperlocomotion). Total activity and time course data (means $\pm$ SEM) were analyzed by one- and two-way ANOVA, respectively, and *post hoc* comparisons were made by Bonferroni's or Newman–Keuls test.

Forced Swim Test

To determine if the partial mGlu₅ NAMs could induce antidepressant- and anxiolytic-like effects to a similar degree as full mGlu₅ NAMs, we compared the effects of M-5MPEP and Br-5MPEPy with full mGlu₅ NAM MTEP across preclinical measures of antidepressant- (forced swim and tail suspension tests) and anxiolytic-like (stress-induced hyperthermia and marble-burying assay) activity (Borsini *et al*, 2002; Yan *et al*, 2010). In the forced swim test, rats (275–325 g) were exposed to a habituation and a test session separated by 24 h. For habituation, rats were placed in swim tanks (20 cm diameter, filled with ~30 cm of water (23–25 °C)) for 15 min. On the test day, rats were administered vehicle, M-5MPEP or Br-5MPEPy (10–56.6 mg/kg), or MTEP (0.3–10 mg/kg), and 30 min later placed in swim tanks for 6 min. The test sessions were video-recorded and manually scored for the time animals exhibited immobility (defined as minimal amount of movement to stay afloat).

Tail Suspension Test

Mice were injected with vehicle, M-5MPEP or Br-5MPEPy (18–56.6 mg/kg), or MPEP (1–10 mg/kg) and tested 30 min later in the tail suspension test as described by Crowley *et al* (2005); (See Supplementary Methods). Mice were suspended for 6 min from a vertical bar of the tail suspension apparatus (Med Associates) and movements were detected and digitally recorded by a strain gauge. Data were expressed as total duration of immobility.

Stress-Induced Hyperthermia

Mice were housed individually and habituated to the testing room for 2 h before testing. Immediately after a basal temperature (T1) reading using a digital rectal thermometer, mice were injected with vehicle, M-5MPEP or Br-5MPEPy (5.6–32 mg/kg), or MTEP (2.5–10 mg/kg). A second temperature reading (T2) was taken 30 min later and the change in temperature (T2 – T1) was calculated.

Marble-Burying

Mice were administered vehicle, M-5MPEP or Br-5MPEPy (5.6–32 mg/kg) and 30 min later individually placed into Plexiglas cages containing 12 evenly distributed marbles (Rodriguez *et al*, 2010). At the end of the 30-min test, the number of buried marbles—those covered $\geq 2/3$ by bedding—was determined.

Data from forced swim, tail suspension, stress-induced hyperthermia, and marble-burying studies were analyzed using one-way ANOVA followed by Dunnett's *post hoc* comparison.

[³H]-Phosphoinositol Accumulation

To determine whether the partial mGlu₅ NAMs have inverse agonist activity similar to fenobam, MPEP, and MTEP (Gasparini *et al*, 1999; Roppe *et al*, 2004; Porter *et al*, 2005), we examined the effects of M-5MPEP, Br-5MPEPy, and MTEP on [³H]-phosphoinositol ([³H]IP) accumulation. Effects of mGlu₅ NAMs on [³H]-phosphoinositol ([³H]IP) accumulation were assessed in HEK293 cells stably expressing hemagglutinin (HA)-tagged N-terminally truncated rat mGlu₅ receptors (Goudet *et al*, 2004; Noetzel *et al*, 2013). This receptor lacks the orthosteric glutamate binding site and is, therefore, unresponsive to glutamate (Goudet *et al*, 2004) and [³H]IP accumulated in this assay represents constitutive mGlu₅ receptor activity (for details see Supplementary Methods).

RESULTS

M-5MPEP (Figure 1a) and Br-5MPEPy (Figure 1b) rapidly entered the brain ($T_{\max} = 0.5$ h) and reached brain levels suitable for *in vivo* dosing. Brain-to-plasma ratios for M-5MPEP and Br-5MPEPy were 2.3 and 1.3, respectively. On the basis of brain homogenate binding data (fraction unbound: 0.010 (M-5MPEP) and 0.030 Br-5MPEPy), estimated unbound brain concentrations at 0.5 h were 55 and 331 nM (M-5MPEP) and 132 and 274 nM (Br-5MPEPy), following administration of 10 and 56.6 mg/kg, respectively.

Figure 1 Concentration profiles for 10 mg/kg (mean $\pm$ SD) of (a) M-5MPEP and (b) Br-5MPEPy in plasma (triangle) or brain homogenate (circles) in rats ($n = 2$ /compound) at 0.25, 0.5, 1, 3, and 6 h. (c) mGlu₅ receptor occupancy (mean $\pm$ SEM) by the partial NAMs M-5MPEP and Br-5MPEPy and the full mGlu₅ NAM MTEP in the rat hippocampus ($n = 8$ –10/group).

Unbound brain concentrations after a dose of 56.6 mg/kg were slightly above and below the *in vitro* IC₅₀ for M-5MPEP and Br-5MPEPy, respectively (see Table 1; Rodriguez *et al*, 2005).

M-5MPEP and Br-5MPEPy Dose-Dependently Increase mGlu₅ Receptor Occupancy

Systemic administration of M-5MPEP, Br-5MPEPy and MTEP dose-dependently reduced the binding of [³H] methoxy-PEPy to mGlu₅ in rats, reflecting increasing mGlu₅ receptor occupancy (see Figure 1c). Doses calculated to produce 50% receptor occupancy (RO₅₀) were 16.5, 18.9, and 0.9 mg/kg for M-5MPEP, and Br-5MPEPy and MTEP, respectively. Occupancy of >80% were achieved by 56.6 mg/kg M-5MPEP and 3 mg/kg MTEP while 56.6 mg/kg Br-5MPEPy achieved ~70% occupancy.

M-5MPEP and Br-5MPEPy Reduce Cocaine Self-Administration

As shown in Figure 2a, M-5MPEP and Br-5MPEPy dose-dependently attenuated the number of cocaine infusions, similar to the full mGlu₅ NAM MTEP (M-5MPEP: ($F_{(4,31)} = 14.617$, $p < 0.001$); Br-5MPEPy: ($F_{(4,29)} = 11.014$, $p < 0.001$); MTEP: ($F_{(5,26)} = 11.646$, $p < 0.001$)). Consistent with a decreased number of reinforcers, all mGlu₅ NAMs dose-dependently reduced response rate (Supplementary Figure S1).

M-5MPEP Attenuates the Discriminative Stimulus Effects of Cocaine in Operant Drug Discrimination

As shown in Figure 2b, cocaine dose-dependently increased percent responding to nearly 100% on the

cocaine-appropriate lever. The partial mGlu₅ NAMs M-5MPEP and Br-5MPEPy produced ~20% or less responding on the cocaine-appropriate lever, with one exception. The 56.6 mg/kg dose of M-5MPEP produced ~40% responding on the cocaine-appropriate lever, and although not significant, was associated with a 40% reduction in response rate (See Supplementary Figure S2). The full mGlu₅ NAM MTEP demonstrated weak partial substitution for cocaine, producing ~20–40% responding on the cocaine-appropriate lever. MTEP, but not M-5MPEP or Br-5MPEPy, dose-dependently decreased response rates (See Supplementary Figure S2).

As shown in Figure 2c and d, M-5MPEP and MTEP attenuated the discriminative stimulus effects of cocaine as demonstrated by a rightward shift in the dose-response curve and an increase in the cocaine dose necessary to engender 50% cocaine-like responding. ED₅₀s were 3.14 ± 0.66 mg/kg (cocaine), 5.88 ± 2.35 mg/kg (cocaine+32 mg/kg M-5MPEP), and 6.01 ± 4.15 mg/kg (cocaine+1 mg/kg MTEP). M-5MPEP and MTEP did not alter response rates in combination with cocaine (see Supplementary Figure S2).

M-5MPEP Blocks Cocaine-Induced Place-Preference

A dose-response curve was obtained to determine whether M-5MPEP or MTEP produced CPP when administered alone (Figure 2e and f). The partial mGlu₅ NAM M-5MPEP did not induce CPP as indicated by lack of effects of dose ($F_{(4,55)} = 2.211$, $p = 0.080$), test day ($F_{(4,55)} = 1.323$, $p = 0.255$), or dose by test day interaction (Figure 2e). As shown in Figure 2f, the full mGlu₅ NAM MTEP produced a significant CPP when administered alone with significant effects of dose ($F_{(3,43)} = 4.819$, $p = 0.006$) and differences between

Table 1 Comparison of the *In vivo* and *In vitro* Effects of M-5MPEP, Br-5MPEPy, and MTEP

Assay	Species	Conc./Dose	Compound		
			M-5MPEP	Br-5MPEPy	MTEP
<i>In vitro</i>					
Potency in Ca ²⁺ assay [IC ₅₀] ^a	Rat	nM	130	428	19
Blockade of [³ H]IP accumulation	Rat	nM	> 30 000	> 30 000	10 000
<i>In vivo</i>					
Unbound brain concentration					
10 mg/kg i.p., t = 30 min	Rat	nM	55	132	ND
56.6 mg/kg i.p., t = 30 min	Rat	nM	331	274	ND
Occupancy—RO ₅₀	Rat	mg/kg	16.5	18.9	0.9
Occupancy—RO ₈₀	Rat	mg/kg	56.6	> 56.6	3
Blockade of cocaine self-administration—MED	Rat	mg/kg	32	18	3.2
Drug discrimination					
Substitution for cocaine	Rat		< 40%	< 20%	< 45%
Shift of cocaine dose response curve	Rat		Right	ND	Right
Substitution for PCP	Rat		< 20%	< 25%	< 35%
Shift of PCP dose-response curve	Rat		NE	ND	Left
Conditioned place preference					
Induction of conditioned place preference	Rat	mg/kg	NE	ND	1.8
Blockade of cocaine-induced CPP	Rat	mg/kg	56	ND	3.2
Potentiation of PCP-induced hyperlocomotion	Rat	mg/kg	NE	NE	5.6
Inhibition of spontaneous locomotor activity	Rat	mg/kg	NE	NE	5
Reversal of immobility					
Forced swim test—MED	Rat	mg/kg	32	32	3
Tail suspension test—MED	Mouse	mg/kg	32	NE	3
Blockade of stress-induced hyperthermia—MED	Mouse	mg/kg	10	10	5
Inhibition of marble burying—MED	Mouse	mg/kg	18	18	10 ^b

Abbreviations: Conc., concentration; CPP, conditioned place preference; MED, minimal effective dose; ND, not determined; NE, no effect.

^aFrom Rodriguez et al (2005) and unpublished data.^bFrom Rodriguez et al (2010).

pre- and post-conditioning tests ($F_{(1,43)} = 23.014$, $p < 0.001$), but no dose by test day interaction. Holm-Sidak *post hoc* tests found that both 1.8 and 5.6 mg/kg MTEP produced significant increases in time spent in the drug-paired chamber. Interestingly, on Day 1 during the pre-test condition, animals in the 3.2 mg/kg MTEP group spent a larger amount of time in the compartment that was subsequently paired with MTEP compared with the other pretest measures for the other groups and this difference at baseline was likely responsible for the inability of MTEP to induce CPP at this dose (ie, a ceiling effect). These studies elucidated subtle differences in the subjective effects of full and partial mGlu₅ NAMs that were not identified from operant drug discrimination studies.

Next, we evaluated whether the partial mGlu₅ NAM M-5MPEP could attenuate a cocaine-associated CPP similar to full mGlu₅ NAMs (Herzig and Schmidt, 2004). Each compound was given in combination with 10 mg/kg cocaine. As shown in Figure 2g, a two-way ANOVA revealed no significant effect of treatment ($F_{(3,66)} = 1.519$, $p = 0.218$), but significant effects of test day ($F_{(1,66)} = 9.645$, $p = 0.003$), and a dose by test day interaction ($F_{(3,66)} = 3.711$, $p = 0.016$). Animals treated with vehicle+10 mg/kg cocaine developed CPP as they spent more time in the cocaine-paired chamber on the test than on the pre-test day, whereas animals treated with M-5MPEP and MTEP in combination with 10 mg/kg cocaine did not develop CPP, indicating an attenuation of the cocaine-induced CPP.

Figure 2 (a) Effects of M-5MPEP, Br-5MPEPy, and MTEP on number of cocaine infusions (0.5 mg/kg/infusion) in rats ($n=8-10$ /group). (b) Effects of M-5MPEP, Br-5MPEPy, and MTEP alone on percent drug-lever (DL) responding in rats trained to discriminate 10 mg/kg cocaine from saline ($n=12$ /group). Effects of (c) 32 mg/kg M-5MPEP or (d) 1 mg/kg MTEP pretreatment on percent DL responding across ascending doses of cocaine in rats trained to discriminate 10 mg/kg cocaine from saline ($n=6$ /group). Time spent in the non-preferred compartment on Day 1 before drug pairing (pretest, P) for rats treated with (e) M-5MPEP or (f) MTEP and on Day 6 post drug pairing (test, T). (g) Time spent in the non-preferred compartment on Day 1 (Pretest, P; open bars) before drug pairing with 10 mg/kg cocaine or cocaine+56 mg/kg M-5MPEP or 3.2 mg/kg MTEP and on Day 6 post training (Test, T; $n=10$ /group). All values represent the mean ($\pm$ SEM); * $p<0.05$ vs respective vehicle-treated (V) groups; + $p<0.05$, +++ $p<0.001$ vs respective pretest condition.

M-5MPEP Does Not Potentiate the Discriminative Stimulus Effects of PCP

As shown in Figure 3a, increasing doses of PCP increased responding on the PCP-appropriate lever, producing full substitution at the training dose and higher. Neither the partial nor full mGlu₅ NAM substituted for PCP. See Supplementary Figure S3 for effects on response rates.

As shown in Figure 3b, 32 mg/kg M-5MPEP did not alter the PCP ED₅₀ (PCP: 1.06 ± 0.23 mg/kg; PCP+32 mg/kg M-5MPEP: 0.83 ± 0.29 mg/kg) and thus did not affect the discriminative stimulus effects of PCP. In contrast, the full

mGlu₅ NAM MTEP produced a leftward shift in the dose-response curve by a quarter-log unit (Figure 3c), and decreased the ED₅₀ (PCP: 1.06 ± 0.23 mg/kg; PCP+1 mg/kg MTEP: 0.55 ± 0.24 mg/kg).

M-5MPEP and Br-5MPEPy Do Not Potentiate PCP-Induced Hyperlocomotion

Pretreatment with the partial mGlu₅ NAMs M-5MPEP or Br-5MPEPy did not alter PCP-induced hyperlocomotion. In contrast, the full mGlu₅ NAM MTEP significantly increased PCP-induced hyperlocomotion (see Figure 3d)

Figure 3 (a) Effects of M-5MPEP, Br-5MPEPy, and MTEP alone on percent drug lever (DL) responding in rats trained to discriminate 1.8 mg/kg PCP from saline ($n = 12/\text{group}$). Effects of (b) 32 mg/kg M-5MPEP or (c) 1 mg/kg MTEP on percent DL responding across ascending doses of PCP in rats trained to discriminate 1.8 mg/kg PCP from saline ($n = 6/\text{group}$). (d, e) Effects of 56.6 mg/kg of M-5MPEP or Br-5MPEPy, or 5 mg/kg MTEP on the locomotor effects of 2.5 mg/kg PCP expressed as the mean number of beam breaks per (d) 5 min and (e) 60 min bins ($n = 8-11/\text{group}$). (f, g) Effects of M-5MPEP, Br-5MPEPy, and MTEP on spontaneous locomotor activity expressed as the mean number of beam breaks per (f) 5 min and (g) 30 min bins ($n = 8/\text{group}$). All values represent mean ($\pm$ SEM); * $p < 0.05$ vs vehicle-PCP group; *** $p < 0.001$ vs respective vehicle-treated (V) group.

with significant effects of treatment ($F_{(4,1512)} = 66.72$, $p < 0.001$), time ($F_{(35,1512)} = 43.89$, $p < 0.001$), and treatment by time interaction ($F_{(140,1512)} = 3.102$, $p < 0.001$). Potentiation of PCP-induced hyperlocomotion by MTEP was observed in the third block (120–180 min) of the experiment, corresponding to the time period 60–120 min after PCP administration ($F_{(4,45)} = 12.97$, $p < 0.05$; see Figure 3e).

M-5MPEP and Br-5MPEPy Do Not Affect Spontaneous Locomotor Activity

As shown in Figure 3f, the partial mGlu₅ NAMs M-5MPEP and Br-5MPEPy did not affect motor activity, whereas the full mGlu₅ NAM MTEP reduced locomotor activity (significant effects of treatment ($F_{(7,672)} = 13.87$, $p < 0.001$),

Figure 4 Effects of M-5MPEP, Br-5MPEPy, and MTEP on the immobility duration in the (a) forced swim test in rats ($n=9-12$ /group) and (b) in the tail suspension test in mice ($n=8-12$ /group). (c) Effects of M-5MPEP ($n=8$), Br-5MPEPy ($n=8$), and MTEP ($n=6-8$ /group) on change in body temperature in a stress-induced hyperthermia assay in mice and (d) on the number of marbles buried in mice ($n=8-12$ /group). All values represent mean ($\pm$ SEM); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs the respective vehicle-treated groups.

time ($F_{(11,672)}=70.07$, $p < 0.001$), and a treatment by time interaction ($F_{(77,692)}=1.699$, $p < 0.001$). MTEP significantly reduced locomotor activity during the first ($F_{7,63}=3.677$, $p < 0.01$; see Figure 3g), but not the second, 30-min block ($F_{7,63}=0.828$, NS). These data suggests that, at doses resulting in ~80% receptor occupancy, M-5MPEP and Br-5MPEPy do not cause sedation.

M-5MPEP and Br-5MPEPy Demonstrate Antidepressant- and Anxiolytic-Like Activity

Antidepressant-like activity. As shown in Figure 4a, treatment with both partial mGlu₅ NAMs M-5MPEP and Br-5-MPEPy ($F_{7,105}=3.567$, $p < 0.01$) and full mGlu₅ NAM MTEP ($F_{4,46}=29.11$, $p < 0.001$), dose-dependently reduced the duration of immobility in the forced swim test. In addition, M-5MPEP ($F_{6,70}=2.362$, $p < 0.05$) and MTEP ($F_{3,32}=13.60$, $p < 0.001$), but not Br-5MPEPy, reduced immobility in the tail suspension test (see Figure 4b).

Anxiolytic-like activity. As shown in Figure 4c, treatment with M-5MPEP prevented the stress-induced increase in core body temperature, whereas Br-5MPEPy reduced, but did not completely inhibit, the hyperthermia response ($F_{8,71}=17.73$, $p < 0.001$). MTEP also significantly reduced stress-induced

hyperthermia ($F_{3,28}=4.45$, $p < 0.05$). In the marble-burying assay (Figure 4d), both partial mGlu₅ NAMs M-5MPEP and Br-5-MPEPy ($F_{8,76}=15.90$, $p < 0.001$) dose-dependently reduced the number of marbles buried, similar to previously reported effects by the full mGlu₅ NAM MTEP (Rodriguez *et al*, 2010).

M-5MPEP and Br-5MPEPy Do Not Produce Inverse Agonist Activity

At a concentration of 10 and 30 μ M, M-5MPEP and Br-5MPEPy did not alter [³H]IP accumulation, indicating that these compounds lack inverse agonist activity as assessed in this assay (see Figure 5). However, similar to previous reports, 10 and 30 μ M MTEP significantly decreased [³H]IP accumulation ($F_{6,144}=44.27$; $p < 0.001$).

DISCUSSION

Partial mGlu₅ NAMs may represent a novel approach for treating cocaine abuse and comorbid conditions, including anxiety and depression, by providing a broader therapeutic window than previously reported full mGlu₅ NAMs. In the present study, M-5MPEP and Br-5MPEPy demonstrated a

Figure 5 Effects of 10 and 30 μM M-5MPEP, Br-5MPEPy or MTEP on percent IP accumulation expressed as mean (± SEM) percentage of the basal [³H]IP accumulation from three independent experiments performed with three to six replicates per treatment. ****p* < 0.001 vs vehicle.

dose-dependent attenuation of cocaine self-administration in a dose range that also showed anxiolytic- and antidepressant-like activity comparable with full mGlu₅ NAMs (Kenny *et al*, 2005; Paterson and Markou, 2005; Amato *et al*, 2013; Hughes *et al*, 2013; Keck *et al*, 2014). Dose-dependent effects of partial and full mGlu₅ NAMs in these assays corresponded with increasing *in vivo* mGlu₅ occupancy, demonstrating an orderly occupancy-to-efficacy relationship (see Table 1). These exciting findings suggest that partial inhibition of mGlu₅ may be sufficient to provide efficacy for multiple symptoms observed in patients with cocaine addiction without induction of adverse side effects.

Within the present study, partial mGlu₅ NAM activity at doses correlating with ~80% occupancy, did not induce psychotomimetic-like effects compared with full mGlu₅ NAM activity. Psychotomimetic effects associated with full mGlu₅ inhibition have been observed in clinical and preclinical studies (see Introduction), representing a major hurdle for the clinical development of full mGlu₅ NAMs. Although the exact nature of the psychotomimetic-like activity of full mGlu₅ NAMs remains unclear, one possible mechanism may involve the enhanced downstream inhibition of NMDARs (Mannaioni *et al*, 2001; Pisani *et al*, 2001; Marino and Conn, 2002; Kinney *et al*, 2003). The partial mGlu₅ NAMs did not potentiate the discriminative stimulus effects of PCP or affect PCP-induced locomotor activity. In contrast, the full mGlu₅ NAM MTEP potentiated the discriminative stimulus effects and, consistent with the full mGlu₅ NAM MPEP (Kinney *et al*, 2003; Pietraszek *et al*, 2004), potentiated the locomotor effects induced by PCP. Collectively, the PCP discrimination and potentiation of hyperlocomotion studies demonstrate that partial NAM activity, at doses correlating with ~80% mGlu₅ receptor occupancy, does not elicit or exacerbate psychotomimetic-like activity associated with antagonism of NMDARs.

The lack of psychotomimetic-like activity of the partial mGlu₅ NAMs relative to full mGlu₅ NAMs may also be attributed to a differential signaling bias. In previous studies, the full mGlu₅ NAMs MTEP and MPEP were reported to produce robust inverse agonist activity in cell-based assays that has been hypothesized to account for their adverse effects (present data; Gasparini *et al*, 1999; Porter *et al*, 2005; Keck *et al*, 2012). In contrast, the partial mGlu₅ NAMs

M-5MPEP and Br-5MPEPy exhibited no inverse mGlu₅ agonist activity *in vitro* at concentrations calculated to exceed 80% mGlu₅ occupancy *in vivo*. Although these findings are promising, it will be important to evaluate future mGlu₅ NAMs with varying degrees of partial mGlu₅ NAM activity to determine whether increased levels of inhibition of the glutamate response, inverse agonist activity or effects via alternate signaling pathways are responsible for beneficial vs adverse effects of mGlu₅ NAMs.

Both the partial mGlu₅ NAMs M-5MPEP and Br-5MPEPy and the full mGlu₅ NAM MTEP decreased cocaine self-administration. However, with only a single dose of cocaine examined in self-administration studies, the present findings could be interpreted as a potentiation (eg, leftward shift) of the reinforcing effects of cocaine. In contrast with this potential interpretation, previous studies have reported that multiple full mGlu₅ NAMs, including MTEP at the same dose (3 mg/kg) tested in the current studies, shifted the entire cocaine dose-response curve (0.06–0.5 mg/kg cocaine) downward, suggesting a reduction in the reinforcing effects of cocaine across doses spanning the entire ascending limb and peak of the cocaine dose-response curve (Keck *et al*, 2013, 2014). Moreover, in rats trained to self-administer cocaine, MTEP alone did not engender responding above saline-maintained levels of responding (eg, did not function as a reinforcer; Swedberg *et al*, 2014). When administered before cocaine self-administration sessions, both partial mGlu₅ NAMs M-5MPEP and Br-5MPEPy engendered responding comparable to the patterns of responding observed when saline was substituted for cocaine suggesting an attenuation of the reinforcing effects of cocaine. M-5MPEP and Br-5MPEPy also had no effect on spontaneous locomotor activity or response rates during the drug discrimination studies within the dose range that decreased cocaine self-administration, indicating these effects were not a result from sedation. Taken together, these findings are consistent with the hypothesis that partial mGlu₅ NAMs can attenuate cocaine-mediated behaviors to a similar degree as full mGlu₅ NAMs.

In contrast to the current and previous studies with MTEP, under specific experimental conditions other studies have reported that the full mGlu₅ NAM MPEP maintained responding above saline-maintained levels of responding (van der Kam *et al*, 2009), induced CPP in combination with a sub-threshold dose of cocaine (Rutten *et al*, 2011), and increased extracellular dopamine levels at high concentrations via intrastratial infusions consistent with other drugs that produce reinforcing effects (Golembiowska *et al*, 2003). In the present study, MTEP produced CPP when administered alone and the top dose of M-5MPEP engendered ~40% cocaine-like responding suggesting that antagonism of mGlu₅ may produce weak cocaine-like subjective effects. Unfortunately, limited physiochemical properties (eg, poor solubility) of the partial mGlu₅ NAMs precluded further characterization at higher doses and direct assessment of abuse liability in the self-administration paradigm. In addition, the highest dose of Br-5MPEPy tested (56.6 mg/kg) achieved only ~70% central mGlu₅ occupancy in rats. Although this degree of occupancy with Br-5MPEPy was sufficient to produce antidepressant-like effects in the rat forced swim test, comparable antidepressant-like effects were not observed in the mouse tail suspension assay with this

compound. The reasons for these differences remain unclear. However, a higher degree of occupancy may be necessary in mice to achieve similar antidepressant-like activity. Future studies using highly optimized partial mGlu₅ NAMs with better physiochemical properties will be needed to further examine the efficacy and abuse liability of full vs partial mGlu₅ NAMs.

Alterations in glutamatergic function are common in patients with substance use disorders, anxiety disorders, and depression (for review, see Terbeck *et al*, 2015); inhibition of mGlu₅ has exciting potential to treat these often co-morbid conditions. In contrast to anxiety disorders and depression, no current medications are approved for the treatment of cocaine abuse. In general, clinically available antidepressants and anxiolytics have produced equivocal effects in studies involving treatment-seeking cocaine users (Brady *et al*, 2007). Further, antidepressants may not be as efficacious in depressed patients with a history of cocaine use, and benzodiazepines are associated with the additional risk of abuse liability (Brady *et al*, 2007). Treatment of cocaine abuse, as well as comorbid conditions or symptoms associated with withdrawal (eg, anxiety, anhedonia, sleep disturbances), may provide additional therapeutic benefit and translate into increased abstinence rates. Partial mGlu₅ NAMs, by combining similar antidepressant-like, anxiolytic-like, and anti-cocaine abuse profiles with a broader therapeutic window compared with previously reported full mGlu₅ NAMs, may represent a promising novel approach for treating cocaine abuse and its comorbid conditions. Future studies will assess behavioral effects of full and partial mGlu₅ NAMs in more complex models integrating cocaine self-administration and abstinence paradigms with models of anxiety and/or depression.

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