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Metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) microinfusions into the nucleus accumbens shell or ventral tegmental area attenuate the reinforcing effects of nicotine in rats

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Abstract

Systemic administration of the mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) was previously shown to selectively attenuate nicotine self-administration without affecting food-maintained responding in rats. Glutamatergic neurotransmission in the ventral tegmental area (VTA) and nucleus accumbens (NAcc) shell plays an important role in the reinforcing effects of nicotine. To determine the brain sites that may mediate the systemic effects of MPEP on nicotine self-administration, the present study investigated the effects of MPEP microinfusions into the VTA or the NAcc shell on nicotine and food self-administration in separate groups of rats. Administration of low MPEP doses (0, 0.5, 1, and 2 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) microinfused into the NAcc shell had no effect on nicotine self-administration, whereas higher MPEP doses (0, 10, 20, and 40 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) microinfused into the NAcc shell dose-dependently attenuated nicotine self-administration without affecting food-maintained responding. Microinfusions of MPEP into the VTA (0, 10, 20, and 40 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) significantly decreased both nicotine and food self-administration at 20 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$ but did not affect responding for either reinforcer at 40 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$. This lack of effect of 40 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$ MPEP on either nicotine or food self-administration when administered into the VTA may be attributable either to actions of MPEP at presynaptic mGlu5 receptors or at targets other than mGlu5 receptors. Importantly, anatomical control injections 2 mm above the NAcc shell or the VTA using the most effective MPEP dose in the two regions did not result in attenuation of nicotine self-administration. In conclusion, MPEP microinfusions in the VTA or NAcc shell attenuates the reinforcing effects of nicotine possibly via blockade of mGlu5 receptors located in these regions.

1. Introduction

Tobacco dependence in the form of cigarette smoking is a major public health problem that results in significant morbidity and mortality (Murray and Lopez, 1997). The reinforcing effects of nicotine, a major component of tobacco smoke, greatly contribute to the maintenance of the tobacco smoking habit in humans (Goldberg and Henningfield, 1988;

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Stolerman and Jarvis, 1995). However, the neural substrates that mediate the reinforcing effects of nicotine have not been completely identified.

The excitatory neurotransmitter glutamate partially mediates the reinforcing effects of nicotine (Kenny and Markou, 2004; Liechti and Markou, 2008; Markou, 2008). Nicotine enhances glutamatergic transmission via activation of excitatory nicotinic receptors located on presynaptic glutamatergic terminals in the mesocorticolimbic reward pathway (Mansvelder and McGehee, 2002; Schilström et al., 2000). Blockade of glutamatergic transmission, via systemic administration of the noncompetitive selective metabotropic glutamate 5 (mGlu5) receptor antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) (Gasparini et al., 1999; Kuhn et al., 2002) dose-dependently (1-9 mg/kg) decreased nicotine self-administration (Liechti and Markou, 2007; Palmatier et al., 2008; Paterson et al., 2003; Tessari et al., 2004; Tronci et al., 2010). However, the brain sites involved in the blockade of the reinforcing effects of nicotine after systemic MPEP administration remain largely unknown.

The nucleus accumbens (NAcc) shell and ventral tegmental area (VTA) are nuclei of the mesocorticolimbic reward pathway (Koob and Volkow, 2010) and play important roles in mediating the reinforcing effects of nicotine (Corrigall et al., 1994; Corrigall et al., 1992; Ikemoto et al., 2006; Kenny et al., 2009; Liechti et al., 2007; Wang et al., 2007). In addition, both mesolimbic dopaminergic neuronal activity at the level of the VTA and dopamine release in the NAcc shell are regulated by extensive corticolimbic glutamatergic inputs to the VTA and NAcc shell (Geisler and Wise, 2008; Imperato et al., 1990; Mitrano et al., 2010; Overton and Clark, 1997; Taber and Fibiger, 1995). Importantly, mGlu5 receptors are expressed in both the NAcc shell and VTA (Mitrano et al., 2010; Mitrano and Smith, 2007; Prisco et al., 2002; Renoldi et al., 2007; Romano et al., 1995). The present study evaluated the effects of bilateral MPEP microinfusions into the NAcc shell and VTA on intravenous nicotine self-administration in rats.

In separate groups of rats, the effects of MPEP microinfusions in the VTA and NAcc shell were also assessed on food self-administration to determine the specificity of the effects of MPEP on nicotine reward *vs.* natural rewards. The fixed-ratio (FR) schedule of reinforcement used for the food self-administration experiments was identical to that used for nicotine self-administration. These additional groups also helped exclude the possible effects of MPEP microinfusions on motoric and cognitive (e.g., learning and memory) deficits that could potentially interfere with operant responding. Based on the selective attenuation of nicotine self-administration assessed under the FR5 timeout 20 s (TO20 s) schedule after systemic MPEP injections (Paterson et al., 2003), MPEP microinfusions into the NAcc shell and VTA were hypothesized to decrease nicotine, but not food, self-administration.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Raleigh, NC), weighing 300-350 g at the beginning of the experiments, were housed two per cage prior to surgery and singly housed after surgery in an environmentally controlled vivarium on a reversed 12 h/12 h light/dark cycle throughout the experiment. All behavioral testing occurred during the dark phase of the light/dark cycle. Rats had *ad libitum* access to food and water initially and were subsequently put on a food-restricted diet of 20 g rat chow per day during behavioral training and testing. All procedures were conducted in accordance with the guidelines from the National Institutes of Health and the Association for the Assessment and Accreditation of Laboratory Animal Care and were approved by the Institutional Animal Care and Use Committee.

2.2. Apparatus: Testing chambers

Testing chambers, constructed of metal and Plexiglas and housed in sound-attenuated boxes, were used for intravenous nicotine and food self-administration (24 cm × 30 cm × 28 cm; Med Associates, St. Albans, VT). One of the walls of the chamber had two metal retractable levers (3 cm × 1.8 cm each, one active and one inactive) mounted 6.5 cm above the metal grid floor with a stimulus light located above each lever. A food receptacle was located between the two levers. A house light was located on the opposite wall. Food was dispensed via a food dispenser (Med Associates). 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 the other end to the catheter base mounted in the midscapular region of the animal. All self-administration sessions (nicotine and food) and data collection were controlled by a computer using Med PC IV software (Med Associates).

2.3. Drugs

(-)-Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was dissolved in saline (0.9%) and pH-adjusted to 7.4 (± 0.5) with 1 M sodium hydroxide solution. Nicotine doses are reported as freebase concentrations. 2-methyl-6-(phenylethynyl)-pyridine (MPEP) was custom synthesized by American Radiolabeled Chemicals (St. Louis, MO). MPEP doses are reported as the salt based on conventions in the literature. Drug was dissolved in sterile normal saline and microinfused bilaterally into specific brain sites as described below. Brevital sodium (500 mg methohexital sodium; JHP Pharmaceuticals, Rochester, MI) was used to assess the patency of the intravenous catheters.

2.4. Food training

One week after arrival in the laboratory and before intravenous catheterization (for the rats that later self-administered nicotine) and intracranial cannula placement surgery (for all rats), rats were trained to lever press for food (45 mg Noyes food pellets). Training started on an FR1 TO1 s schedule of reinforcement. The schedule was gradually increased over 4 days from FR1 TO1 s to FR1 TO10 s, FR2 TO20 s, and FR5 TO20 s, with sessions lasting 60 min. Animals moved through the sequence only after successful acquisition of the previous schedule (defined as earning 50 pellets during a session). The training period lasted 5 days. An identical training procedure was used for both the food-responding animals and animals that were later allowed to self-administer nicotine.

2.5. Intravenous catheter and Intracranial cannula placement surgery

After the initial food training, all rats underwent surgery for either intracranial cannula implantation (food self-administering rats) or intracranial cannula implantation and intravenous catheterization (nicotine self-administering rats; both cannulae and catheters implanted during the same surgical session). All surgeries were conducted under isoflurane anesthesia (1-3% isoflurane in oxygen mixture). Intravenous catheters were prepared and implanted in the right jugular vein as described previously (Liechti and Markou, 2007). Bilateral intracranial guide cannulae (26 gauge; Plastics One, Roanoke, VA) were implanted 2 mm above the NAcc shell (from bregma: anterior/posterior, 1.7 mm; medial/lateral, ± 0.8 mm; dorsal/ventral, -5.0 mm from dura) or the posterior VTA (from bregma: anterior/posterior, -6.0 mm; medial/lateral, ± 0.6 mm; dorsal/ventral, -5.5 mm from dura; flat skull coordinates; Paxinos and Watson 1998) using a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Four stainless steel screws and dental acrylic held the cannulae in place. To prevent blockage of the intracranial cannulae, stylets of appropriate length (cut flush with the tip of the guide cannulae) were inserted into the cannulae and then covered with dust caps. After surgery, rats with intravenous catheters received daily intravenous infusion (0.1

cc) with the antibiotic Timentin (100 mg/kg/day, intravenous; GlaxoSmithKline, Research Triangle Park, NC) and heparin (30 units/ml) to prevent postoperative infections and to ensure the patency of the catheters until the end of the experiment. Rats with only intracranial cannulae received one intramuscular injection of the antibiotic Baytril (22.7 mg/kg, intramuscular; Bayer Healthcare, Shawnee Mission, KS) immediately after the surgery. Catheter patency was tested with Brevital sodium (500 mg methohexital sodium; JHP Pharmaceuticals, Rochester, MI) whenever an animal not receiving drug pretreatment displayed self-administration behavior outside baseline performance. Animals with patent catheters exhibit pronounced loss of muscle tone within 2 s of an intravenous injection of 0.2 ml Brevital sodium (10 mg/ml).

2.6. Nicotine and food self-administration sessions

After at least 1 week of recovery from surgery, rats were allowed to self-administer either food or nicotine during 1 h sessions on an FR5 TO20 s schedule of reinforcement 5 days/week. Both levers were available during the sessions. Pressing the active lever five times (FR5) resulted in the delivery of either food (45 mg food pellet) or a nicotine solution (0.03 mg/kg/0.1 ml over 1 s) intravenously. In contrast, lever presses on the inactive lever were recorded but had no consequences. A cue-light located above the active lever was lit simultaneously with the initiation of a delivery of either reinforcer and remained illuminated throughout the 20 s timeout period that followed the delivery of the reinforcer. During the timeout period, responding was recorded but not reinforced. MPEP microinfusions (see below) into the NAcc shell or VTA were performed after stable nicotine or food self-administration was achieved. Stable nicotine or food self-administration was defined as active lever presses more than twice the number of inactive lever presses and at least six infusions for the nicotine self-administering rats or 50 food pellets for the food-responding rats during a test session, with less than 20% variability in the number of reinforcements earned over three consecutive daily 1 h session. Animals achieved stable responding within 1 week but were allowed to self-administer for an additional 2 weeks to allow stabilization of nicotine self-administration. Food-responding rats had as many training/test sessions as the nicotine self-administering rats.

2.7. Intracranial MPEP microinfusions

One week prior to the first microinfusions of MPEP or saline into the NAcc shell and VTA, rats were habituated to the microinfusion procedure. These habituation injections were performed after the daily self-administration session until the rats appeared calm during this procedure, which required at least two such mock injections. These habituation injections involved the insertion of a 33 gauge intracranial microinjector (Plastics One) into the intracranial guide cannulae that terminated at the lowermost tip of the intracranial guide cannulae and did not extend beyond. The injectors were connected to an empty 10 μ l Hamilton syringe (Fisher Scientific) placed on a microinfusion pump (model 975; Harvard Apparatus, Holliston, MA) via polyethylene-50 tubing (Plastics One). The pump was switched on for 1 min, although the animals did not receive any infusions during the habituation process. After 1 min, the pump was switched off, but the injectors were left in place for an additional 1 min to mimic the actual infusion procedure.

During the actual drug microinfusions, the 33 gauge microinjectors were extended 2 mm below the tip of the guide cannulae, and MPEP/saline was infused bilaterally into either the NAcc shell or VTA in a volume of 0.5 μ l per side over 1 min using the Harvard microinfusion pump. Injectors were left in place for an additional 1 min after the infusion. Animals were then returned to the home cage for 10 min before being placed into the operant chamber for nicotine or food self-administration sessions. An anatomical control injection was made 2 mm above the actual target site with the most effective MPEP dose in

rats self-administering either nicotine or food after completion of the Latin-square design (see below).

2.8. Experimental design

2.8.1. Experiment 1: Effect of low-dose MPEP microinfusions into the NAcc shell on nicotine self-administration—MPEP (0, 0.5, 1, and 2 $\mu\text{g}/0.5\text{ }\mu\text{l/side}$) was bilaterally microinfused into the NAcc shell of nicotine ($n = 7$) self-administering rats using a within-subjects Latin-square design with a minimum of 3-4 sessions between injections. As none of the doses affected responding for nicotine (see Results), the highest dose (2 $\mu\text{g}/0.5\text{ }\mu\text{l/side}$) was used for the anatomical control injection in nicotine self-administering rats after the completion of the Latin-square design. Because these relatively low doses of MPEP did not affect nicotine self-administration, for the remaining experiments we evaluated the effects of administration of higher MPEP doses into the NAcc shell and VTA on nicotine and food self-administration.

2.8.2. Experiment 2: Effect of high-dose MPEP microinfusions into the NAcc shell on nicotine and food self-administration—MPEP (0, 10, 20, and 40 $\mu\text{g}/0.5\text{ }\mu\text{l/side}$) was bilaterally microinfused into the NAcc shell of food ($n = 8$) and nicotine ($n = 8$) self-administering rats using a within-subjects Latin-square design with a minimum of 3-4 sessions between injections. The 40 $\mu\text{g}/0.5\text{ }\mu\text{l/side}$ dose was the most effective dose in reducing responding for nicotine (see below) and therefore was used for the anatomical control injection. The same dose was also used for the anatomical control injection in animals self-administering food because none of the doses had any effect on food responding (see below).

2.8.3. Experiment 3: Effect of high-dose MPEP microinfusion into the posterior VTA on nicotine and food self-administration—MPEP (0, 10, 20, and 40 $\mu\text{g}/0.5\text{ }\mu\text{l/side}$) was administered bilaterally into the posterior VTA in food ($n = 7$) and nicotine ($n = 7$) self-administering rats using a within-subjects Latin-square design with a minimum of 3-4 sessions between injections. The 20 $\mu\text{g}/0.5\text{ }\mu\text{l/side}$ produced the most pronounced decrease in responding for both food and nicotine (see below); therefore, this dose was used for the anatomical control injection.

2.9. Cannula placement verification and histology

At the end of the experiment, rats were anaesthetized with pentobarbital, and Evans blue dye (0.5 $\mu\text{l/side}$) was microinjected via the intracranial cannulae. Rats were then decapitated, and the brains were removed and fixed in 10% formalin. Coronal sections were sliced on a cryostat to verify the infusion sites.

2.10. Statistical analyses

Data for nicotine and food self-administration were converted into percentage of baseline number of rewards earned, in which baseline was defined as the mean number of rewards earned over the 3 days prior to each MPEP/saline test session. Data were analyzed separately for each brain region using two-factor analyses of variance (ANOVAs; *Reinforcer* $\times$ *MPEP Dose*), with *Dose* as the repeated-measure. *Post hoc* comparisons were made with Newman-Keul's *post hoc* test after statistically significant effects were found in the ANOVA. Value of $p < 0.05$ was considered statistically significant. A paired *t*-test was used to compare the response to the most effective dose in decreasing the number of reinforcers earned when injected into the NAcc shell and VTA with the respective anatomical control injections administered 2 mm dorsal to the primary injection sites. Prism 4.0 (GraphPad, San Diego, CA) was used for graphing and statistical analyses.

3. Results

Fig. 1A and B shows the histologically confirmed locations of microinfusions into the NAcc shell and VTA. Only data from animals with cannulae localized in the NAcc shell or posterior VTA were included in the analyses. Two animals (NAcc: $n = 2$) were excluded for misplaced cannulae, and five animals (NAcc: $n = 3$; VTA: $n = 2$) were excluded because their intravenous catheters did not maintain patency throughout the experiment. Table 1 shows the mean $\pm$ SEM bodyweights at the beginning and end of the experiment for the different experimental groups during the period when MPEP microinfusions were performed. All groups showed increasing bodyweights, suggesting that MPEP microinfusions did not adversely affect weight gain in animals regardless of the region of injection.

3.1. Effect of low-dose MPEP microinfusions into the NAcc shell on nicotine self-administration

A one-way ANOVA indicated no significant differences in baseline nicotine infusions obtained prior to each microinfusion of MPEP or saline. The overall mean $\pm$ SEM of the basal nicotine infusions was 11.17 ± 0.41 . A one-way ANOVA on the drug effect data showed no significant effect of the low doses of MPEP on nicotine self-administration (Table 2).

3.2. Effect of high-dose MPEP microinfusions into the NAcc shell on nicotine self-administration and food-reinforced responding

A one-way ANOVA indicated no significant differences in baseline rewards for either nicotine or food prior to each microinfusion of MPEP or saline. The overall means $\pm$ SEM of the basal nicotine and food rewards were 11.15 ± 0.23 and 109.25 ± 2.72 , respectively. A two-way ANOVA on the drug effect data transformed into percentage change of baseline showed a significant main effect of *Reinforcer* ($F_{1,48} = 9.2$, $p < 0.01$), a main effect of *Dose* ($F_{3,48} = 2.96$, $p < 0.05$), and an interaction effect ($F_{3,48} = 4.03$, $p < 0.01$; Fig. 2A). The Newman-Keuls *post hoc* test showed a significant percentage decrease in responding for nicotine compared with food at $40 \mu\text{g}/0.5\mu\text{l}/\text{side}$ ($p < 0.01$). Further *post hoc* tests showed a significant percentage decrease in responding for nicotine at the $40 \mu\text{g}/0.5\mu\text{l}/\text{side}$ dose compared with the saline and $10 \mu\text{g}/0.5\mu\text{l}/\text{side}$ conditions. A paired *t*-test also revealed significant difference ($p < 0.05$) between the percentage change from baseline for nicotine reward at the $40 \mu\text{g}/0.5\mu\text{l}/\text{side}$ dose compared with the anatomical control injection of the same dose 2 mm above the NAcc shell site.

Analysis of the actual number of nicotine infusions and food rewards earned after MPEP microinfusion in the NAcc shell (i.e., data without percentage change transformation) yielded similar results to those described above for the percentage data. A one-way ANOVA on the number of nicotine infusions after microinjection of different MPEP doses in the NAcc shell showed a significant effect of *Dose* ($F_{3,24} = 3.901$, $p < 0.01$). The Newman-Keuls *post hoc* test showed a significant decrease in responding for nicotine at $40 \mu\text{g}/0.5\mu\text{l}/\text{side}$ compared with the saline condition ($p < 0.01$). A one-way ANOVA on the number of food rewards showed no significant effect of MPEP microinfusions in the NAcc shell on food responding at any dose.

3.3. Effect of MPEP microinfusions into the VTA on nicotine self-administration and food-reinforced responding

A one-way ANOVA indicated no significant differences in baseline rewards for either nicotine or food prior to each microinfusion of MPEP or saline in the Latin-square design. The overall means $\pm$ SEM of the basal nicotine and food rewards were 10.52 ± 0.53 and

99.18 $\pm$ 2.72, respectively. A two-way ANOVA showed no significant effect of *Reinforcer* or *Dose* and no *Reinforcer* $\times$ *Dose* interaction (Fig. 3). However, visual examination of the data revealed a possible dose-dependent-like decrease in the number of both food and nicotine reinforcers earned at the lowest MPEP doses tested. Therefore, a one-way, repeated-measures ANOVA that compared the percentage change from baseline of reinforcers earned after saline and the two lowest MPEP doses was performed. This ANOVA revealed a significant percentage decrease in nicotine reinforcement after administration of MPEP microinfusions in the VTA ($F_{2,12} = 4.16$, $p < 0.05$). The Newman-Keuls *post hoc* test showed that this effect was attributable to a significant percentage decrease in nicotine reinforcement at the 20 $\mu\text{g}/0.5\mu\text{l}/\text{side}$ dose compared with the saline control condition. A one-way, repeated-measure ANOVA using all MPEP doses did not show any significant percentage change in food responding after microinfusion of MPEP into the VTA. A paired *t*-test showed a significant percentage decrease in responding for both nicotine and food after administration of the 20 $\mu\text{g}/0.5\mu\text{l}/\text{side}$ dose compared with their respective anatomical control injections of the same dose ($p < 0.05$).

Analysis of the raw number of nicotine infusions and food rewards after MPEP microinfusions in the VTA (i.e., data without percentage change transformation) yielded similar results to those described above. A one-way, repeated-measures ANOVA using all MPEP doses showed no significant effect on the number of nicotine infusions after microinfusion of MPEP in the VTA. However, a paired *t*-test indicated a significant decrease ($p < 0.05$) in the number of nicotine infusions after 20 $\mu\text{g}/0.5\mu\text{l}/\text{side}$ MPEP compared with number of nicotine infusions earned after saline microinfusions. A oneway, repeated-measures ANOVA using all MPEP doses showed no significant effect of MPEP microinfusions in the VTA on food responding. However, a paired *t*-test indicated a significant decrease in the number of food rewards after MPEP 20 $\mu\text{g}/0.5\mu\text{l}/\text{side}$ compared to the saline condition ($p < 0.05$).

4. Discussion

The main finding of this study was that administration of the mGlu5 receptor antagonist MPEP directly into the NAcc shell dose-dependently decreased nicotine self-administration without affecting food self-administration. In contrast, MPEP microinfusion into the VTA decreased responding for both nicotine and food at the two lower doses tested but had no effect on responding for either reinforcer at the highest tested dose.

In the present study, low MPEP doses (0.5-2 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) did not decrease nicotine-self-administration when administered into the NAcc shell (Table 2). In a previously published study, MPEP (3-10 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) microinfused into the NAcc shell attenuated ethanol self-administration (Besheer et al., 2009). Neuroadaptations in mGlu5 receptor expression have been reported after chronic exposure to psychostimulant drugs, such cocaine and nicotine (Ghasemzadeh et al., 1999; Kenny et al., 2003). Thus, it is possible that neuroadaptations in mGlu5 receptors after chronic nicotine exposure contributed to the lack of effects of low doses (0.5-2 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) of MPEP microinfusions in the NAcc shell on nicotine self-administration in rats with chronic nicotine self-administration experience. Furthermore, the highest effective MPEP dose (10 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) in the Besheer and colleagues (2009) study, was not effective in attenuating nicotine self-administration when infused into the NAcc shell of rats with chronic nicotine self-administration experience in the present study (Experiment 2; Fig. 2). Therefore, subsequent experiments in this study used higher MPEP doses (10-40 $\mu\text{g}/0.5\mu\text{l}/\text{side}$).

The selective and dose-dependent decrease in nicotine self-administration after microinfusion of higher MPEP doses (10-40 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) into the NAcc shell is consistent

with similar effects seen with systemic administration of MPEP on nicotine self-administration (Liechti and Markou, 2007; Paterson and Markou, 2005; Paterson et al., 2003). These findings also extend our previous findings, which showed attenuation of nicotine self-administration after a decrease of glutamatergic transmission in the NAcc shell via activation of predominantly presynaptic inhibitory mGlu2/3 receptors (Liechti et al., 2007). Importantly, anatomical control injections 2 mm above the NAcc shell into the lateral septal nucleus using the MPEP dose that was most effective in decreasing nicotine self-administration when administered into the NAcc shell (40 µg/0.5µl/side) did not result in attenuation of nicotine self-administration compared to the vehicle condition.

MPEP microinfusions into the NAcc shell did not affect food-maintained responding under the FR5 TO20 s schedule of reinforcement at doses that affected nicotine self-administration. Systemically administered MPEP (Paterson et al., 2003) or microinfusions of another mGlu5 receptor antagonist, 3-(2-methyl-1,3-thiazol-4-yl)ethynylpyridine (MTEP; Gass and Olive, 2009) into the NAcc shell also did not affect food-maintained responding under the same FR5 TO20 s schedule of reinforcement. Finally, consistent with our present findings, a decrease in glutamatergic transmission in the NAcc shell by blocking presynaptic mGlu2/3 receptors using the mGlu2/3 receptor agonist LY379268 also had no impact on food self-administration at doses that decreased nicotine self-administration (Liechti et al., 2007).

This reinforcer-selective decrease in nicotine self-administration suggests that the attenuation of nicotine self-administration was not attributable to an anhedonia- or dysphoria-like state or nonspecific motor- or cognitive-impairing effects induced by MPEP administration. Interestingly, few studies have reported that MPEP may have reinforcing effects on its own (van der Kam et al., 2009a, b). Thus, the decrease in nicotine self-administration could be attributable to a substitution of the reinforcing effects of nicotine by MPEP. However, this interpretation is unlikely because MPEP microinfusions into the NAcc shell did not affect food self-administration. Furthermore, the majority of the currently available literature shows that MPEP has no reinforcing effect on its own when tested using the conditioned place preference procedure (Aoki et al., 2004; Herzig et al., 2005; Herzig and Schmidt, 2004; Popik and Wrobel, 2002). In addition, systemic administration of MPEP did not lower brain reward thresholds, suggesting that MPEP does not have reward-enhancing properties (Harrison et al., 2002) that are commonly induced by drugs that have intrinsic reinforcing properties of their own (Kornetsky and Esposito, 1979). In fact, at the highest doses tested, MPEP elevated brain reward thresholds, suggesting a mild dysphoric effect (Harrison et al., 2002). Finally, the aforementioned reinforcing effect of MPEP in the conditioned place preference procedure has only been seen after intravenous MPEP administration and not after intraperitoneal or intracranial administration into discrete brain sites.

The VTA is a heterogeneous structure and can be broadly divided into anterior and posterior regions (Ikemoto, 2007). In the present study, MPEP microinfusions were made into the posterior VTA because rats have been shown to self-administer nicotine in the posterior VTA and not anterior VTA (Ikemoto et al., 2006). Furthermore, blockade of glutamatergic transmission in the posterior VTA by either activating inhibitory presynaptic mGlu2/3 receptors (Liechti et al. 2007) or blocking postsynaptic *N*-methyl-D-aspartate (NMDA) receptors (Kenny et al. 2009) attenuated nicotine self-administration. Importantly, fibers from the posterior VTA project to the NAcc shell (Ikemoto, 2007), which is the other brain target assessed in the present study. Nevertheless, the anterior VTA may also be involved in nicotine dependence. Animals chronically exposed to nicotine showed decreased sensitivity of dopamine D₁-like, but not dopamine D₂-like, receptors in the anterior VTA (Bruijnzeel and Markou, 2005).

In the present study, MPEP microinfusions into the posterior VTA had similar effects on nicotine and food self-administration. MPEP decreased nicotine and food self-administration after bilateral administration of either 10 or 20 $\mu\text{g}/0.5\mu\text{l}/\text{side}$, whereas no effect was observed on responding for either reinforcer after administration of 40 $\mu\text{g}/0.5\mu\text{l}/\text{side}$. These data suggest that mGlu5 receptors in the VTA are critical for maintaining reward-dependent instrumental responding. Consistent with these data, a decrease in glutamatergic transmission in the VTA by administration of the amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainate (AMPA) receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) decreased both nicotine and sucrose self-administration in rats (Wang et al., 2008). In contrast, blockade of glutamatergic activity via activation of predominantly presynaptic mGlu2/3 receptors in the VTA selectively attenuated nicotine self-administration and did not affect food self-administration (Liechti et al., 2007). Furthermore, blockade of glutamatergic transmission by blockade of ionotropic NMDA receptors in the VTA, similar to blockade of mGlu5 receptors in the same area, dose-dependently attenuated nicotine self-administration (D'Souza and Markou, unpublished observations; Kenny et al., 2009). These previous studies, however, did not assess the effects of NMDA antagonist microinfusions into the VTA on food self-administration. Importantly, anatomical control injections 2 mm above the VTA into the red nucleus using the most effective MPEP dose in decreasing nicotine self-administration when administered into the VTA (20 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) did not result in attenuation of nicotine self-administration compared to the vehicle condition. Altogether, these data suggest that glutamatergic neurotransmission in the VTA is critical for the reinforcing effects of nicotine. However, the decrease in food self-administration seen after blockade of postsynaptic glutamatergic receptors suggests that glutamatergic activity via these postsynaptic receptors may be required for the maintenance of operant-seeking regardless of the type of reinforcer.

The lack of effects of the highest tested MPEP dose (40 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) on nicotine and food self-administration when microinjected into the VTA could be due to activity of MPEP at off-site targets or an interaction between mGlu5 receptors and other receptors. MPEP, although relatively selective for mGlu5 receptors, also binds to other targets, such as the mGlu4 and NMDA receptors and the norepinephrine transporter (Heidbreder et al., 2003; Mathiesen et al., 2003; O'Leary et al., 2000). Furthermore, interactions between mGlu5 receptors and other receptor subtypes, including NMDA (glutamate), D_1 (dopamine), and CB_1 (endocannabinoids), are also known (Martin et al., 1997; Robbe et al., 2002; Schotanus and Chergui, 2008). These interactions of MPEP with receptors/transporters other than mGlu5 receptors may have neutralized the attenuating effects of MPEP on nicotine and food self-administration. Another possible reason for the lack of effects of MPEP infusions (40 $\mu\text{g}/0.5\mu\text{l}/\text{side}$) into the VTA on nicotine and food reinforcement could be due to action of MPEP at presynaptic mGlu5 receptors in the VTA. Although mGlu5 receptors are predominantly postsynaptic, some evidence suggests that mGlu5 receptors may be located on axon terminals and therefore could act as presynaptic autoreceptors also (Romano et al., 1995). Blockade of presynaptic mGlu5 receptors by MPEP could theoretically have opposite effects to those induced by blockade of postsynaptic mGlu5 receptors.

Nicotine (0.03 mg/kg/infusion) self-administration in rats is attenuated after intraperitoneal administration of 9 mg/kg MPEP administered 30 min prior to a nicotine self-administration session (Paterson et al., 2003). The precise concentrations of MPEP after systemic administration of this dose in the NAcc and VTA have not been determined. One study has reported MPEP concentrations in the hippocampus 1 h after systemic intraperitoneal administration of 3 mg/kg MPEP to be in the range of $0.8 \pm 0.05\mu\text{M}$ (Cosford et al., 2003). As mentioned above, in the present study, infusions of 3-7 μM MPEP concentration in the NAcc did not have any effect on nicotine self-administration; MPEP was effective in decreasing nicotine self-administration only when infused at higher concentration (70-300

μM) in the NAcc shell or VTA. Thus, the concentrations of MPEP required to decrease nicotine self-administration appear to be higher than those obtained in the brain after systemic MPEP injections. However, it must be pointed out that systemically administered MPEP blocks mGlu5 receptor activity in several brain regions at the same time, and the aggregate of these multisite effects likely leads to the observed behavioral effects. In contrast, localized microinjections of MPEP blocks mGlu5 receptor activity in only a specific brain region, such as the NAcc shell or VTA, and therefore complete blockade of all mGlu5 receptors and/or off target activity of MPEP at this specific brain site may be required to induce the behavioral effect. Furthermore, it should be noted that injection concentrations may be much higher than concentrations achieved at the synapse. Taken together, the findings of the present study therefore suggest that while the NAcc shell and VTA play an important and sufficient role in mediating the effects of systemically administered MPEP, other brain regions are also possibly involved in mediating the effects of systemically administered MPEP.

In summary, MPEP microinfusions in the NAcc shell selectively attenuated nicotine self-administration without affecting responding for a food reinforcer, whereas MPEP microinfusions in the VTA had similar effects on both nicotine and food self-administration. These effects of MPEP are possibly due to blockade of mGlu5 receptor activity in mesolimbic brain sites, such as the VTA and NAcc shell, although offsite effects of MPEP cannot be precluded. Thus, our study extends the existing body of literature on the role of mGlu5 receptors in the reinforcing effects of nicotine by identifying brain sites that mediate the actions of the mGlu5 receptor antagonist MPEP. The present data also suggest that mGlu5 receptors in the VTA possibly mediate the reinforcing effects of both drug and natural reinforcers and may be critical for the maintenance of motivated operant responding.

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Abbreviations

VTA	ventral tegmental area
NAcc	nucleus accumbens
MPEP	2-methyl-6-(phenylethynyl)pyridine
AMPA	amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainate
NMDA	<i>N</i> -methyl-D-aspartate
FR5 TO20s	fixedratio 5 timeout 20 s
CPP	conditioned place preference

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Research Highlights

Mesolimbic mGlu5 receptors play a role in nicotine reinforcement

Blockade of mGlu5 receptors in the NAcc attenuates nicotine intake

mGlu5 receptors in the VTA regulate both nicotine and food intake

Diagrams of coronal sections from the rat brain depicting injection sites in the NAcc shell

B

Diagrams of coronal sections from the rat brain depicting injection sites in the

VTA**Figure 1.**

Diagrams of coronal sections from the rat brain (Paxinos and Watson, 1998) that depict injection sites in the NAcc shell (A) and ventral tegmental area (B) from the rats whose data are shown in Fig. 2 and 3. Black filled circles and empty circles indicate histologically confirmed locations of the injector tips in nicotine and food self-administering animals, respectively. Numbers next to the respective coronal section indicate distance from the bregma in millimeters.

Effect of intra NAcc shell MPEP administration on nicotine and food self-administration

Figure 2.

Effects of microinfusions of the mGlu5 receptor antagonist MPEP into the NAcc shell on nicotine ($n = 8$) and food self-administration ($n = 8$) as assessed under a fixed-ratio 5 timeout 20 s (FR5 TO20 s) schedule of reinforcement in rats. Data are expressed as the percentage of the number of rewards earned under baseline conditions (mean $\pm$ SEM), in which baseline is defined as the mean number of rewards earned over the 3 days prior to each MPEP test session. $*p < 0.01$, significant difference between the food and nicotine self-administering groups at 40 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$; $+p < 0.05$, significant difference between saline and the 40 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$ condition; $##p < 0.01$, significant difference between the 10 and 40 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$ conditions; $@p < 0.05$, significant decrease in responding for nicotine compared with the corresponding anatomical control injection of the same MPEP dose performed 2 mm above the actual site.

Effect of intra VTA MPEP administration on nicotine and food self-administration

Figure 3.

Effects of microinfusions of the mGlu5 receptor antagonist MPEP into the VTA on nicotine ($n = 7$) and food self-administration ($n = 7$) assessed under a fixed-ratio 5 timeout 20 s (FR5 TO20 s) schedule of reinforcement in rats. Data are expressed as the percentage of the number of rewards earned under baseline conditions (mean $\pm$ SEM), in which baseline is defined as the mean number of rewards earned over the 3 days prior to each MPEP test session. * $p < 0.05$, significant decrease in responding for nicotine between the 20 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$ and saline control conditions; # $p < 0.05$, significant difference between the food and nicotine self-administering groups at 20 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$ and their respective anatomical control injections.

Table 1

Bodyweights of rats at the beginning and end of the experiments for each of the experimental groups during the period when MPEP microinfusions were performed (mean $\pm$ SEM).

Group	Bodyweight at the beginning of the experiment (g)	Bodyweight at the end of the experiment (g)
NAcc shell nicotine	398 $\pm$ 7.7	418 $\pm$ 7.05
NAcc shell food	352.2 $\pm$ 4.14	418 $\pm$ 6.05
VTA nicotine	399.57 $\pm$ 8.40	418.14 $\pm$ 6.52
VTA food	341.66 $\pm$ 5.92	402.83 $\pm$ 7.89

NAcc shell, nucleus accumbens shell; VTA, ventral tegmental area.

Table 2

Effects of microinfusions of low doses of MPEP into the NAcc shell on intravenous nicotine self-administration in rats (baseline nicotine infusions = 11.17 ± 0.41 ; $n = 7$).

MPEP dose ($\mu\text{g}/0.5\mu\text{l}/\text{side}$)	Percentage of baseline (mean $\pm$ SEM)
Saline	93.3 ± 7.70
0.5	97.20 ± 13.83
1	94.02 ± 9.96
2	97.54 ± 11.77
Anatomical control (2)	100.08 ± 13.38