Dissociation of the Effects of MTEP [3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]piperidine] on Conditioned Reinstatement and Reinforcement: Comparison between Cocaine and a Conventional Reinforcer

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ABSTRACT

To advance understanding of the potential of metabotropic glutamate receptor (mGluR) 5 as treatment targets for cocaine addiction, the effects of MTEP [3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]piperidine] (a selective mGluR5 antagonist) on conditioned reinstatement of cocaine seeking were examined. To test whether modification of conditioned reinstatement by MTEP is selective for drug-directed behavior or reflects general actions on motivated behavior, effects of MTEP on reinstatement induced by a stimulus conditioned to palatable conventional reward, sweetened condensed milk (SCM), were also evaluated. Previous data suggest that mGluR manipulations preferentially interfere with conditioned reinstatement compared with cocaine self-administration. Therefore, the effects of MTEP on cocaine self-administration were compared with MTEP's effects on SCM-reinforced behavior using the same cocaine doses and SCM concentrations employed for establishing conditioned reinstatement. Male Wistar rats were trained

to associate a discriminative stimulus (S^D) with response-contingent availability of cocaine or SCM and subjected to reinstatement tests after extinction of cocaine or SCM-reinforced behavior. MTEP (0.3–10 mg/kg i.p.) dose-dependently attenuated the response-reinstating effects of both the cocaine S^D and SCM S^D. MTEP also decreased cocaine self-administration without a clear graded dose-response profile and did not modify SCM-reinforced responding. The findings implicate mGluR5-regulated glutamate transmission in appetitive behavior controlled by reward-related stimuli but without selectivity for cocaine seeking. However, the data suggest a differential role for mGluR5 in the acute reinforcing effects of cocaine versus conventional reward. These observations identify mGluR5 as potential treatment targets for cocaine relapse prevention, although the profile of action of mGluR5 antagonists remains to be more closely examined for potential anhedonic effects.

Drugs of abuse increase glutamatergic excitatory transmission throughout the brain reward and incentive motive circuit (e.g., Kalivas and Duffy, 1998; Wolf, 2003). Increases in glutamatergic signaling contribute to the reinforcing actions of addictive drugs (e.g., Kenny and Markou, 2004) and the development of drug-induced behavioral sensitization (Pierce et al., 1996; Kalivas and Duffy, 1998; Vanderschuren and Kalivas, 2000). Moreover, altered glutamate transmis-

sion has been implicated in symptoms of drug withdrawal (e.g., Kalivas et al., 2003).

Glutamate transmission is regulated by both ionotropic and G-protein-coupled or metabotropic glutamate receptors (mGluRs). The role of mGluRs in the reinforcing and addictive actions of drugs of abuse and in withdrawal states has received particular attention in recent years (Chiamulera et al., 2001; Kenny et al., 2003; Paterson et al., 2003; Lee et al., 2005; Rasmussen et al., 2005). Because of their specific synaptic and circuitry locations, mGluRs have been implicated in the fine-tuning of synaptic efficacy and control of the accuracy and "sharpness" of glutamatergic transmission (Spooren et al., 2003). Therefore, abnormalities in the functioning of these receptors or their signaling cascades are thought to play important roles in psychiatric disorders, including drug addiction (Schoepp and Conn, 1993; Neuge-

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ABBREVIATIONS: mGluR, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]piperidine; SCM, sweetened condensed milk; FR, fixed ratio; TO, time-out; S^D, discriminative stimulus; ANOVA, analysis of variance; COC, cocaine; NMDA, *N*-methyl-D-aspartate; LY379268, 1*R*,4*R*,5*S*,6*R*-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate.

bauer, 2002; Spooren et al., 2003; Kenny and Markou, 2004). For the same reasons, these receptors may offer unique pharmacotherapeutic opportunities for targeting specific alteration in glutamatergic transmission underlying such disorders (Chiamulera et al., 2001; Swanson et al., 2005).

Among the eight subtypes of mGluRs identified to date, mGluR5 has received particular attention regarding a role in drug addiction (Kenny and Markou, 2004). mGlu5 receptors reside principally on postsynaptic neurons, where they are coupled to G-proteins and positively modulate neural excitability (e.g., Conn and Pin, 1997; Hermans and Challiss, 2001; Kenny and Markou, 2004). Evidence exists that implicates mGlu5 receptors in the positive reinforcing actions of addictive drugs. For instance, mGluR5-deficient mice do not acquire cocaine self-administration (Chiamulera et al., 2001), and pharmacological blockade of mGluR5 attenuates cocaine self-administration in rodents and primates (Chiamulera et al., 2001; Kenny et al., 2003; Lee et al., 2005), nicotine (Kenny et al., 2003; Paterson et al., 2003), and ethanol (Olive et al., 2005; Schroeder et al., 2005) in rodents. Accumulating evidence suggests that mGlu5 receptors have a role in the conditioned reinforcing effects of drugs of abuse. The mGluR5 antagonist MPEP attenuated expression of morphine-induced conditioned place preference in mice (Popik and Wróbel, 2002), behavior maintained by a stimulus conditioned to cocaine in rats trained under a second order reinforcement contingency (Bäckström and Hyttiä, 2006), cocaine seeking induced by a cocaine priming dose in monkeys (Lee et al., 2005), recovery of ethanol seeking induced by concurrent ethanol priming and cue manipulations (Bäckström et al., 2004), and conditioned reinstatement of nicotine seeking in rats (Bespalov et al., 2005).

The present set of experiments was designed to extend understanding of the role of mGlu5 receptors in addiction-relevant conditioned effects of cocaine and of the treatment target potential of this receptor for relapse prevention. For this purpose, the effects of the selective mGluR5 antagonist MTEP were tested on cocaine seeking in a conditioned reinstatement model of relapse. To establish whether blockade of mGlu5 receptors preferentially modifies drug-directed behavior or exerts general suppressant effects on motivated behavior, the effects of MTEP were tested on responding induced by stimuli conditioned to a potent conventional reinforcer, sweetened condensed milk (SCM). This was considered essential because: 1) selectivity for cocaine seeking would identify mGlu5 receptors as a potential neural substrate for the maladaptive and distinctly compulsive nature of cocaine seeking compared with behavior motivated by natural reward essential for survival, well being, and "healthy" hedonic pursuits; and 2) selective interference with drug seeking would be indicative of superior treatment target potential of mGluR5 antagonists. Previous work with an mGluR2/3 agonist (LY379268), which, like MTEP, reduces glutamate-mediated neural excitability (albeit via different mechanisms of action) revealed a significant preferential inhibitory action of mGluR2/3 blockade on conditioned reinstatement compared with cocaine self-administration (Baptista et al., 2004). A secondary objective, therefore, was to examine the effects of MTEP on cocaine self-administration to determine whether mGluR5 blockade produces a similar differential profile of effects, using identical experimental

conditions as in Baptista et al. (2004). Moreover, to determine whether mGluR5 blockade selectively interferes with drug reinforcement, the effects of MTEP on cocaine versus SCM self-administration were examined using the same cocaine doses and SCM concentrations employed for establishing conditioned reinstatement.

Materials and Methods

Subjects. One hundred thirty-three male Wistar rats (Charles River Laboratories, Inc., Wilmington, MA; 200–250 g upon arrival) were housed two to three in a cage in a temperature- and humidity-controlled vivarium on a reverse 12/12-h light/dark cycle with ad libitum access to food and water. All procedures were conducted in strict adherence to the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Drugs. Cocaine hydrochloride (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile physiological saline. Cocaine or saline vehicle was infused intravenously in a volume of 0.1 ml over 4 s. MTEP was generously provided by Merck (Whitehouse Station, NJ), dissolved in 10% (v/v) Tween 80, and administered intraperitoneally in a volume of 1 ml/kg, 60 min before behavioral testing.

Apparatus. Animals were trained and tested in standard 29-cm (length) $\times$ 24-cm (width) $\times$ 19.5-cm (height) operant conditioning chambers, located inside ventilated sound-attenuating cubicles (MED Associates, St. Albans, VT). All chambers were equipped with two retractable levers (6 cm above the grid floor), a white cue light above each lever, and a house light located at the top of the chamber's front panel. Auditory stimuli consisted of a 70-dB white noise produced by a white noise generator (MED Associates) presented via an 80- Ω speaker located in the center of the chamber's front panel just below the house light, and an intermittent tone (7 kHz, 70 dB) generated by a tone source (Sonalert with volume control; MED Associates) also positioned in the center of the chamber's front panel just above the speaker. Intravenous cocaine infusions were administered by a syringe pump (Razel Scientific Instruments, Stamford, CT) located outside the sound-attenuating cubicles. SCM was delivered by a syringe pump into drinking reservoirs located on the front panel centered between the active and inactive levers, 4 cm above the grid floor. Testing equipment and data collection were controlled by an IBM-compatible computer.

Self-Administration Training. Behavioral training and testing were conducted as described previously (Baptista et al., 2004). In brief, rats designated for cocaine reinstatement and self-administration tests were surgically prepared with jugular catheters and given 7 days of recovery before commencing self-administration training. Rats designated for testing with SCM were not subjected to surgical procedures. Self-administration of cocaine (0.25 mg/0.1 ml i.v. delivered over 4 s) or SCM [2:1 (v/v) in distilled water; 0.1 ml delivered into a 0.2-ml receptacle] began on a fixed-ratio (FR) 1 schedule of reinforcement in daily 120-min (cocaine) or 40-min (SCM) sessions, 5 days/week. Responses at the right, active lever were reinforced and followed by a 20-s time-out (TO) period signaled by illumination of a cue light above the active lever. During this time, the lever remained inactive to prevent accidental overdosing with cocaine. To maintain identical training and experimental conditions, the signaled TO period was in effect also during SCM self-administration. Responses at the left, inactive lever had no scheduled consequences.

After 2 weeks of cocaine or SCM self-administration training, a contingency was introduced whereby responses at the active lever were differentially reinforced in the presence of distinct discriminative stimuli (S^D) that signaled reinforcer availability versus non-availability. A constant 70-dB white noise served as a discriminative stimulus (S^+) for availability of the reinforcer (cocaine or SCM), whereas illumination of a 2.8-W house light located at the top of the

chamber's front panel served as a discriminative stimulus (S^-) signaling nonavailability of the reinforcer (i.e., saline solution instead of cocaine or no consequence instead of SCM). Sessions were initiated by extension of the levers into the chambers and concurrent onset of the respective S^D , which remained present until termination of the session by retraction of the levers. In the presence of the S^+ , responses at the right, active lever were reinforced by cocaine or SCM on an FR1 schedule and, as during training, followed by a 20-s TO period signaled by illumination of a cue light above the lever. In the presence of the S^- , depression of the right active lever was followed by an intermittent tone during which the lever remained inactive for 20 s. Three daily sessions (each lasting 1 h for the cocaine group and 20 min for the SCM group) separated by 30-min intervals were conducted, with two "reward" sessions and one "nonreward" session sequenced in random order. SCM conditioning sessions were restricted to 20 min to avoid satiety by excessive ingestion of SCM and to ensure that levels of responding during the first and second SCM sessions were comparable (Baptista et al., 2004). Sessions were initiated by presentation of the respective S^D and extension of the levers. The S^D remained present until termination of the session by retraction of the levers. After 8 training days (i.e., a total of 16 reward and eight nonreward sessions), both the cocaine and SCM groups were placed on extinction conditions in daily 1-h sessions, during which the reinforcers and S^D were withheld until a criterion of less than four responses/session for 3 consecutive days was reached. Reinstatement tests then began under extinction conditions, but with reintroduction of the S^D similar to the conditioning phase. To verify that reinstatement was selectively controlled by the reward-paired S^D (i.e., S^+), rats were tested in the presence of the S^D paired with nonreward (i.e., S^-) on the 1st day of the reinstatement phase. Two days later, tests of MTEP effects on S^+ -induced reinstatement began. MTEP was administered 1 h before the onset of sessions at doses of 0, 0.3, 1, 3, or 10 mg/kg i.p. Each animal was tested with only one dose of MTEP according to a between-subjects design.

Self-Administration Testing. After acquisition of cocaine- or SCM-reinforced responding on an FR1 schedule (without being subjected to conditioning procedures), rats received further daily 120-min access to cocaine on an FR5 schedule or 30-min access to SCM on an FR5 schedule until stable intake was established ($\pm 10\%$ over three consecutive sessions). The effects of MTEP (0, 0.3, 1, 3, and 10 mg/kg i.p.) on cocaine ($n = 7$) or SCM ($n = 7$) self-administration then were determined. Each rat was tested with all MTEP doses according to a Latin square design. Drug tests were preceded by five baseline cocaine or SCM self-administration sessions.

Spontaneous Locomotion. These tests were conducted in a separate group of drug- and experimentally naive rats ($n = 48$). Locomotor activity was measured in 16 identical metal wire hanging cages, each measuring 36 (length) $\times$ 25 (width) $\times$ 20 (height) cm. Each cage contained two sets of infrared emitter-detector photocells positioned along the long axis, 1 cm above the grid floor and 8 cm from the front and back of the cage. Movement within the cages produced photocell interruptions that were automatically recorded by an IBM-compatible computer. Rats were injected with MTEP (0, 0.3, 1, 3, or 10 mg/kg; $n = 12$ /dose) 1 h before being placed into the locomotor cages, and locomotor activity was monitored for a total of 3 h.

Statistical Analysis. Differences in responding at the active lever between the respective reward and nonreward conditions during the last day of the training/conditioning phase were analyzed by paired Student's t tests. Differences in the number of responses between the extinction and reinstatement phases in vehicle-treated rats of the cocaine and SCM groups were analyzed separately by one-way within-subjects ANOVA. The effects of MTEP on reinstatement responses were analyzed separately for the cocaine and SCM groups by one-way between-subjects ANOVA. Effects of MTEP on responses reinforced directly by cocaine or SCM were analyzed separately by one-way within-subjects ANOVA. Locomotor activity data

were expressed as the total number of beam breaks during the 3-h recording period and analyzed by a one-way ANOVA. The cumulative number of responses for conditioned reinstatement and self-administration and the cumulative number of beam breaks were analyzed by mixed-factorial ANOVA, followed by simple effects analysis. Significant main effects or interactions were confirmed by Newman-Keuls post hoc tests.

Results

Effects of MTEP on Conditioned Reinstatement

Cocaine. All rats ($n = 31$) acquired cocaine-reinforced responding, maintained stable cocaine self-administration during the conditioning phase, and showed a significant reduction of responding during nonreward (saline) sessions ($p < 0.01$; Fig. 1A, left). After initiation of the extinction contingency, rats required on average 9.3 ± 0.9 (mean $\pm$ S.E.M.) sessions to reach the criterion. During subsequent reinstatement tests, the cocaine S^+ but not the saline-associated S^- elicited strong recovery of responding in MTEP vehicle-treated rats ($p < 0.01$, Newman-Keuls post hoc tests after ANOVA: $F_{2,12} = 18.0$; $p < 0.001$; Fig. 1A, center and right). MTEP dose-dependently reduced the response-reinstatement induced by the S^+ (Fig. 1A, right) with significant effects at the 1, 3, and 10 mg/kg doses ($p < 0.05$, Newman-Keuls post hoc tests after ANOVA: $F_{4,26} = 5.2$; $p < 0.01$). In addition, a complete reversal of responding to extinction levels was measured at the two highest doses tested. Further examination of the temporal profile of MTEP effects on responding (as measured by the cumulative number of responses) revealed that the mGlu5 antagonist reduced responding associated with the presentation of the S^+ per unit time (Fig. 1B). As illustrated by the progressive decrease in the cumulative number of responses with increasing MTEP doses, these effects were dose-dependent (Fig. 1B). This dose-dependent decrease of reinstatement was reflected by a main effect for MTEP doses ($F_{4,26} = 6.2$; $p < 0.01$) and a dose $\times$ time (10-min intervals) interaction ($F_{20,130} = 3.1$; $p < 0.001$).

Fig. 1. Effects of MTEP on reinstatement induced by discriminative stimuli associated with cocaine. A, left, active lever responses during conditioning sessions in the presence of stimuli paired with cocaine (COC/ S^+) versus nonavailability of cocaine (SAL/ S^-). ***, paired Student's t test, $t_{30} = 10.6$; $p < 0.001$ versus COC/ S^+ . Center, extinction (EXT) responses at criterion and responses during an initial reinstatement test in the presence of the stimulus paired with reward nonavailability (S^-). &, $p < 0.01$ versus S^+ . Right, reinstatement responses in the presence of the stimuli previously associated with cocaine availability (S^+) in vehicle-treated rats (0) and modification of conditioned reinstatement across doses of MTEP. *, $p < 0.05$ versus vehicle. B, cumulative number of responses (per 10-min intervals) throughout the 60-min reinstatement periods (error bars omitted for clarity). Simple effects: *, $p < 0.05$ vehicle versus 1, 3, and 10 mg/kg (for details, see Results).

Moreover, this analysis indicated that MTEP decreased responding at all time points compared with vehicle-treated rats, except at the 0.3 mg/kg dose (simple effects, $p < 0.05$; Fig. 1B). Inactive lever responses remained negligible ($\leq$ two responses) throughout the experiment and unaltered by MTEP (data not shown).

SCM. Similar to cocaine, all rats ($n = 40$) acquired robust SCM-reinforced responding, maintained stable responding during the conditioning phase, and showed only negligible responding during nonreward sessions ($p < 0.001$; Fig. 2A, left). The number of sessions required to reach the extinction criterion was 7.2 ± 0.6 (mean $\pm$ S.E.M.) sessions and statistically indistinguishable from that in the cocaine group (unpaired Student's t test: $t_{69} = 1.9$; N.S.). In MTEP vehicle-injected rats, presentation of the SCM S^+ , but not the S^- , produced significant reinstatement ($p < 0.01$, Newman-Keuls post hoc tests after ANOVA: $F_{2,16} = 22.3$; $p < 0.001$, Fig. 2A, center and right). In vehicle-treated rats, the mean total number of reinstatement responses induced by the COC S^+ was somewhat greater than responses induced by the SCM S^+ , but this difference was not statistically significant (unpaired Student's t test: $t_{14} = 1.3$; N.S.; compare Fig. 1A, right with Fig. 2A, right). Analysis of the time course of responses, however, showed that the profile of responding induced by the SCM S^+ and the COC S^+ was different (Figs. 1B and 2B). Although the COC S^+ induced responding throughout the 1-h reinstatement test, the majority of responses in the presence of the SCM S^+ occurred during the first 20 min of testing. This difference was confirmed statistically by a significant group (cocaine or SCM) $\times$ time (10-min intervals) interaction ($F_{5,70} = 9.6$; $p < 0.001$).

MTEP dose-dependently attenuated the S^+ -induced response reinstatement with significant effects at all doses ($p < 0.01$, Newman-Keuls post hoc tests after ANOVA: $F_{4,35} = 14.2$; $p < 0.001$, Fig. 2A). Examination of MTEP's effects on cumulative responses confirmed that the mGluR5 antagonist modified the cumulative response profile at all doses, re-

Fig. 2. Effects of MTEP on reinstatement induced by discriminative stimuli associated with SCM. A, left, active lever responses during conditioning sessions in the presence of stimuli paired with cocaine SCM (SCM/ S^+) versus nonavailability of the reinforcer (non-SCM/ S^-). ***, paired Student's t test, $t_{39} = 91.4$; $p < 0.001$ versus SCM/ S^+ . Center, extinction (EXT) responses at criterion and responses during an initial reinstatement test in the presence of the stimulus paired with reward nonavailability (S^-). &, $p < 0.001$ versus S^+ . Right, reinstatement responses in the presence of the stimuli previously associated with SCM availability (S^+) in vehicle-treated rats (0) and modification of conditioned reinstatement across doses of MTEP. **, $p < 0.01$ versus vehicle; †, $p < 0.05$ versus MTEP 0.3 mg/kg. B, cumulative number of responses (per 10-min intervals) throughout the 60-min reinstatement periods (error bars omitted for clarity). Simple effects: *, $p < 0.05$ vehicle versus 0.3, 1, 3, and 10 mg/kg (for details, see Results).

flected by a main effect of MTEP dose ($F_{4,35} = 3.5$; $p < 0.05$) and a dose $\times$ time (10-min intervals) interaction ($F_{20,175} = 2.6$; $p < 0.001$). Moreover, simple effects analyses confirmed that MTEP decreased responding at all time points across all doses ($p < 0.05$; Fig. 2B). Responses at the inactive lever remained negligible ($\leq$ five responses) throughout training and testing and were not modified by MTEP (data not shown).

Effects of MTEP on Cocaine- and SCM-Reinforced Behavior

Cocaine. All rats ($n = 7$) acquired stable cocaine self-administration on an FR5 schedule of reinforcement with an average baseline of 35.0 ± 2.4 (mean $\pm$ S.E.M.) responses/2-h session. MTEP significantly, although not completely, decreased responding at the 3 and 10 mg/kg doses ($p < 0.05$; Newman-Keuls post hoc tests after ANOVA: $F_{4,24} = 5.3$; $p < 0.01$; Fig. 3A). Responding remained unaltered in vehicle-treated rats. Analysis of the cumulative number of responses (Fig. 3B) confirmed that MTEP decreased the cumulative number of responses per unit time reflected by a main effect of MTEP dose ($F_{4,24} = 6.7$; $p < 0.001$) and a dose $\times$ time (10-min intervals) interaction ($F_{44,264} = 4.8$; $p < 0.001$). Moreover, simple effects analyses indicated that the 3 and 10 mg/kg MTEP doses decreased responding at all time points compared with vehicle-treated rats ($p < 0.05$; Fig. 3B). Responses at the inactive lever were negligible ($\leq$ two responses) throughout testing and were not modified by MTEP (data not shown).

SCM. All rats ($n = 7$) acquired stable SCM-reinforced responding on an FR5 schedule with an average baseline of 64.5 ± 4.5 (mean $\pm$ S.E.M.) responses/30-min session. MTEP did not alter the reinforcing effects of SCM at any dose ($F_{4,24} = 0.9$; $p > 0.05$; Fig. 4A). Further scrutiny of the time course of responding (i.e., the cumulative number of responses, Fig. 4B) confirmed that MTEP did not alter SCM intake at any time throughout the 30-min session. Responses at the inactive lever were low ($\leq$ four responses) throughout testing and not modified by MTEP (data not shown).

Effect of MTEP on Spontaneous Locomotion

After administration of MTEP, animals in all five dose groups showed overall similar spontaneous locomotion ($F_{4,43} = 0.2$; $p > 0.05$; Fig. 5A). However, examination of the time course of locomotor activity in terms of the cumulative

Fig. 3. Effects of MTEP on cocaine self-administration. A, cocaine-reinforced responses after vehicle (0) versus MTEP administration (*, $p < 0.05$ versus vehicle). B, cumulative number of responses throughout the 2-h self-administration session (error bars omitted for clarity). Simple effects: *, $p < 0.05$ vehicle versus 3 and 10 mg/kg (for details, see Results).

Fig. 4. Effects of MTEP on SCM self-administration. A, SCM-reinforced responses after vehicle (0) versus MTEP administration. B, cumulative number of responses (per 10-min intervals) during the 30-min self-administration session (error bars omitted for clarity).

Fig. 5. Spontaneous locomotor activity during the 3-h monitoring period after MTEP injection (A) and cumulative number of beam breaks (B; per 10-min intervals) during the 3-h monitoring period (error bars omitted for clarity). Simple effects: 10 mg/kg versus vehicle, *, $p < 0.05$ (for details, see Results).

number of beam breaks (Fig. 5B) revealed a small but statistically significant decrease in locomotion during the first 80 min of the test at the highest dose of MTEP (Fig. 5B), reflected by a dose $\times$ time (10-min intervals) interaction ($p < 0.01$; simple effects after ANOVA: $F_{68,731} = 1.9$; $p < 0.001$).

Discussion

The mGluR5 antagonist MTEP dose-dependently reduced reinstatement induced by a cocaine-related contextual stimulus and by a stimulus conditioned to highly palatable natural reward (SCM). Thus, pharmacological blockade of mGluR5 produced general suppressant effects on appetitive behavior motivated by reward-related stimuli without selectivity for cocaine. MTEP also decreased cocaine self-administration at the two highest doses without affecting SCM-reinforced responding at any dose, suggesting that mGluR5 receptors participate in mediating the reinforcing actions of cocaine but not a palatable natural reward such as SCM.

MTEP had no overall effect on spontaneous locomotion over the 3-h test but produced a small reduction during the first 80 min of the test (i.e., the time interval corresponding to the reinstatement and self-administration tests) at the highest (10 mg/kg) dose. Thus, the attenuation of cocaine or SCM seeking and cocaine self-administration by MTEP may have been the result of locomotor impairment as opposed to specific effects on reinstatement or reinforcement, although

several considerations argue against this interpretation. MTEP did not alter SCM-reinforced responding at any dose. Therefore, the inhibitory effects of MTEP on conditioned reinstatement and cocaine self-administration cannot be explained by interference with the ability to perform operant lever-press responses despite MTEP's mild locomotor effects. Also supporting this interpretation are findings that mGluR5 blockade by an "earlier generation" mGluR5 antagonist, MPEP, did not interfere with responding for food under fixed-ratio schedules in mice and rats (Chiamulera et al., 2001; Paterson et al., 2003). Moreover, it is known that MTEP, at the dose range tested, does not interfere with motor coordination as measured by the Rotorod test in rodents (Varty et al., 2005; Belozertseva et al., 2007). Thus, at the dose range used here, the effects of MTEP on conditioned reinstatement and cocaine self-administration cannot be attributed to nonspecific locomotor impairment or sedation.

Another issue relevant for the interpretation of the findings concerning the comparison of MTEP effects on behavior motivated by cocaine versus SCM are the differences in baseline levels of responding during self-administration that were considerably higher with SCM than cocaine. It is well established, however, that reinforcing efficacy and rate of responding on fixed-ratio schedules are not necessarily correlated (Richardson and Roberts, 1996; Stafford et al., 1998). The concentration of SCM used in the present study has been shown to maintain breaking points (an index of reinforcing efficacy) under a progressive-ratio schedule comparable with those measured with cocaine at the present dose (Hodos, 1961; Roberts, 1993; Martin-Fardon and Weiss, 2002). Moreover, the magnitude of the response reinstatement induced by the cocaine and SCM S^+ and the level of responses per 10-min intervals during the reinstatement tests were statistically identical, suggesting that under the present conditions, reliable and comparable conditioning effects occurred for the cocaine and SCM reinforcers, consistent with previous reports (Hodos, 1961; Baptista et al., 2004; Martin-Fardon et al., 2007). Most importantly, the similar dose-response profile of MTEP's effects paired with the full reversal of responding to extinction levels at the highest MTEP dose of both cocaine S^+ and SCM S^+ -induced behavior confirms that cocaine and SCM were equivalent in terms of their reinforcing efficacy and strength of conditioning they produced despite the difference in baseline levels of responding maintained by these reinforcers.

With the above considerations in mind, the results indicate that pharmacological blockade of mGluR5 attenuates appetitive behavior motivated by reward-related stimuli regardless of their association with a specific primary reinforcer (i.e., drug versus conventional reinforcer). The present findings extend previous reports showing that antagonizing mGluR5 blocks conditioned drug-seeking responses across wide range of abused substances, including nicotine, cocaine, alcohol, and morphine (Popik and Wróbel, 2002; Bäckström et al., 2004; Bessalov et al., 2005; Bäckström and Hyttiä, 2006). MTEP attenuated conditioned SCM seeking in a manner almost identical to its effects on cocaine S^+ -induced reinstatement in terms of magnitude or efficacy. A possible explanation for the equivalent effects of MTEP on conditioned cocaine and SCM seeking, although speculative and not tested in the present study, is that these effects may be related to an indirect action by MTEP on reward-related

memory and, thus, occasion setting by the S^+ because of putative interactions between mGlu5 and *N*-methyl-D-aspartate (NMDA) receptors (Doherty et al., 1997; Alagarsamy et al., 1999). Reciprocal positive feedback interactions occur between mGlu5 and NMDA receptors in that activation of NMDA receptors can activate mGlu5-mediated neural responses and vice versa (Doherty et al., 1997; Alagarsamy et al., 1999). Although the role of mGlu5 receptors in memory retrieval is not fully understood, NMDA receptors have been implicated in memory retrieval in animal models of fear conditioning (Melik et al., 2006; Matus-Amat et al., 2007) and spatial working memory (Yoshihara and Ichitani, 2004). Thus, functional blockade of mGlu5 may have impeded NMDA receptor-mediated effects on the retrieval of reward-related memory, thereby resulting in the observed decrease produced by MTEP of conditioned reinstatement induced by both the cocaine and SCM S^+ .

With respect to MTEP's effects on the conditioned behavioral effects of conventional reinforcers, MPEP has been shown to be ineffective in altering conditioned reinstatement of food seeking in an earlier study (Bespalov et al., 2005). One possible explanation for the discrepancy between these and the present findings is that MPEP and MTEP differ in their effects on behavior motivated by natural reward because of MPEP's action at other receptors, specifically its lower selectivity for mGlu5 over mGlu1, NMDA, and kainate receptors (Cosford et al., 2003; Lea and Faden, 2006), and the lower levels of receptor occupancy produced by MPEP (Anderson et al., 2003). A more likely explanation is that contextual reinstatement, as in the present study, is mediated in part by different neural substrates than reinstatement induced by discrete conditioned stimuli (e.g., Ghitza et al., 2003; McLaughlin and See, 2003; Fuchs et al., 2004; Fuchs et al., 2005), a procedure utilized in the earlier study with MPEP (Bespalov et al., 2005).

MTEP blocks not only cocaine conditioned reinstatement but also cocaine self-administration, although in contrast to the reduction of conditioned reinstatement, these effects were significant only at the two highest MTEP doses and not characterized by a compelling dose-response relationship. Nonetheless, in contrast to its nonselective attenuation of conditioned reinstatement, MTEP failed to alter SCM self-administration at any dose. The effects of MTEP on primary cocaine reinforcement in drug self-administering rats are consistent with previous findings that implicate mGlu5 receptors in cocaine reinforcement (Chiamulera et al., 2001; Kenny et al., 2003) and, in addition, reveal that antagonizing mGlu5 receptors interferes selectively with cocaine's reinforcing effects. These findings have positive implications for the treatment target potential of mGlu5 receptors. The criteria for effective pharmacotherapies for cocaine addiction include prevention of withdrawal symptoms, normalization of physiological functions disrupted by chronic cocaine use, and prevention of craving that represents a critical risk factor for relapse (Kreek, 1997). It is important that an ideal agent would also block cocaine's reinforcing actions without affecting behavior motivated by conventional reinforcers and have minimal side effects. Several of these characteristics apply to MTEP because it blocked cocaine seeking and self-administration without affecting SCM self-administration and only minimal effects on spontaneous locomotion. The treatment drug potential of MTEP is augmented further by

the recent finding that the drug effectively prevented drug seeking (i.e., reinstatement) associated with another risk factor for relapse, exposure to stress (Martin-Fardon et al., 2008). However, what remains to be understood is whether the non-selective reversal of cocaine and SCM seeking may reflect an undesirable "anhedonic" side effect, blunting motivation not only for cocaine but also for hedonically motivated behaviors essential for normal behavioral functioning. It will also be important to understand at the functional, neurocircuitry, and molecular levels the differential regulation of cocaine versus SCM self-administration by mGlu5 and, thus, the apparent differential role of these receptors in the rewarding effects of drugs of abuse versus natural rewards.

The finding that MTEP reversed conditioned reinstatement of both cocaine and SCM seeking was somewhat surprising in light of previous data showing that an mGluR2/3 agonist (LY379268) selectively reverses the reinstatement elicited by the cocaine S^+ (Baptista et al., 2004), suggesting a preferential role of mGluR2/3 in drug-directed behavior. MTEP and LY379268, while acting at different synaptic sites, were expected to produce the same behavioral effects because the net effect of both agents is to dampen glutamate-mediated neural excitability. Understanding of the neural basis for the differential behavioral effects of interference with glutamatergic transmission by mGlu5 versus mGluR2/3 manipulation remains for future research. On the other hand, the effects of MTEP observed here were comparable with those of LY379268 with respect to suppressing conditioned reinstatement of cocaine seeking more effectively than cocaine self-administration and with respect to the lack of effects on SCM self-administration, suggesting that the pharmacological actions of both agents are selective for drug-maintained reinforcement and do not extend to the reinforcing actions of natural reward.

In conclusion, the results implicate mGlu5-regulated glutamatergic transmission in the incentive-motivational effects of stimuli conditioned to drugs of abuse (cocaine), although this function is not selective for drugs of abuse and extends to behavior motivated by stimuli conditioned to potent natural reward (SCM). However, with respect to primary reinforcement, the results reveal that pharmacological blockade of mGlu5 receptors attenuates cocaine self-administration but leaves intact SCM-reinforced behavior, implicating a differential role for mGlu5 receptors in drug versus conventional reward. Lastly, the findings identify selective mGlu5 antagonists as potential treatment medications for cocaine addiction and relapse prevention, although the behavioral profile of the "antiaddictive" actions of these compounds remains to be more systematically established, including verification of the absence of potential anhedonic effects.

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