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Interactive effects of the mGlu5 receptor antagonist MPEP and the mGlu2/3 receptor antagonist LY341495 on nicotine self-administration and reward deficits associated with nicotine withdrawal in rats

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Abstract

Stimulatory actions of nicotine on mesocorticolimbic dopamine transmission are partly mediated by nicotine-induced glutamate release acting on ionotropic and metabotropic glutamate (mGlu) receptors. Because both presynaptic inhibitory mGlu2/3 and postsynaptic excitatory mGlu5 receptors provide potential targets for treatment of aspects of nicotine dependence, we examined interacting effects of mGlu5 (2-methyl-6-(phenylethynyl)-pyridine, MPEP) and mGlu2/3 (LY341495) receptor antagonists on nicotine self-administration and brain reward threshold elevations associated with spontaneous nicotine withdrawal in rats. We hypothesized that increasing glutamate transmission by blocking presynaptic inhibitory mGlu2/3 autoreceptors would antagonize MPEP-induced decreases in nicotine self-administration. We also hypothesized that blocking postsynaptic actions of glutamate on mGlu5 receptors would exacerbate nicotine withdrawal-induced reward deficits, and that this effect would be attenuated by co-administration of the mGlu2/3 receptor antagonist LY341495. MPEP selectively decreased nicotine, but not food, self-administration in rats. LY341495 slightly decreased both nicotine and food self-administration. Co-administration of LY341495 with MPEP attenuated the effectiveness of MPEP in decreasing nicotine intake, although MPEP was still effective. Spontaneous nicotine withdrawal induced somatic signs of withdrawal and reward threshold elevations indicating reward deficits. MPEP increased somatic signs and reward deficits in both nicotine- and saline-withdrawing rats. Thus, while mGlu5 receptor antagonists may be therapeutically useful in decreasing tobacco smoking, they worsen nicotine withdrawal. Co-administration of LY341495 reduced MPEP-induced reward deficits in both nicotine- and saline-withdrawing rats. Thus, increasing glutamate transmission via mGlu2/3 autoreceptor blockade reduces the effects of mGlu5 receptor blockade on nicotine self-administration and MPEP-induced exacerbation of brain reward deficits associated with nicotine withdrawal.

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Keywords

LY341495; MPEP; metabotropic glutamate receptor; nicotine; self-administration; intracranial self-stimulation; reward; dependence; withdrawal

1. Introduction

Nicotine is one of the major components of tobacco responsible for the tobacco smoking habit in humans (Stolerman and Jarvis, 1995). Nicotine acts as a reinforcer that is intravenously self-administered by humans (Harvey et al., 2004; Henningfield and Goldberg, 1983; Rose et al., 2003) and experimental animals (Corrigall and Coen, 1989; Donny et al., 1995; Goldberg et al., 1981; Henningfield and Goldberg, 1983; Watkins et al., 1999). Nicotine exerts its reinforcing effects primarily via activation of nicotinic acetylcholine receptors on mesolimbic dopamine neurons projecting from the ventral tegmental area to the nucleus accumbens (Kalivas, 1993; Maskos et al., 2005; Picciotto and Corrigall, 2002; Watkins et al., 2000a). While the role of dopamine in the effects of nicotine is well established, recent evidence suggests a contributing role for other neurotransmitter systems, including glutamate (Kenny and Markou, 2004; Mansvelder et al., 2002; Picciotto and Corrigall, 2002). Nicotine activates nicotinic acetylcholine receptors on presynaptic glutamate terminals in the ventral tegmental area and increases glutamate input to dopamine neurons (Grillner and Svensson, 2000; Mansvelder and McGehee, 2000; Schilstrom et al., 2000), thereby enhancing dopamine release in the nucleus accumbens (Kalivas, 1993). Glutamate acts via ionotropic and metabotropic glutamate (mGlu) receptors. mGlu receptors are classified in three groups (Pin and Duvoisin, 1995). Group I mGlu receptors (mGlu1 and mGlu5) are coupled to phospholipase C activation, and are located primarily postsynaptically where they positively mediate the excitatory effects of glutamate (Schoepp, 2001). By contrast, the group II (mGlu2 and mGlu3) mGlu receptors are mainly located presynaptically outside the active axon terminals where they function as inhibitory autoreceptors that regulate glutamate transmission (Schoepp, 2001). Accordingly, stimulation of mGlu2/3 receptors decreased extracellular glutamate and blockade of mGlu2/3 receptors increased extracellular glutamate in the nucleus accumbens (Xi et al., 2002). Similarly, the Glu2/3 receptor agonist LY354740 decreased extracellular dopamine levels and the mGlu2/3 receptor antagonist MGS0039 increased dopamine levels in the nucleus accumbens shell (Karasawa et al., 2006).

Postsynaptic mGlu5 receptors are involved in various effects of abused drugs, including the reinforcing effects of nicotine. Mice lacking mGlu5 receptors do not acquire intravenous cocaine self-administration (Chiamulera et al., 2001). Further, the relatively specific mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reduced self-administration of cocaine (Chiamulera et al., 2001; Kenny et al., 2005; Kenny et al., 2003b; Paterson and Markou, 2005) and alcohol (Schroeder et al., 2005) in rodents. In addition, MPEP decreased the conditioned rewarding effects of cocaine in mice (McGeehan and Olive, 2003), of morphine in mice (Aoki et al., 2004; Popik and Wrobel, 2002) and rats (Herzig and Schmidt, 2004), of methamphetamine in mice (Miyatake et al., 2005) and of amphetamine in rats (Herzig et al., 2005), as measured by the conditioned place preference procedure. In addition, MPEP selectively decreased nicotine- but not food-maintained responding in rats (Paterson et al., 2003; Tessari et al., 2004), and the motivation to self-administer nicotine, as assessed with the progressive ratio schedule of reinforcement (Paterson and Markou, 2005). Further, MPEP reduced nicotine-, cue- and schedule-induced reinstatement of nicotine seeking in rats (Bespalov et al., 2005; Tessari et al., 2004). Thus, blockade of mGlu5 receptors decreases the reinforcing effects of nicotine and may lead to decreases in tobacco smoking in humans.

Data on mGlu2/3 receptor function in nicotine dependence are limited. Nicotine withdrawal precipitates an aversive abstinence syndrome in human smokers that is thought to contribute to the persistence of the smoking habit and relapse during abstinence (Hughes and Hatsukami, 1986). Similarly, spontaneous nicotine withdrawal is associated with deficits in brain reward function in rats, measured by elevations in reward thresholds for intracranial self-stimulation, similar to those observed in rats undergoing withdrawal from other major drugs of abuse (Epping-Jordan et al., 1998). Presynaptic mGlu2/3 receptors are implicated in the development of reward deficits associated with nicotine withdrawal. Activation of mGlu2/3 receptors by systemic administration or directly into the ventral tegmental area precipitated intracranial self-stimulation reward threshold elevations in nicotine-dependent, but not control, rats (Kenny et al., 2003a); this effect is similar to that seen when nAChR antagonists are administered to nicotine dependent rats (Epping-Jordan et al., 1998; Watkins et al., 2000b). These data indicate that mGlu2/3 receptor activity is increased in nicotine dependent rats and that such increased negative feedback may result in decreased glutamate transmission when nicotine administration stops (Markou, 2006). Decreased glutamate transmission may therefore contribute to the depression-like state observed during nicotine withdrawal. In fact, blockade of presynaptic inhibitory mGlu2/3 receptors with LY341495 attenuated elevations in intracranial self-stimulation thresholds in rats undergoing spontaneous nicotine withdrawal (Kenny et al., 2003a). These findings are also consistent with an antidepressant-like effect of the mGlu2/3 antagonists LY341495 and MGS0039 in the rat forced swim test and the mouse tail suspension test (Chaki et al., 2004) and of MGS0039 in the learned helplessness paradigm in rats (Yoshimizu et al., 2006). Together, these data indicate that LY341495 may be used to treat the depressive or dysphoric state associated with the early phase of nicotine withdrawal (Kenny and Markou, 2004; Markou, 2006).

In summary, mGlu5 receptors appear to play a role in the reinforcing and motivational effects of acute nicotine and relapse to drug use after abstinence from nicotine. mGlu2/3 receptors may take part in the modulation of early withdrawal symptoms leading to drug relapse by the development of plastic changes in their activity during chronic nicotine exposure. Thus, mGlu5 receptor antagonists and mGlu2/3 receptor antagonists may offer treatment options for specific phases of nicotine dependence and withdrawal. That is, mGlu5 receptor antagonists may be indicated as an aid to smoking cessation in humans, while mGlu2/3 receptor antagonists may ameliorate the depressive or dysphoric aspects of early nicotine withdrawal. Due to medication compliance issues for human smokers, it appears easier to provide a single pill formulation that would deliver both the medication to lead to smoking cessation (mGlu5 receptor antagonist) and the medication to treat the depression-like aspects of early nicotine withdrawal (mGlu2/3 receptor antagonist) that may appear when the individual reduces smoking and upon smoking cessation. However, increased glutamatergic neurotransmission by blockade of presynaptic inhibitory mGlu2/3 receptors could potentially counteract the effects of postsynaptic mGlu5 receptor blockade on the reinforcing effects of nicotine. Conversely, mGlu5 blockade may antagonize the effects of mGlu2/3 receptor blockade on reversing intracranial self-stimulation threshold elevations associated with nicotine withdrawal. Indeed, it has been shown previously that MPEP elevates intracranial self-stimulation reward thresholds under baseline conditions (Harrison et al., 2002), an effect that is opposite to the mGlu2/3 receptor antagonist-induced reversal of the threshold elevations associated with nicotine withdrawal (Kenny et al., 2003a). Such possible pharmacodynamic interactions have not been investigated.

Thus, the present study first examined possible interactions of the mGlu5 receptor antagonist MPEP and the mGlu2/3 receptor antagonist LY341495 on nicotine self-administration in rats. Second, we assessed the effects of repeated MPEP administration on intracranial self-stimulation reward threshold elevations and somatic signs associated with spontaneous nicotine withdrawal. Third, we evaluated the effects of MPEP and LY341495 co-

administration on intracranial self-stimulation threshold elevations induced by spontaneous nicotine withdrawal. We hypothesized that LY341495 would antagonize the effects of MPEP on both nicotine self-administration and intracranial self-stimulation threshold elevations associated with nicotine withdrawal.

2. Materials and Methods

2.1. Subjects

Male Wistar rats (N=150, Charles River, Raleigh, NC) weighing 300-350 g upon arrival in the laboratory were group housed (two per cage) in a temperature- and humidity-controlled vivarium on a 12 hr reverse light-dark cycle (lights off at 8 a.m.). All behavioral testing took place during the dark phase of the light-dark cycle. After arrival in the vivarium, animals were allowed to habituate to their new environment for one week and were handled twice during this week. Rats had unrestricted access to water (except during testing) and were food-restricted to 20 g per day throughout the nicotine and food self-administration experiments except during recovery from surgery and the initial habituation period. Animal care and experimental protocols were in accordance with the NIH guidelines and the Association for the Assessment of Accreditation of Laboratory Animal Care (AAALAC), and approved by the institutional committee.

2.2. Drugs

(-)-Nicotine hydrogen tartrate was purchased from Sigma (St. Louis, MO), dissolved in saline and pH adjusted to 7 (± 0.5) with sodium hydroxide. The solution was filtered through a 0.22 μ m syringe filter for sterilization purposes. Nicotine doses are reported as free base concentrations. 2-methyl-6-(phenylethynyl)pyridine (MPEP) hydrochloride was kindly donated by Novartis (Basel, Switzerland) or purchased from ANAWA (Wangen, Switzerland) and dissolved in saline. LY341495 ((2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-[xanth-9-yl] propionic acid) was purchased from Tocris Cookson (Ballwin, MO), dissolved in saline, and pH-adjusted to 7.0 (± 0.5) with NaOH. Pretreatment time for MPEP and LY341495 was 30 min before testing and the route of administration was intraperitoneal (IP).

2.3. Apparati

2.3.1. Intravenous nicotine self-administration and food-maintained responding

—Intravenous nicotine self-administration and food-maintained responding took place in 20 Plexiglas experimental chambers (24 $\times$ 30 $\times$ 28 cm; Med Associates, St. Albans, VT) each housed in a sound-attenuated box. In each chamber, one wall contained two metal retractable levers (each 3 $\times$ 1.8 cm; active and inactive) mounted 6.5 cm above the metal grid floor of the chamber. Intravenous infusions were delivered by an infusion pump (Razel, Scientific Instruments, Stamford, CT) through Tygon tubing protected by a spring lead that was connected on one end to a swivel to allow free movement of the animal and on the other end to the catheter base mounted in the midscapular region of the animal.

2.3.2. intracranial self-stimulation operant-testing chambers—Intracranial self-stimulation training and testing took place in 16 sound-attenuated Plexiglas experimental chambers (30.5 $\times$ 30 $\times$ 17 cm; Med Associates). One wall contained a metal wheel manipulandum (5 cm wide), which required 0.2 N force to rotate it a quarter turn. Brain stimulation was delivered by constant current stimulators (Stimtech 1200; San Diego Instruments, San Diego, CA). Subjects were connected to the stimulation circuit through flexible bipolar leads (Plastics One) attached to gold-swivel commutators (model SL2C; Plastics One, Roanoke, VA). The stimulation parameters, data collection, and all test session functions were controlled by a microcomputer.

2.4. Surgical procedures

2.4.1. Intravenous catheter placement—Catheters were constructed from a 14 cm long piece of silastic laboratory tubing (0.30 mm inside diameter $\times$ 0.64 mm outside diameter; Dow Corning Corp., Midland, MI) attached to a 22 gauge stainless steel guide cannula (Plastics One) bent at a right angle and encased in a molded base of dental cement (Teets Methyl Methacrylate Denture Material; Co-Oral-Lite Mfg. Co., Diamond Springs, CA) anchored with a 2.5 cm² marlex mesh (Small Parts Inc., Miami Lakes, FL) as previously described (Caine and Koob, 1993). Animals were anaesthetized with an isoflurane/oxygen mixture (1-3% isoflurane). The catheter tubing was passed subcutaneously from the animal's midscapular region to the right jugular vein where it was secured with suture thread. After surgery, animals were given a 10-day course of antibiotic treatment (Timentin, 20 mg IV). In addition, catheters were flushed daily with 0.2 ml heparinized saline (30 IE/ml) to ensure patency.

2.4.2. Intracranial self-stimulation electrode implantation—Rats to be prepared with intracranial self-stimulation electrodes were anaesthetized with an isoflurane/oxygen mixture and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set 5 mm above the interaural line. Stainless steel bipolar electrodes (model MS303/2 Plastics One), 11 mm in length, were implanted into the medial forebrain bundle at the level of the posterior lateral hypothalamus (AP -0.5 mm from bregma; ML $\pm$ 1.7 mm; DV -8.3 mm from dura) (Paxinos and Watson, 1998). Animals were allowed at least seven days to recover from surgery prior to being tested.

2.4.3. Osmotic mini-pump implantation and removal—Subjects were anesthetized with an isoflurane/oxygen vapor mixture and an osmotic pump (Alzet model 2ML1, 10 μ l/h, 7 days; Durect Corporation, Cupertino, CA) was inserted subcutaneously (back of the animal, parallel to the spine) with the flow moderator directed posteriorly. Pumps were surgically removed seven days later under isoflurane anesthesia.

2.5. Food Training and Testing

One week after arrival in the laboratory and prior to intravenous catheterization surgery, animals were trained to lever press for food. Training started on a fixed-ratio 1 time-out 1 s (FR1TO1) schedule of reinforcement. The schedule was then progressively changed from FR1TO1 to FR1TO10, FR2TO20, and FR5TO20, with sessions lasting 30 min. Animals moved through the sequence only after successful acquisition of the previous schedule (defined as earning 50 pellets during the session). The training period lasted approximately 5 days. An identical training procedure was used for the food-control subjects. These animals were then allowed to respond for food (45 mg Noyes food pellets) on a FR5TO20 schedule with sessions lasting for one hour, with all parameters identical to the nicotine self-administration session parameters.

2.6. Nicotine Self-Administration

After recovery from intravenous catheter implantation, rats were allowed to self-administer nicotine (0.03 mg/kg free base/infusion) by switching the delivery of a food pellet for the delivery of a nicotine infusion. The lever previously paired with food delivery was paired with the delivery of nicotine infusion (active lever). Responses on the other lever (inactive lever) were recorded but had no consequences. Responding on the active lever resulted in the delivery of the nicotine solution in a volume of 0.1 ml over a 1 s period. The delivery of an infusion was paired with a cue light located above the active lever, which was lit simultaneously with the initiation of the nicotine infusion and remained illuminated throughout the 20 s time-out period, during which responding was recorded but not reinforced. The delivery of an infusion was earned by responding five times on the active lever (FR5TO20s). Rats were considered to

have acquired stable nicotine self-administration when they pressed the active lever more than twice the number of times they pressed the inactive lever and received a minimum of six infusions/1 h session, with less than 20% variance in the number of infusions earned per session over three consecutive sessions. Animals were allowed to self-administer nicotine 5 days/week.

2.7. intracranial self-stimulation reward threshold procedure

The discrete-trial current-threshold procedure used was a modification of a task initially developed by Kornetsky and Esposito (1979). The rats were initially trained to turn the wheel manipulandum on a fixed-ratio 1 (FR1) schedule of reinforcement. Each quarter turn of the wheel resulted in the delivery of a 500 ms train of 0.1 ms cathodal square-wave pulses at a frequency of 100 Hz. After the successful acquisition of responding for stimulation on this FR1 schedule, defined as 100 reinforcements within 10 min, the rats were trained gradually on the discrete-trial current-threshold procedure (Kornetsky and Esposito, 1979; Markou and Koob, 1992). Each trial began with the delivery of a non-contingent electrical stimulus, followed by a 7.5 s response window within which the subject could make a response to receive a second contingent stimulus identical in all parameters to the initial non-contingent stimulus. A response during this time window was labeled a positive response, while the lack of a response was labeled a negative response. During a 2 s period immediately after a positive response, additional responses had no consequences (extra responses). The inter-trial interval (ITI), which followed either a positive response or the end of the response window (in the case of a negative response), had an average duration of 10 s (ranging from 7.5 s to 12.5 s). Responses that occurred during the ITI were labeled time-out responses and resulted in a further 12.5 s delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the ITI and delay periods induced by time-out responses were gradually increased until animals performed consistently for a fixed stimulation intensity at standard test parameters. The animals were subsequently tested on the current-threshold procedure in which stimulation intensities were varied according to the classical psychophysical method of limits. A test session consisted of four alternating series of descending and ascending current intensities starting with a descending series. Blocks of three trials were presented to the subject at a given stimulation intensity, and the intensity changed by steps of 5 μ A between blocks of trials. The initial stimulus intensity was set at approximately 40 μ A above the baseline current-threshold for each animal. Each test session typically lasted 30 min and provided two dependent variables for behavioral assessment: threshold and response latency.

Threshold—The current-threshold for each series was defined as the midpoint in microamperes between the current intensity level at which the animal made two or more positive responses out of the three stimulus presentations and the level where the animal made less than two positive responses, with two successive stimulus intensities that led to this outcome. The animal's estimated current threshold for each test session was the mean of the four series thresholds.

Response latency—The time interval between the initiation of the non-contingent stimulus and a positive response was recorded as the response latency. The response latency for each session was defined as the mean response latency on all trials during which a positive response occurred.

2.8. Experimental Design

2.8.1. Effects of co-administration of MPEP and LY341495 on nicotine self-administration and food-maintained responding—After three to four weeks of training, subjects acquired stable nicotine self-administration or food-maintained responding, and drug testing was initiated. Each dose of MPEP (0, 1, 6 or 9 mg/kg) was administered to one of four groups of nicotine- (N=10/group) or food-trained rats (N=5/group) according to a

between-subjects design. LY341495 (0, 0.5, 1 and 3 mg/kg) was administered according to a within-subjects Latin square design. Thus, each subject received one dose of MPEP four times and all four doses of LY341495. Drugs were administered only when the animals had demonstrated stable self-administration behavior between injections, with at least 6 days between each injection day.

2.8.2. Effects of repeated MPEP administration on elevations in intracranial self-stimulation thresholds and somatic signs associated with spontaneous

nicotine withdrawal—Naïve rats were trained in the intracranial self-stimulation procedure until stable baseline responding was achieved, defined as <10% variation in the thresholds for five consecutive days. Establishment of stable intracranial self-stimulation thresholds required approximately three to four weeks of daily training and testing. One group of animals (N=13) was then prepared with subcutaneous osmotic minipumps delivering saline and a second group (N=16) with minipumps delivering 9 mg/kg/day nicotine hydrogen tartrate (3.16 mg/kg/day nicotine free base) for 7 days. This time period of exposure to nicotine was sufficient to produce robust elevations in thresholds in nicotine-treated, but not vehicle-treated, rats upon removal of the minipumps (i.e. spontaneous withdrawal) (Epping-Jordan et al., 1998; Harrison et al., 2001; Kenny et al., 2003a). During these 7 days, intracranial self-stimulation thresholds continued to be measured daily. The minipumps were surgically removed on the seventh day after pump implantation. Subjects were then tested in the intracranial self-stimulation procedure at 6, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h after pump removal. Somatic signs of the nicotine withdrawal syndrome were assessed at 6, 24, 48, 72, 96, 168, 192 and 240 h after pump removal. Based on the intracranial self-stimulation thresholds obtained at the 6 h time point, subjects were allocated to two drug treatment groups such that there were no differences in the magnitude of reward threshold elevations between the two nicotine-withdrawing groups, and between the two “saline-withdrawing” groups. These two “saline-withdrawing” groups exhibited stable thresholds upon removal of the saline-containing minipumps. One group of nicotine-withdrawing rats (N=9) and one group of saline-withdrawing rats (N=7) were then injected daily with MPEP (3 mg/kg) 30 min before the 24 - 168 h time points. The two other groups (nicotine-withdrawing N=7 and saline-withdrawing N=6) were injected with vehicle before each daily session. The dose of MPEP (3 mg/kg) used was selected because it was shown previously that this dose decreased self-administration of 0.01 mg/kg nicotine free base/infusion and tended to decrease self-administration of 0.03 mg/kg/infusion (Paterson et al., 2003), while it induced small threshold elevations under baseline conditions in both nicotine- and saline-treated rats (Harrison et al., 2002; Kenny et al., 2003a). Thus, this MPEP dose appeared to be the optimal dose to use in this study as it “balances” the desirable effects of MPEP on intravenous nicotine self-administration without inducing potentially large “negative” effects on baseline brain reward function. MPEP was administered repeatedly to mimic the hypothesized situation of a patient who stops smoking but continues to take an mGlu5 receptor antagonist.

Somatic signs were assessed immediately after the intracranial self-stimulation test sessions at 6, 24, 48, 72, 96, 168, 192, and 240 h after pump removal. Rats were placed individually in transparent plastic cylindrical containers (30 × 38 cm) in which they could move freely. Subjects were habituated to the containers for 5 min each day over 4 days before the first test session. During the test sessions the rats were observed by an experienced observer, who was blind to experimental group assignment, for 10 min. The frequencies of the following signs were recorded based on a checklist of nicotine abstinence signs (Hildebrand et al., 1999; Malin, 2001; Watkins et al., 2000b): chews, cheek tremors, eye blinks, foot licks, gasps, genital licks, scratches, teeth chattering, writhes and yawns. Multiple successive counts of any sign required a distinct pause between episodes. For statistical analyses, the total number of somatic signs was defined as the sum of individual occurrences of the above mentioned withdrawal signs.

2.8.4. Effects of acute co-administration of MPEP and LY341495 on elevations in intracranial self-stimulation thresholds associated with spontaneous nicotine withdrawal—Naïve rats were trained in the intracranial self-stimulation paradigm until stable baseline responding was achieved. One group of animals (N=25) was then prepared with subcutaneous osmotic minipumps delivering saline and a second group (N=30) with minipumps delivering 9 mg/kg/day nicotine hydrogen tartrate for 7 days. The minipumps were removed on the seventh day after pump implantation. Subjects were then tested in the intracranial self-stimulation procedure at 6, 12, 24, 48, 72, 96 and 120 h after pump removal. Based on the reward thresholds obtained at the 6 h time point, subjects were allocated to two drug treatment groups so that there were no differences in the magnitude of reward threshold elevations between the two nicotine-withdrawing groups, and between the two “saline-withdrawing” groups. The “saline-withdrawing” groups exhibited stable thresholds upon removal of the saline-containing minipumps. One group of nicotine-withdrawing rats (N=15) and one group of saline-withdrawing rats (N=13) were then injected once with the MPEP (3 mg/kg) + LY341495 (1 mg/kg) combination 30 min prior to the 12 h test session, and the two other groups (nicotine-withdrawing N=15 and saline-withdrawing N=11) were injected with vehicle. The dose of LY341495 used was a dose previously shown to significantly reverse intracranial self-stimulation threshold elevations in rats undergoing spontaneous nicotine withdrawal (Kenny et al., 2003a), while having no effect on thresholds of control rats. The dose of MPEP used was the same as that used in the previous study for the reasons outlined above. MPEP and LY341495 were co-administered once during nicotine withdrawal to test whether LY341495 would reduce intracranial self-stimulation threshold elevations induced by MPEP and nicotine withdrawal.

2.9. Statistical analyses

Nicotine infusions and food pellets earned on test days are expressed as percentage of baseline responding defined as the mean number of rewards earned during the last three days before the test day. Percent values are presented to allow for direct comparison of responding for the two rewards (i.e., nicotine and food). A two-way ANOVA was conducted with MPEP dose (1-9 mg/kg) as the between-subjects factors and LY341495 dose (0.5-3 mg/kg) as the within-subjects factor for each reward condition separately. Dunnett's tests were used to compare the effects of specific doses of MPEP or LY341495 with the corresponding vehicle condition. For the spontaneous nicotine withdrawal experiments, percentage changes from baseline reward thresholds were calculated by expressing the threshold values obtained at each time point during withdrawal as a percentage of thresholds for each rat on the day immediately before minipump removal. These percentages of baseline scores were subjected to three-way repeated measures ANOVAs. The within-subjects factor was time after mini-pump removal, and the two between-subjects factors were pump content (nicotine or vehicle) and acute drug treatment (MPEP, MPEP + LY341495, or vehicle). A separate follow-up ANOVA was performed on the 24 h time-point data. Response latency data were analyzed in the same manner as the threshold data. P values of <0.05 were considered statistically significant.

3. Results

3.1. Effects of co-administration of MPEP and LY341495 on nicotine self-administration and food-maintained responding

Table 1 shows the weights and baseline absolute numbers of nicotine infusions and food pellets earned for all experimental groups prior to treatment. The effects of MPEP and LY341495 on nicotine self-administration and food-maintained responding are shown in Figures 1A and 1B, respectively. MPEP reduced the number of nicotine infusions earned [main effect of MPEP: $F(3,36)=21.5$, $P<0.001$] at the 6 and 9 mg/kg doses ($P<0.01$ and $P<0.001$, respectively). ANOVA revealed a significant MPEP $\times$ LY341495 interaction [$F(9,108)=2.87$; $P<0.01$],

indicating that LY341495 alone (0 mg/kg MPEP) reduced nicotine intake ($P<0.05$), but partly reversed the reduction in nicotine self-administration induced by MPEP. MPEP had no effect on food-maintained responding. LY341495 reduced the number of food pellets earned [main effect of LY341495: $F(3,48)=17.45$; $P<0.001$] at the 3 mg/kg dose in the 0 ($P<0.05$), 6 ($P<0.001$), and 9 ($P<0.05$) mg/kg MPEP dose groups.

3.2. Effects of MPEP on intracranial self-stimulation threshold elevations and somatic signs associated with nicotine withdrawal

Mean $\pm$ S.E.M. absolute reward thresholds prior to minipump removal for nicotine-treated and control animals were 118.8 ± 6.1 and 115.6 ± 10.6 , respectively. Withdrawal from chronic nicotine treatment induced intracranial self-stimulation threshold elevations (Fig. 2A), as indicated by a significant Pump content $\times$ Time interaction [$F(10,250)=5.12$; $P<0.001$]. Treatment with MPEP 30 min before each of the 24 – 168 h measurements significantly elevated intracranial self-stimulation thresholds in both the nicotine-withdrawing and control rats, as shown by a significant Drug $\times$ Time interaction [$F(10,250)=2.02$, $P<0.05$]. The effect of MPEP on intracranial self-stimulation thresholds was the same in the nicotine- and saline-withdrawing rats as confirmed by the absence of a Drug $\times$ Pump content $\times$ Time interaction in the ANOVA. Similarly, a follow-up ANOVA for the 24 h time point (after the first MPEP or vehicle injection) revealed both significant main effects of Pump [$F(1,25)=5.05$; $P<0.05$] and Drug [$F(1,25)=6.44$; $P<0.05$], but no Pump $\times$ Drug interaction (see Figure 4A). MPEP had no effect on response latencies (data not shown).

Nicotine-exposed rats exhibited an increased number of somatic signs of withdrawal compared to saline-exposed animals (Fig. 2B), as confirmed by a significant Pump content $\times$ Time interaction effect [$F(7,175)=7.30$; $P<0.001$]. MPEP non-selectively increased somatic signs in both nicotine- and saline-exposed rats [$F(7,175)=2.18$; $P<0.038$]. There was no Pump content $\times$ Drug $\times$ Time interaction.

3.4. Effects of co-administration of MPEP and LY341495 on intracranial self-stimulation threshold elevations associated with nicotine withdrawal

Mean $\pm$ S.E.M. absolute reward thresholds prior to minipump removal for nicotine-treated and control animals were 115.7 ± 7.9 and 121.5 ± 8.0 , respectively. Withdrawal from chronic nicotine treatment induced intracranial self-stimulation threshold elevations peaking at 12 h after pump removal (Fig. 3) as indicated by a significant Pump content $\times$ Time interaction [$F(6,300)=7.93$; $P<0.001$]. Co-treatment of MPEP with LY341495 30 min before the 12 h testing time-point after pump removal had no effect on intracranial self-stimulation threshold elevations in nicotine-withdrawing rats or control rats (no Drug $\times$ Time or Drug $\times$ Pump content $\times$ Time interaction). Thus, when MPEP and LY341495 were co-administered there was no increase in intracranial self-stimulation thresholds as was observed when MPEP was administered alone. This effect was confirmed by a follow-up ANOVA for the 12 h time point (after the MPEP/ LY341495 or vehicle injection): main effect of Pump [$F(1,51)=9.72$; $P<0.01$] but no main effect of Drug and no Pump $\times$ Drug interaction (see Figure 4B). MPEP and LY341495 had no effect on response latencies at any time point after the MPEP + LY341495 combination, indicating that the drug combination did not affect test performance (data not shown). Somatic signs associated with nicotine withdrawal were not assessed in this study.

4. Discussion

4.1. Effects of co-administration of MPEP and LY341495 on nicotine self-administration and food-maintained responding

The mGlu5 receptor antagonist MPEP (6 and 9 mg/kg) selectively reduced nicotine self-administration in rats while having no effect on food maintained responding. The mGlu2/3

receptor antagonist LY341495 alone significantly reduced food-maintained responding and nicotine self-administration. Further, the results indicated that LY341495 did not further reduce the number of nicotine infusions in animals pretreated with 6 or 9 mg/kg of MPEP. In contrast and as predicted, LY341495 significantly attenuated the MPEP-induced reduction in nicotine-intake at the highest doses of LY341495 tested. Thus, when MPEP and LY341495 were co-administered, LY341495 significantly reduced the effectiveness of MPEP in decreasing nicotine self-administration.

The presently observed interacting effects of LY341495 and MPEP on nicotine self-administration are consistent with the role of the mGlu2/3 receptors as modulatory inhibitory autoreceptors (Schoepp, 2001). By blocking these autoreceptors, glutamate release is increased (Baker et al., 2002) possibly acting at other postsynaptic glutamate receptors such as NMDA receptors. This increased glutamate transmission at other glutamate receptors may account for the slightly decreased effectiveness of mGlu5 receptor blockade with MPEP in decreasing nicotine self-administration. However, MPEP, as a postsynaptic non-competitive mGlu5 receptor antagonist, was still highly effective under conditions of blocked mGlu2/3 autoreceptors. The present results suggest that an mGlu5 receptor antagonist may help in decreasing smoking. In addition, mGlu5 and mGlu2/3 receptor antagonists can potentially be co-administered without the mGlu2/3 receptor antagonist having a major effect on the actions of the mGlu5 receptor antagonist on nicotine self-administration.

The effects of MPEP on nicotine self-administration are not likely to be due to motoric impairments since MPEP did not affect food-maintained responding. In addition, an even higher dose of MPEP (10 mg/kg) was without effect on locomotor and exploratory behavior (Henry et al., 2002; Tessari et al., 2004). The present finding that LY341495 (3 mg/kg) reduced both nicotine and food intake in a non-specific manner contrasts with a previous observation that LY341495 had no effect on ethanol self-administration at doses as high as 10 mg/kg in rats (Schroeder et al., 2005). Motor impairments are unlikely to explain our findings, since LY341495 had no effect on locomotor activity (Linden et al., 2005) or even increased spontaneous locomotion in mice (O'Neill et al., 2003). In addition, the mGlu2/3 receptor antagonist MGS0039 did not affect mouse or rat spontaneous locomotor activity at doses that were behaviorally active (Chaki et al., 2004). Finally, the co-administration of MPEP and LY341495 had no effect on response latencies, a measure of motor performance in the intracranial self-stimulation task. We would not expect LY341495 to affect test performance by interfering with learning and memory processes either, since LY341495 improved spatial learning in the Morris water maze (Higgins et al., 2004). However, LY341495 increased anxiety-like behavior measured in the elevated plus maze in the mouse (Linden et al., 2005). It is therefore possible that anxiogenic effects of LY341495 may have affected operant behavior in animals responding for food and nicotine but not in animals responding for alcohol, which has anxiolytic properties.

4.2. Effects of MPEP alone and co-administration of MPEP and LY341495 on intracranial self-stimulation threshold elevations associated with nicotine withdrawal

The induction of spontaneous nicotine withdrawal by the removal of nicotine-containing osmotic minipumps induced elevations in intracranial self-stimulation thresholds indicating deficits in brain reward function. We found that the mGlu5 receptor antagonist MPEP exacerbated the intracranial self-stimulation threshold elevations associated with nicotine withdrawal, indicating a worsening of the reward deficits. Similar magnitudes of threshold elevations were also seen in the “saline-withdrawing” rats. MPEP also significantly increased somatic signs of the nicotine withdrawal syndrome in nicotine-withdrawing rats, and had the same magnitude of effect in “saline-withdrawing” rats. Consistently, MPEP has previously been shown to elevate intracranial self-stimulation reward thresholds in both chronically

nicotine- and saline-treated rats (Kenny et al., 2003a) at the same dose used in the present study (3 mg/kg). This pattern of results suggests that MPEP has detrimental effects on both brain reward function and somatic signs independent of prior drug history of the subject. Thus, independent of whether the effects of MPEP appear to be orthogonal or interactive with nicotine withdrawal, it appears that MPEP aggravates nicotine withdrawal. Based on these results, potential treatment with an mGlu5 antagonist to assist people in smoking cessation would have to be discontinued after smoking cessation to prevent negative effects of the mGlu5 antagonist on mood and somatic symptoms.

Co-administration of LY341495 and MPEP did not result in changes in intracranial self-stimulation thresholds. This lack of effect when the two drugs are co-administered is in contrast to the MPEP-induced threshold elevations when MPEP was administered alone (see above), and the threshold lowering seen after the administration of LY341495 alone in rats undergoing nicotine withdrawal (Kenny et al., 2003a). Thus, it appears that MPEP and LY341495 have opposite additive effects on brain reward function, as measured by intracranial self-stimulation thresholds. As predicted based on mechanisms of actions of these two drugs, the “undesirable” threshold-elevating effect of MPEP on reward function could be counteracted via LY341495 administration at a dose that did not attenuate the effectiveness of MPEP in decreasing nicotine self-administration. Conversely, MPEP blocked any ameliorative effects of LY341495 on nicotine withdrawal-induced threshold elevations. The proposed therapeutic potential of mGlu2/3 receptor antagonists, such as LY341495, for smoking cessation is based on the effectiveness of LY341495 in reversing elevated intracranial self-stimulation reward thresholds associated with nicotine withdrawal (Kenny et al., 2003a) and on its antidepressant-like effects in animal models of depression and drug abuse (Chaki et al., 2004; Kenny and Markou, 2004). An mGlu5 receptor antagonist, such as MPEP, may therefore antagonize the potential therapeutic effects of an mGlu2/3 receptor antagonist, such as LY341495, on early depressive symptoms associated with nicotine withdrawal.

In summary, the present study provides information on the interaction of the mGlu2/3 receptor antagonist LY341495 and the mGlu5 receptor antagonist MPEP on nicotine self-administration and the depression-like aspects of nicotine withdrawal. The results indicated that blockade of mGlu5 receptors decreased nicotine self-administration in the presence of mGlu2/3-mediated enhancement of glutamate release. mGlu5 receptor blockade exacerbated intracranial self-stimulation reward threshold elevations associated with spontaneous nicotine withdrawal, while concomitant mGlu2/3 and mGlu5 receptor blockade had no effect on the magnitude of the nicotine withdrawal effect. Thus, the present and previously published data suggest that administration of an mGlu5 receptor antagonist alone or in combination with a mGlu2/3 receptor antagonist would decrease nicotine intake via mGlu5 blockade, while subsequent administration of a mGlu2/3 receptor antagonist alone would ameliorate nicotine withdrawal via mGlu2/3 blockade (Kenny et al., 2003a). Further, the present data suggest that while mGlu5 receptor antagonists may potentially decrease tobacco smoking, they exacerbate the nicotine withdrawal syndrome and antagonize the proposed therapeutic effects of mGlu2/3 receptor antagonists in alleviating depressive symptoms associated with nicotine withdrawal. Nonetheless, mGlu2/3 blockade did not antagonize significantly the suppressant effect of mGlu5 blockade on nicotine intake, while it did attenuate the mGlu5-mediated exacerbation of nicotine withdrawal. Thus, co-administration of mGlu5 and mGlu2/3 antagonists would allow the maintenance of the anti-smoking effects of mGlu5 blockade without a loss of compliance due to mGlu5-mediated exacerbation of nicotine withdrawal. In contrast, if an mGlu2/3 receptor antagonist is administered for the alleviation of the depression-like aspects of the early nicotine withdrawal phase, it is recommended that mGlu5 receptor antagonist treatment be discontinued to not antagonize the potentially therapeutic effects of the mGlu2/3 receptor antagonist.

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References

- Aoki T, Narita M, Shibasaki M, Suzuki T. Metabotropic glutamate receptor 5 localized in the limbic forebrain is critical for the development of morphine-induced rewarding effect in mice. *Eur. J. Neurosci* 2004;20:1633–1638. [PubMed: 15355330]
- Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW. The origin and neuronal function of in vivo nonsynaptic glutamate. *J. Neurosci* 2002;22:9134–9141. [PubMed: 12388621]
- Bespalov AY, Dravolina OA, Sukhanov I, Zakharova E, Blokhina E, Zvartau E, Danysz W, van Heeke G, Markou A. Metabotropic glutamate receptor (mGluR5) antagonist MPEP attenuated cue- and schedule-induced reinstatement of nicotine self-administration behavior in rats. *Neuropharmacology* 2005;49(Suppl 1):167–178. [PubMed: 16023685]
- Caine, B.; Koob, GF. Intravenous drug self-administration techniques in animals, in: Behavioural neuroscience: a practical approach. Sahgal, A., editor. IRL Press; Oxford: 1993. p. 117-143.
- Chaki S, Yoshikawa R, Hirota S, Shimazaki T, Maeda M, Kawashima N, Yoshimizu T, Yasuhara A, Sakagami K, Okuyama S, Nakanishi S, Nakazato A. MGS0039: a potent and selective group II metabotropic glutamate receptor antagonist with antidepressant-like activity. *Neuropharmacology* 2004;46:457–467. [PubMed: 14975669]
- Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, Corsi M, Orzi F, Conquet F. Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat. Neurosci* 2001;4:873–874. [PubMed: 11528416]
- Corrigall WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. *Psychopharmacology (Berl)* 1989;99:473–478. [PubMed: 2594913]
- Donny EC, Caggiula AR, Knopf S, Brown C. Nicotine self-administration in rats. *Psychopharmacology (Berl)* 1995;122:390–394. [PubMed: 8657839]
- Epping-Jordan MP, Watkins SS, Koob GF, Markou A. Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* 1998;393:76–79. [PubMed: 9590692]
- Goldberg SR, Spealman RD, Goldberg DM. Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science* 1981;214:573–575. [PubMed: 7291998]
- Grillner P, Svensson TH. Nicotine-induced excitation of midbrain dopamine neurons in vitro involves ionotropic glutamate receptor activation. *Synapse* 2000;38:1–9. [PubMed: 10941135]
- Harrison AA, Gasparini F, Markou A. Nicotine potentiation of brain stimulation reward reversed by DH beta E and SCH 23390, but not by eticlopride, LY 314582 or MPEP in rats. *Psychopharmacology (Berl)* 2002;160:56–66. [PubMed: 11862374]
- Harrison AA, Liem YT, Markou A. Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. *Neuropsychopharmacology* 2001;25:55–71. [PubMed: 11377919]
- Harvey DM, Yasar S, Heishman SJ, Panlilio LV, Henningfield JE, Goldberg SR. Nicotine serves as an effective reinforcer of intravenous drug-taking behavior in human cigarette smokers. *Psychopharmacology (Berl)* 2004;175:134–142. [PubMed: 14997277]
- Henningfield JE, Goldberg SR. Nicotine as a reinforcer in human subjects and laboratory animals. *Pharmacol. Biochem. Behav* 1983;19:989–992. [PubMed: 6657732]
- Henry SA, Lehmann-Masten V, Gasparini F, Geyer MA, Markou A. The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. *Neuropharmacology* 2002;43:1199–1209. [PubMed: 12527469]
- Herzig V, Capuani EM, Kovar KA, Schmidt WJ. Effects of MPEP on expression of food-, MDMA- or amphetamine-conditioned place preference in rats. *Addict. Biol* 2005;10:243–249. [PubMed: 16109585]

- Herzig V, Schmidt WJ. Effects of MPEP on locomotion, sensitization and conditioned reward induced by cocaine or morphine. *Neuropharmacology* 2004;47:973–984. [PubMed: 15555632]
- Higgins GA, Ballard TM, Kew JN, Richards JG, Kemp JA, Adam G, Woltering T, Nakanishi S, Mutel V. Pharmacological manipulation of mGlu2 receptors influences cognitive performance in the rodent. *Neuropharmacology* 2004;46:907–917. [PubMed: 15081787]
- Hildebrand BE, Panagis G, Svensson TH, Nomikos GG. Behavioral and biochemical manifestations of mecamylamine-precipitated nicotine withdrawal in the rat: role of nicotinic receptors in the ventral tegmental area. *Neuropsychopharmacology* 1999;21:560–574. [PubMed: 10481840]
- Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. *Arch. Gen. Psychiatry* 1986;43:289–294. [PubMed: 3954551]
- Kalivas PW. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain. Res. Brain. Res. Rev* 1993;18:75–113. [PubMed: 8096779]
- Karasawa J, Yoshimizu T, Chaki S. A metabotropic glutamate 2/3 receptor antagonist, MGS0039, increases extracellular dopamine levels in the nucleus accumbens shell. *Neurosci. Lett* 2006;393:127–130. [PubMed: 16233956]
- Kenny PJ, Boutrel B, Gasparini F, Koob GF, Markou A. Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology (Berl)* 2005;179:247–254. [PubMed: 15602687]
- Kenny PJ, Gasparini F, Markou A. Group II metabotropic and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate glutamate receptors regulate the deficit in brain reward function associated with nicotine withdrawal in rats. *J. Pharmacol. Exp. Ther* 2003a;306:1068–1076. [PubMed: 12805481]
- Kenny PJ, Markou A. The ups and downs of addiction: role of metabotropic glutamate receptors. *Trends. Pharmacol. Sci* 2004;25:265–272. [PubMed: 15120493]
- Kenny PJ, Paterson NE, Boutrel B, Semenova S, Harrison AA, Gasparini F, Koob GF, Skoubis PD, Markou A. Metabotropic glutamate 5 receptor antagonist MPEP decreased nicotine and cocaine self-administration but not nicotine and cocaine-induced facilitation of brain reward function in rats. *Ann. N. Y. Acad. Sci* 2003b;1003:415–418. [PubMed: 14684476]
- Kornetsky C, Esposito RU. Euphorogenic drugs: effects on the reward pathways of the brain. *Fed. Proc* 1979;38:2473–2476. [PubMed: 488370]
- Linden AM, Bergeron M, Schoepp DD. Comparison of c-Fos induction in the brain by the mGlu2/3 receptor antagonist LY341495 and agonist LY354740: evidence for widespread endogenous tone at brain mGlu2/3 receptors in vivo. *Neuropharmacology* 2005;49(Suppl 1):120–134. [PubMed: 16023151]
- Malin DH. Nicotine dependence: studies with a laboratory model. *Pharmacol. Biochem. Behav* 2001;70:551–559. [PubMed: 11796153]
- Mansvelder HD, Keath JR, McGehee DS. Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron* 2002;33:905–919. [PubMed: 11906697]
- Mansvelder HD, McGehee DS. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* 2000;27:349–357. [PubMed: 10985354]
- Markou A. Metabotropic glutamate receptor antagonists: Novel therapeutics for nicotine dependence and depression? *Biol Psychiatry*. 2006in press
- Markou A, Koob GF. Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiol. Behav* 1992;51:111–119. [PubMed: 1741436]
- Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, Dufour N, Cloez-Tayarani I, Bemelmans AP, Mallet J, Gardier AM, David V, Faure P, Granon S, Changeux JP. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* 2005;436:103–107. [PubMed: 16001069]
- McGeehan AJ, Olive MF. The mGluR5 antagonist MPEP reduces the conditioned rewarding effects of cocaine but not other drugs of abuse. *Synapse* 2003;47:240–242. [PubMed: 12494407]
- Miyatake M, Narita M, Shibasaki M, Nakamura A, Suzuki T. Glutamatergic neurotransmission and protein kinase C play a role in neuron-glia communication during the development of methamphetamine-induced psychological dependence. *Eur. J. Neurosci* 2005;22:1476–1488. [PubMed: 16190901]

- O'Neill MF, Heron-Maxwell C, Conway MW, Monn JA, Ornstein P. Group II metabotropic glutamate receptor antagonists LY341495 and LY366457 increase locomotor activity in mice. *Neuropharmacology* 2003;45:565–574. [PubMed: 12941370]
- Paterson NE, Markou A. The metabotropic glutamate receptor 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology (Berl)* 2005;179:255–261. [PubMed: 15619120]
- Paterson NE, Semenova S, Gasparini F, Markou A. The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. *Psychopharmacology (Berl)* 2003;167:257–264. [PubMed: 12682710]
- Paxinos, G.; Watson, C. The rat brain in stereotactic coordinates. Academic; San Diego: 1998.
- Picciotto MR, Corrigall WA. Neuronal systems underlying behaviors related to nicotine addiction: neural circuits and molecular genetics. *J. Neurosci* 2002;22:3338–3341. [PubMed: 11978809]
- Pin JP, Duvoisin R. The metabotropic glutamate receptors: structure and functions. *Neuropharmacology* 1995;34:1–26. [PubMed: 7623957]
- Popik P, Wrobel M. Morphine conditioned reward is inhibited by MPEP, the mGluR5 antagonist. *Neuropharmacology* 2002;43:1210–1217. [PubMed: 12527470]
- Rose JE, Behm FM, Westman EC, Bates JE. Mecamylamine acutely increases human intravenous nicotine self-administration. *Pharmacol. Biochem. Behav* 2003;76:307–313. [PubMed: 14592683]
- Schilstrom B, Fagerquist MV, Zhang X, Hertel P, Panagis G, Nomikos GG, Svensson TH. Putative role of presynaptic $\alpha 7^*$ nicotinic receptors in nicotine stimulated increases of extracellular levels of glutamate and aspartate in the ventral tegmental area. *Synapse* 2000;38:375–383. [PubMed: 11044884]
- Schoepp DD. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J. Pharmacol. Exp. Ther* 2001;299:12–20. [PubMed: 11561058]
- Schroeder JP, Overstreet DH, Hodge CW. The mGluR5 antagonist MPEP decreases operant ethanol self-administration during maintenance and after repeated alcohol deprivations in alcohol-preferring (P) rats. *Psychopharmacology (Berl)* 2005;179:262–270. [PubMed: 15717208]
- Stolerman IP, Jarvis MJ. The scientific case that nicotine is addictive. *Psychopharmacology (Berl)* 1995;117:2–10. [PubMed: 7724697]discussion 14–20
- Tessari M, Pilla M, Andreoli M, Hutcheson DM, Heidbreder CA. Antagonism at metabotropic glutamate 5 receptors inhibits nicotine- and cocaine-taking behaviours and prevents nicotine-triggered relapse to nicotine-seeking. *Eur. J. Pharmacol* 2004;499:121–133. [PubMed: 15363959]
- Watkins SS, Epping-Jordan MP, Koob GF, Markou A. Blockade of nicotine self-administration with nicotinic antagonists in rats. *Pharmacol. Biochem. Behav* 1999;62:743–751. [PubMed: 10208381]
- Watkins SS, Koob GF, Markou A. Neural mechanisms underlying nicotine addiction: acute positive reinforcement and withdrawal. *Nicotine. Tob. Res* 2000a;2:19–37. [PubMed: 11072438]
- Watkins SS, Stinus L, Koob GF, Markou A. Reward and somatic changes during precipitated nicotine withdrawal in rats: centrally and peripherally mediated effects. *J Pharmacol. Exp. Ther* 2000b; 292:1053–1064. [PubMed: 10688623]
- Xi ZX, Baker DA, Shen H, Carson DS, Kalivas PW. Group II metabotropic glutamate receptors modulate extracellular glutamate in the nucleus accumbens. *J. Pharmacol. Exp. Ther* 2002;300:162–171. [PubMed: 11752112]
- Yoshimizu T, Shimazaki T, Ito A, Chaki S. An mGluR2/3 antagonist, MGS0039, exerts antidepressant and anxiolytic effects in behavioral models in rats. *Psychopharmacology (Berl)* 2006;186:587–593. [PubMed: 16612616]

Figure 1.

The effects of the mGlu5 receptor antagonist MPEP and the mGlu2/3 receptor antagonist LY341495 on nicotine- and food-maintained responding in rats ($n=10$ for each of the four MPEP doses for the nicotine groups, $n=5$ for each of four MPEP doses for the food groups). MPEP alone significantly reduced nicotine self-administration (**A**) but not food-maintained responding (**B**). LY341495 alone reduced nicotine self-administration (**A**) and food self-administration (**B**). Interestingly, LY341495 attenuated the decreases in nicotine self-administration seen after administration of 6 or 9 mg/kg MPEP (**A**), as demonstrated by a significant LY341495 $\times$ MPEP dose interaction (# for $P<0.01$; see text for details). Data are expressed as mean $\pm$ S.E.M. percent of baseline (mean of last three days of baseline nicotine or

food responding). Asterisks indicate significant differences from the corresponding vehicle condition (Dunnett's tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

A Effects of MPEP on intracranial self-stimulation threshold elevations associated with nicotine withdrawal

B Effects of MPEP on somatic signs of nicotine withdrawal

Figure 2.

The effects of repeated injections of the mGlu5 receptor antagonist MPEP on intracranial self-stimulation threshold elevations (**A**) and somatic signs (**B**) associated with spontaneous nicotine withdrawal. Rats received MPEP injections (3 mg/kg) or vehicle 30 min before testing at the 24-168 h time points, as indicated by vertical lines over the time-points when injections were administered. **A:** Intracranial self-stimulation thresholds were tested 6, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h after pump removal. There was a significant elevation in intracranial self-stimulation thresholds in the nicotine-withdrawing rats compared to control rats [Pump content $\times$ Time interaction: $F(10,250)=5.12$; $P<0.001$]. Treatment with MPEP elevated intracranial self-stimulation thresholds in both nicotine- and saline withdrawing rats

[Drug $\times$ Time interaction: $F(10,250)=2.02$, $P<0.05$]. Asterisks indicate significant difference from the corresponding saline-vehicle control condition (Dunnett's tests: * $P<0.05$, *** $P<0.001$). Data are expressed as mean $\pm$ S.E.M. percentage change from baseline thresholds prior to removal of nicotine- or saline-containing osmotic mini-pumps. **B:** Somatic signs were assessed immediately following the intracranial self-stimulation test sessions 6, 24, 48, 72, 96, 168, 192 and 240 h after pump removal. Data are expressed as mean $\pm$ S.E.M. of total number of somatic signs observed during the 10 min observation periods. Somatic signs were increased in nicotine-treated compared to saline-treated animals [Pump $\times$ Time interaction: $F(7,175)=7.30$; $P<0.001$]. MPEP increased somatic signs in both nicotine- and saline-treated rats [MPEP $\times$ Time interaction: $F(7,175)=2.18$; $P<0.05$]. Asterisks (Dunnett's test: * $P<0.05$) indicate significant difference from the vehicle-treated control group.

Effects of co-administration of MPEP and LY341495 on intracranial self-stimulation threshold elevations associated with nicotine withdrawal

Figure 3.

The effects of co-administration of the mGlu5 receptor antagonist MPEP and the mGlu2/3 receptor antagonist LY341495 on intracranial self-stimulation threshold elevations associated with spontaneous nicotine withdrawal. Intracranial self-stimulation thresholds were assessed 6, 12, 24, 48, 72, 96 and 120 h after pump removal. Rats received a single co-administration of MPEP (3 mg/kg) and LY341495 (1 mg/kg) or vehicle 30 min before testing at the 12-h time point (indicated by a vertical line). There was a significant elevation in intracranial self-stimulation thresholds in the nicotine-withdrawing rats compared to control rats [Pump content $\times$ Time interaction: $F(6,300)=7.93$; $P<0.001$]. The combined treatment with MPEP and LY341495 had no effect on intracranial self-stimulation elevations induced by the removal of the nicotine-containing pumps and did not affect intracranial self-stimulation thresholds in the control rats. Asterisks (Dunnett's tests: * $P<0.05$, ** $P<0.01$) indicate significant difference from the vehicle-treated control group. Data are expressed as mean $\pm$ S.E.M. percentage change from baseline thresholds prior to removal of nicotine- or saline-containing osmotic minipumps.

A

Effect of first MPEP administration on intracranial self-stimulation thresholds

B

Effect of co-administration of MPEP and LY341495 on intracranial self-stimulation thresholds

Figure 4.

Comparison of the effects of the first MPEP administration and of co-administration of MPEP and LY341495 on intracranial self-stimulation thresholds in nicotine- and saline withdrawing rats. **A.** At the 24 h time point, the first MPEP injection similarly elevated intracranial self-stimulation thresholds in both nicotine- and saline withdrawing rats [main effect of MPEP: $F(1,25)=6.44$; $\#P<0.05$, main effect of Nicotine: $F(1,25)=5.05$; $P<0.05$, no Nicotine $\times$ MPEP interaction]. **B.** At the 12 h time point, co-administration of MPEP and LY341495 had no effect in either nicotine or saline withdrawing rats [no main effect of MPEP/LY341495, NS for non-significant, main effect of Nicotine: $F(1,51)=9.72$; $P<0.01$, no MPEP/LY341495 $\times$ Nicotine interaction]. Data are expressed as mean $\pm$ S.E.M. percentage change from baseline thresholds

prior to removal of nicotine- or saline-containing osmotic minipumps. Data are represent measurements shown in Figures 2A (24 h time-point) and Figure 3 (12 h time-point).

Table 1

Body weights and baseline absolute number of reinforcers (nicotine or food, mean $\pm$ S.E.M.) earned prior to the start of MPEP or LY341495 treatment. Number of rewards is expressed as mean $\pm$ S.E.M. of the last 3 days prior to MPEP or LY341495 treatment.

MPEP dose	Reinforcer	n	Number of rewards earned	Body weight (g)
0 mg/kg	Nicotine	10	12.9 $\pm$ 1.1	426.3 $\pm$ 13
1 mg/kg	Nicotine	10	14.4 $\pm$ 1.4	422.0 $\pm$ 14.7
6 mg/kg	Nicotine	10	13.8 $\pm$ 0.8	387.0 $\pm$ 13.8
9 mg/kg	Nicotine	10	15.2 $\pm$ 1.4	425.0 $\pm$ 14.2
0 mg/kg	Food	5	131.9 $\pm$ 6	421.6 $\pm$ 7.4
1 mg/kg	Food	5	142.7 $\pm$ 2.1	454.4 $\pm$ 10.4
6 mg/kg	Food	5	143.9 $\pm$ 8.5	465.6 $\pm$ 9
9 mg/kg	Food	5	119.1 $\pm$ 8.9	442.8 $\pm$ 15.8