

The Metabotropic Glutamate 2/3 Receptor Agonist LY379268 Blocked Nicotine-Induced Increases in Nucleus Accumbens Shell Dopamine only in the Presence of a Nicotine-Associated Context in Rats

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The metabotropic glutamate 2/3 (mGlu2/3) receptor agonist LY379268 ([–]-2-oxa-4-aminobicyclo [3.1.0] hexane-4,6-dicarboxylate) attenuates both nicotine self-administration and cue-induced nicotine seeking in rats. In this study, the effects of LY379268 (1 mg/kg) or saline pretreatment on nicotine-induced increases in nucleus accumbens (NAcc) shell dopamine were evaluated using *in vivo* microdialysis under different experimental conditions: (i) nicotine (0.4 mg/kg, base) was experimenter-administered subcutaneously to nicotine-naïve rats; (ii) nicotine was experimenter-administered either subcutaneously (0.4 mg/kg) or by a single experimenter-administered infusion (0.06 mg/kg, base) in rats with a history of nicotine self-administration (nicotine experienced) in the absence of a nicotine-associated context (ie, context and cues associated with nicotine self-administration); (iii) nicotine (0.06 mg/kg) was self-administered or experimenter-administered in nicotine-experienced rats in the presence of a nicotine-associated context. In saline-pretreated nicotine-naïve and nicotine-experienced rats, nicotine increased NAcc shell dopamine regardless of the context used for testing. Interestingly, LY379268 pretreatment blocked nicotine-induced increases in NAcc shell dopamine in nicotine-experienced rats only when tested in the presence of a nicotine-associated context. LY379268 did not block nicotine-induced increases in NAcc shell dopamine in nicotine-naïve or -experienced rats tested in the absence of a nicotine-associated context. These intriguing findings suggest that activation of mGlu2/3 receptors negatively modulates the combined effects of nicotine and nicotine-associated contexts/cues on NAcc dopamine. Thus, these data highlight a critical role for mGlu2/3 receptors in context/cue-induced drug-seeking behavior and suggest a neurochemical mechanism by which mGlu2/3 receptor agonists may promote smoking cessation by preventing relapse induced by the combination of nicotine and nicotine-associated contexts and cues.

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INTRODUCTION

The reinforcing effects of nicotine, a major psychoactive component of tobacco smoke, are largely responsible for the maintenance of the tobacco smoking habit in human beings (Goldberg and Henningfield, 1988; Stolerman and Jarvis, 1995). The mesolimbic dopaminergic system, originating in the ventral tegmental area (VTA) and projecting to several cortical and limbic nuclei, including the nucleus accumbens (NAcc) shell, is critically involved in the motivational and

rewarding properties of drugs of abuse (Wise, 1987; Koob and Volkow, 2010), including nicotine (Balfour, 2009; Di Chiara, 2000; Picciotto and Corrigall, 2002; Watkins *et al*, 2000, but also see Laviolette and van der Kooy, 2004). The activity of mesolimbic dopaminergic neurons is regulated by glutamatergic inputs to the VTA from several cortical and subcortical regions (Overton and Clark, 1997; Taber *et al*, 1995; Taber and Fibiger, 1995). Nicotine increases glutamatergic transmission by activating excitatory nicotinic receptors located on presynaptic glutamatergic terminals (Fu *et al*, 2000; Mansvelder and McGehee, 2002; Reid *et al*, 2000). Blockade of glutamatergic neurotransmission by systemic administration of receptor antagonists that act at postsynaptically located ionotropic or metabotropic glutamate (mGlu) receptors attenuated both nicotine self-administration (Kenny *et al*, 2009, 2003; Liechti and Markou, 2007; Palmatier *et al*, 2008; Paterson and Markou, 2005;

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Paterson *et al*, 2003) and nicotine-induced increases in NAcc dopamine release (Fu *et al*, 2000; Kosowski *et al*, 2004; Schilstrom *et al*, 1998; Sziraki *et al*, 2002).

Similarly, activation of predominantly presynaptic metabotropic glutamate 2/3 (mGlu2/3) receptors results in decreased glutamate transmission (Cartmell and Schoepp, 2000; Xi *et al*, 2002a) and had the same effects on nicotine self-administration as blockade of postsynaptic glutamate receptors. Specifically, systemic, intra-VTA or intra-NAcc shell administration of the mGlu2/3 receptor agonist LY379268 ([−]-2-oxa-4-aminobicyclo [3.1.0] hexane-4, 6-dicarboxylate) attenuated nicotine self-administration in rats at doses that had no effect on responding for food (Liechti *et al*, 2007). In addition, systemic LY379268 administration blocked cue-induced reinstatement of both nicotine- and food-seeking behavior in rats (Liechti *et al*, 2007). Because mGlu2/3 receptor activation negatively regulates glutamatergic neurotransmission (Cartmell and Schoepp, 2000), we hypothesized that LY379268 decreases the reinforcing effects of nicotine by attenuating nicotine-induced increases in NAcc shell dopamine. This hypothesis is supported by the observation that mGlu2/3 receptors are expressed in the NAcc shell (Testa *et al*, 1998), and these receptors regulate both basal and psychostimulant-induced NAcc dopamine neurotransmission (Greenslade and Mitchell, 2004; Hu *et al*, 1999; Karasawa *et al*, 2006; Kim *et al*, 2005; Manzoni *et al*, 1997; Uslaner *et al*, 2009; Xi *et al*, 2002a, 2010a, b). Furthermore, activation of mGlu2/3 receptors attenuated the dopamine-dependent psychostimulant-induced enhancement of locomotor activity (Kim *et al*, 2005; Riegel *et al*, 2003).

This study assessed the effects of systemic administration of the mGlu2/3 receptor agonist LY379268 on nicotine-induced increases in dopamine in the NAcc shell using *in vivo* microdialysis in rats. First, the effects of LY379268 pretreatment on experimenter-administered subcutaneous nicotine on NAcc shell dopamine was determined in both nicotine-naïve (ie, rats with no history of nicotine exposure) and nicotine-experienced rats (ie, rats with a history of nicotine self-administration) in an environment never associated with nicotine. The effects of LY379268 in nicotine-experienced animals were evaluated because chronic nicotine self-administration results in functional adaptations in mGlu2/3 receptors in cortical and limbic brain sites, including the VTA and NAcc shell (Liechti *et al*, 2007). Surprisingly, data from these initial experiments indicated that LY379268 did not influence NAcc dopamine increases induced by subcutaneous administration of nicotine. Because differential neurochemical responses have been reported after self- vs experimenter administration of the same drug (Hemby *et al*, 1997; Lecca *et al*, 2007), the effects of LY379268 on NAcc shell dopamine after a single self- or experimenter-administered infusion of nicotine in nicotine-experienced rats was assessed. Findings from these experiments indicated the reversal of nicotine-induced increases in NAcc shell dopamine in LY379268-pretreated rats independent of whether the infusion was self- or experimenter-administered. Because these latter positive findings with LY379268 on nicotine-induced increases in NAcc shell dopamine were seen in rats tested in the presence of a context associated with nicotine self-administration (defined as the combination of both context and cues),

whereas the initial negative findings involved testing the rats in a context never associated with nicotine administration, we hypothesized that the presence of a nicotine-associated context was critical to the effects of LY379268. To test this hypothesis, a final experiment assessed the effects of LY379268 pretreatment on NAcc shell dopamine after a single experimenter-administered infusion of nicotine in nicotine-experienced rats in a context never associated with nicotine self-administration.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River, Raleigh, NC) weighing 275–300 g upon arrival at the laboratory were kept on a reverse 12/12 h light/dark cycle. All procedures were 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.

Drugs

(−)Nicotine hydrogen tartrate (Sigma, St Louis, MO) was dissolved in saline (0.9%) and pH adjusted to 7.4 (\pm 0.5) with 1 M sodium hydroxide (NaOH). Nicotine doses are reported as base concentrations. LY379268 was custom synthesized as described previously (Monn *et al*, 1999) by ANAWA (Wangen, Switzerland). LY379268 (1 mg/kg, salt) was dissolved in 0.9% saline and pH-adjusted to 7.4 (\pm 0.5) with 1 M NaOH.

Apparati

Chambers A: Six custom-made *in vivo* microdialysis chambers (30 × 30 × 35 cm³) had Plexiglas walls, a Plexiglas roof, and a metal grid floor and were housed in sound-attenuating boxes (Kuczenski and Segal, 1989). No levers or lights (stimulus or house lights) were present in these chambers. One of the walls had an opening for food and water 3 cm above the grid floor. The chambers were illuminated by a red light located on one of the inner walls of the sound-attenuating boxes. These chambers were used to conduct microdialysis sessions for nicotine-naïve rats and nicotine-experienced rats in a context not associated with nicotine self-administration.

Chambers B: The operant chambers used for nicotine self-administration and microdialysis sessions in a context associated with nicotine were standard operant chambers (24 × 30 × 28 cm³; Med Associates, St Albans, VT) that had two walls and a roof made of Plexiglas, two walls made of metal, and a metal grid floor. One of the metal walls had two metal retractable levers (each 3 × 1.8 cm², one active and one inactive) mounted 6.5 cm above the metal grid floor with a stimulus/cue light located above each lever. A house light was located in the center of the opposite metal wall 1 cm from the roof of the chamber. These operant chambers were housed in sound-attenuating boxes (Med Associates).

Surgery

An intracranial guide cannula (CMA 12; CMA Microdialysis, Stockholm, Sweden) was implanted 2 mm above the NAcc shell (flat skull coordinates from bregma: anterior/posterior 1.7 mm, medial/lateral \pm 0.8 mm, dorsal/ventral $-$ 4.8 mm from dura; Paxinos and Watson, 1998), using a stereotaxic frame (David Kopf Instruments, Tujunga, CA) under isoflurane (1–3%) anesthesia. Cannulae and a slotted tether screw were anchored to the skull with four stainless-steel screws and dental cement. Self-administering rats (Experiments 2 and 3a–c) were also prepared with intravenous catheters in the jugular vein as described previously (Liechti and Markou, 2007) during the same surgical session. Rats were individually housed after surgery and allowed to recover for at least 1 week before the microdialysis session (Experiment 1) was conducted or before initiating the intravenous nicotine self-administration sessions (Experiments 2 and 3a–c).

Microdialysis Probes

The inlet and outlet of the microdialysis probes (CMA Elite 12; 2 mm active membrane; molecular weight cutoff, 20 000 Da; CMA Microdialysis) were made of Micro-Line Tygon tubing (flexible plastic tubing; 0.020 in inner diameter; 0.060 in outer diameter; Norton Performance Plastics, Norton, OH) and FEP microtubing (CMA Microdialysis; volume, 1.2 μ l/10 cm), respectively. A microinfusion pump (Harvard Apparatus 22) was used to perfuse the probes with artificial cerebrospinal fluid (147 mM NaCl, 1.2 mM CaCl₂, 0.9 mM MgCl₂, 4.0 mM KCl). Probe recovery was performed by sampling a standard dopamine solution *in vitro* (Kuczenski et al, 1997; Kuczenski and Segal, 1997). *In vitro* probe recovery was 8–17% for dopamine, with a mean of $11.49 \pm 0.8\%$.

In Vivo Microdialysis Sampling

Microdialysis sessions were conducted in animals either 1 week after surgery (Experiment 1) or 24 h after the last nicotine self-administration session, which was approximately 4 weeks after surgery (Experiments 2 and 3a–c). Microdialysis probes were introduced into the NAcc shell via the guide cannula, at least 18 h (habituation period) before the collection of the dialysis samples, using either brief isoflurane (Experiments 1 and 2; 1–3%) or methohexital sodium anesthesia (Experiments 3a–c; 0.2 ml of 50 mg/ml solution; that is, approximately 25 mg/kg; 500 mg Brevital sodium; JHP Pharmaceuticals, Rochester, MI). In Experiments 3a–c, the use of methohexital helped with both probe introduction and ensuring catheter patency because the animals received nicotine intravenously during the dialysis session in these experiments. After the introduction of the probes in the NAcc shell, the animals were placed in either Chambers A (Experiments 1, 2, and 3c) or Chambers B (Experiments 3a and b) for the entire habituation period (18 h) and subsequent microdialysis sampling. To allow free movement of the animals during the dialysis session, the animals were attached by a head block tether assembly (M115; Instech Laboratories, Plymouth Meeting, PA) to a swivel (375/22PS; Instech Laboratories) and a counter-

balanced lever arm. During the habituation period, all animals were provided with food and water, and their microdialysis probes were perfused with artificial cerebrospinal fluid (described above) at a rate of 0.2 μ l/min. This procedure was followed in all experiments to allow adequate equilibration of the dialysis probes and acclimatize the rats to the dialysis chamber before dialysate sampling.

The rate at which artificial cerebrospinal fluid was perfused through the probes was increased to 1.5 μ l/min at least 60 min before the collection of the baseline dialysate samples. In Experiments 1 and 2, the dialysate was collected every 20 min outside of the dialysis chamber into 200 μ l conical polyethylene vials (Sun Sri, Rockwood, TN) prefilled with 20 μ l of 0.2 M sodium citrate in 20% methanol, pH 4.0. In Experiments 3a–c, the dialysate was collected every 10 min into 200 μ l conical glass vials (C4012-529L; National Scientific, Rockwood, TN) prefilled with 10 μ l of 0.2 M sodium citrate in 20% methanol, pH 4.0. For the microdialysis sessions, the rats were randomly assigned to saline or LY379268 pretreatment groups, ensuring that rats from each experimental group within an experiment were equally represented in each dialysis session. The saline/LY379268 pretreatment was always experimenter-administered via a subcutaneous injection. All animals were habituated to the subcutaneous injections before the microdialysis sessions. Nicotine administration during the dialysis sessions was determined by the aim of each experiment. Details about the nicotine self-administration sessions (Experiments 2 and 3a–c) and the microdialysis sessions for all experiments are provided in the next subsection.

Experimental Design

Experiment 1: effects of LY379268 pretreatment and subcutaneous experimenter-administered nicotine on NAcc shell dopamine in nicotine-naïve rats. Nicotine-naïve rats were surgically prepared with an intracranial guide aimed 2 mm above the NAcc shell. After at least 1 week of recovery from surgery, a microdialysis probe was inserted through the guide cannula under brief isoflurane anesthesia at least 18 h before the initiation of the dialysis session. The rats were housed in Chambers A (described above) for the habituation period and subsequent dialysate sampling. Initially, three baseline samples were collected during the first hour. Subsequently, rats were randomly assigned to one of five treatment groups, each treated with two subcutaneous injections separated by 20 min: (i) saline + nicotine (0.4 mg/kg); (ii) LY379268 (1 mg/kg) + nicotine (0.4 mg/kg); (iii) LY379268 (1 mg/kg) + saline; (iv) LY379268 (3 mg/kg) + nicotine (0.4 mg/kg); and (v) LY379268 (3 mg/kg) + saline. Samples were collected every 20 min for 3 h after the subcutaneous administration of nicotine.

Experiment 2: effects of LY379268 pretreatment and subcutaneous experimenter-administered nicotine on NAcc shell dopamine in nicotine-experienced rats. As described previously (Liechti et al, 2007), the rats were initially trained to lever press for food (45 mg Noyes food pellets) in Chambers B (these chambers were later used for nicotine self-administration for these animals, but the dialysis

session was not conducted in these chambers, but rather in Chambers A; see below). The rats then underwent a single surgical session during which an intracranial guide cannula was implanted 2 mm above the NAcc shell, and an intravenous catheter was inserted into the jugular vein. After at least 1 week of recovery from surgery, the rats were allowed to self-administer nicotine (0.03 mg/kg per infusion in 0.1 ml over 1 s) under a fixed-ratio 5, timeout 20 s (FR5 TO 20 s) schedule of reinforcement during 1 h sessions in Chambers B for 3 weeks (5 days per week). The delivery of an infusion was paired with a cue light located above the active lever that was lit simultaneously with the initiation of the nicotine infusion and remained illuminated throughout the 20-s timeout period, during which responding was recorded but not reinforced. Responses on the inactive lever were recorded but had no consequences.

Microdialysis probes were inserted in the NAcc shell through the intracranial guide cannula under brief isoflurane anesthesia at least 18 h before the initiation of dialysate sampling. In addition, as in Experiment 1, the animals were housed in Chambers A for the habituation period and subsequent dialysate sampling. Chambers A were never associated with nicotine self-administration, and these animals were not exposed to these chambers before the 18-h habituation period and dialysis session. During the dialysis session, the rats were randomly assigned to one of three treatment groups, each treated with two subcutaneous injections separated by 20 min: (i) saline + nicotine (0.4 mg/kg); (ii) LY379268 (1 mg/kg) + nicotine (0.4 mg/kg); and (iii) LY379268 (1 mg/kg) + saline. Dialysate samples were collected every 20 min as in Experiment 1.

Experiments 3a–c: effects of LY379268 pretreatment and a single intravenous nicotine infusion (self- or experimenter-administered) on NAcc shell dopamine in nicotine-experienced rats either in the presence or absence of a nicotine-associated context. Food training and the surgical procedures were identical to those used in Experiment 2. After recovery from surgery, the animals were allowed to self-administer nicotine in Chambers B. Rats initially self-administered 0.03 mg/kg per infusion nicotine in 0.1 ml over 1 s for 1 week and then 0.06 mg/kg per infusion in 0.1 ml over 1 s for 3 weeks (5 days per week). This self-administration training protocol was used because (i) it ensured an appreciable increase in NAcc dopamine after a single self- or experimenter-administered infusion of nicotine and (ii) allowed the administration of the same dose of nicotine per infusion during the dialysis session as during the nicotine self-administration sessions. This self-administration training protocol was based on previous work in our laboratory, in which the initial training to self-administer 0.03 mg/kg per infusion nicotine for 1 week led to the development of tolerance to the initial aversive effects of high nicotine doses and thus allowed subsequent nicotine self-administration of 0.06 mg/kg per infusion (Watkins *et al*, 1999). Other self-administration parameters, such as fixed-ratio requirement, timeout duration, and the use of a cue light during the nicotine infusion, were identical to those in Experiment 2. After completion of nicotine self-administration training, the animals underwent microdialysis. Three experiments (Experiments 3a–c) resulted from

the experimental manipulations during the microdialysis session. In Experiment 3a, the animals self-administered a single infusion of nicotine (0.06 mg/kg per infusion) in a context associated with nicotine self-administration (Chambers B) during the dialysis session. In Experiment 3b, the animals received a single experimenter-administered infusion of nicotine (0.06 mg/kg per infusion) in a context associated with nicotine self-administration (Chambers B) during the dialysis session. Finally, in Experiment 3c, the animals received a single experimenter-administered infusion of nicotine (0.06 mg/kg per infusion) during the dialysis session in a context never associated with nicotine self-administration (Chambers A). These three experiments were conducted sequentially because the data from each experiment guided the design of the subsequent experiment to allow an integrated interpretation of the data from all of the experiments.

The procedure described below was followed for the microdialysis sessions in Experiments 3a–c. Microdialysis probes were inserted via the intracranial guide cannulae using intravenous methohexital sodium (Brevital sodium) anesthesia at least 18 h before the initiation of collecting the dialysis samples. Rats with the microdialysis probes were housed in either Chambers B (Experiments 3a and b; that is, chambers associated with nicotine administration) or Chambers A (Experiment 3c; that is, chambers not associated with nicotine administration) for the habituation period and subsequent dialysate sampling period. During the dialysis session, samples were collected every 10 min. Initially, three baseline samples were collected for the first 30 min. Subsequently, rats were randomly assigned to two treatment groups: (i) saline + nicotine (0.06 mg/kg per infusion) and (ii) LY379268 (1 mg/kg) + nicotine (0.06 mg/kg per infusion). After the collection of baseline samples, the animals received saline/LY379268 pretreatment. Thirty minutes after the saline/LY379268 pretreatment, the animals received a single nicotine infusion that was either self-administered (Experiment 3a) or experimenter-administered (Experiments 3b and c; see details below). After the administration of nicotine, samples were collected every 10 min for 60 min (six samples). The first sample collection after nicotine administration was delayed for either 2 min (Experiments 3a and b) or 8 min (Experiment 3c) to account for the dead volume of the probe. The description below highlights the differences during the dialysis sessions in Experiments 3a–c.

Experiment 3a: effects of LY379268 pretreatment and a single self-administered intravenous nicotine infusion on NAcc shell dopamine in nicotine-experienced rats in a nicotine-associated context. Thirty minutes after the saline/LY379268 pretreatment, the two levers in Chambers B were introduced (an additional cue associated with nicotine administration in the rats), and the animals had the opportunity to self-administer a single infusion of nicotine by pressing the lever five times. The self-administered nicotine infusion was associated with the illumination of a cue light located above the active lever that remained lit for 20 s after the completion of the FR5 schedule. This was the same cue light that was previously associated with nicotine self-administration. The nicotine infusion parameters were also identical to those used during the

nicotine self-administration sessions, with the exception that the levers retracted permanently after the single infusion of nicotine. The time required for the animals to complete the FR5 to obtain the nicotine infusion was recorded.

Experiment 3b: effects of LY379268 pretreatment and a single experimenter-administered intravenous nicotine infusion on NAcc shell dopamine in nicotine-experienced rats in a nicotine-associated context. Thirty minutes after the saline/LY379268 pretreatment, the two levers in Chamber B were introduced, but in this experiment, unlike Experiment 3a, the animals received a single experimenter-administered intravenous infusion of nicotine. The nicotine infusion was associated with the illumination of the cue light located above the active lever that remained lit for 20 s, similar to Experiment 3a, and during the nicotine self-administration sessions. The two levers in Chambers B retracted after the administration of the nicotine infusion, similar to Experiment 3a.

Experiment 3c: effects of LY379268 pretreatment and a single experimenter-administered intravenous nicotine infusion on NAcc shell dopamine in nicotine-experienced rats in a context never associated with nicotine administration. The animals were housed in Chambers A as described above. Thirty minutes after the saline/LY379268 pretreatment, the animals received a single experimenter-administered intravenous infusion of nicotine, similar to Experiment 3b. Chambers A, as described above, did not have levers or cue lights.

Dopamine and Monoamine Metabolite Assay

Samples were assayed for dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindole acetic acid using reverse-phase high-performance liquid chromatography with electrochemical detection as described previously (Kuczenski *et al*, 1995).

Histology

At the end of the experiments, the rats were anesthetized with sodium pentobarbital and decapitated. The brains were removed and fixed in 10% formalin. The brains were then sliced (60- μ m-thick coronal sections) on a cryostat to determine the location of the probes.

Statistical Analyses

Dopamine and metabolite levels are expressed as a percentage of baseline levels (the average of the three samples that preceded the drug treatments) and were analyzed by repeated-measures analysis of variance (ANOVA), with Treatment as the between-subjects factor and Time as the within-subjects factor. Statistically significant main or interaction effects were followed by the Newman-Keuls *post hoc* test. In Experiment 3a, an unpaired *t*-test was used to analyze the latency to self-administer nicotine in saline- and LY379268-pretreated animals. Cumulative nicotine intake and the number of nicotine infusions 24 h before the microdialysis session were compared using unpaired

t-tests for groups pretreated with LY379268 and saline separately in each of the experiments. Only data from rats with accurate probe placements were included in the analyses.

RESULTS

Table 1 shows the history of nicotine exposure, cumulative nicotine intake in mg/kg (total number of nicotine infusions over all self-administration sessions \times dose of nicotine per infusion), and number of nicotine infusions 24 h before the dialysis session between saline- and LY379268-pretreated groups. Unpaired *t*-tests showed no significant differences in cumulative nicotine intake over the entire self-administration training period between saline- and LY379268-pretreated rats in each experiment. Furthermore, unpaired *t*-tests indicated no significant differences in the number of nicotine infusions self-administered 24 h before the dialysis session between the saline- and LY379268-pretreated rats in each experiment; the observed differences are within the variability usually observed in nicotine self-administration studies. Comparisons of cumulative nicotine intake and the number of nicotine infusions 24 h before the dialysis session between the saline- and LY379268-pretreated groups were conducted only within each experiment and not across experiments. The effects of LY379268/saline on nicotine-stimulated NAcc shell dopamine responses were also compared only within each experiment and not across experiments. Finally, no significant differences in metabolite levels were observed after experimenter- or self-administered nicotine in either saline- or LY379268-pretreated animals in all experiments (Table 2). Figure 1 shows the active membranes of the probes in the animals used in the data analyses.

Experiment 1: LY379268 Pretreatment did not Block the Increases in NAcc Shell Dopamine after Experimenter-Administered Subcutaneous Injection of Nicotine in Nicotine-Naïve Rats

No significant differences were observed in mean baseline NAcc shell dopamine levels between the saline + nicotine (28.44 ± 0.54 fmol/30 μ l), LY379268 + nicotine (25.66 ± 1.84 fmol/30 μ l), and LY379268 + saline (18.24 ± 0.96 fmol/30 μ l) groups. An ANOVA of the percentage data after saline/LY379268 treatment revealed significant main effects of Treatment ($F_{4,28} = 9.70$, $p < 0.001$) and Time ($F_{12,336} = 4.83$, $p < 0.001$) and a significant Treatment \times Time interaction ($F_{48,336} = 3.32$, $p < 0.001$). The Newman-Keuls *post hoc* test indicated a significant increase in nicotine-stimulated NAcc shell dopamine responses in saline-pretreated animals at the 20–40 min ($p < 0.001$), 40–60 min ($p < 0.01$), 80–100 min ($p < 0.05$), and 120–140 min ($p < 0.05$) time-points compared with their respective pretreatment baseline levels (Figure 2a). Similarly, *post hoc* tests indicated a significant increase in nicotine-stimulated NAcc shell dopamine responses in animals pretreated with LY379268 (1 mg/kg) at the 40–60 min ($p < 0.05$) and 60–80 min ($p < 0.05$) time-points compared with their respective pretreatment baseline levels (Figure 2a). In addition, significant increases in nicotine-stimulated NAcc shell dopamine responses were found in the animals pretreated with LY379268 (3 mg/kg) at the 20–40 min

Table 1 Nicotine Intake in all Groups in all Experiments

| Experiment | History of nicotine self-administration | Dialysis context | Dose and mode of nicotine delivery during the dialysis session | Pretreatment | Cumulative self-administered nicotine (mg/kg) over 3 weeks of nicotine self-administration training (mean \pm SEM) | Number of nicotine infusions/h (24 h before dialysis) (mean \pm SEM) |
|------------|--|--|---|---------------------------------|--|--|
| 1 | None | Neutral | Single experimenter-administered subcutaneous injection (0.4 mg/kg) | LY379268 ^a Saline | None None | None None |
| 2 | 0.03 mg/kg per infusion (3 weeks) | Never associated with nicotine self-administration | Single experimenter-administered subcutaneous injection (0.4 mg/kg) | LY379268 Saline | 5.32 \pm 1.20 5.50 \pm 0.66 | 9.20 \pm 0.60 9.40 \pm 1.90 |
| 3a | 0.03 mg/kg per infusion (1 week)+0.06 mg/kg per infusion (3 weeks) | Associated with nicotine self-administration | Single self-administered intravenous nicotine infusion (0.06 mg/kg) | LY379268 Saline | 9.92 \pm 1.06 7.58 \pm 0.37 | 8.0 \pm 1.29 6.37 \pm 0.92 |
| 3b | 0.03 mg/kg per infusion (1 week)+0.06 mg/kg per infusion (3 weeks) | Associated with nicotine self-administration | Single experimenter-administered intravenous nicotine infusion (0.06 mg/kg) | LY379268 Saline | 12.06 \pm 1.5 9.78 \pm 0.60 | 11.08 \pm 1.25 8.60 \pm 0.74 |
| 3c | 0.03 mg/kg per infusion (1 week)+0.06 mg/kg per infusion (3 weeks) | Never associated with nicotine self-administration | Single experimenter-administered intravenous nicotine infusion (0.06 mg/kg) | LY379268 Saline | 7.32 \pm 0.64 9.67 \pm 1.30 | 8.75 \pm 2.03 12.14 \pm 1.76 |

^aValues reported only for 1 mg/kg dose of LY379268.

($p < 0.05$), 40–60 min ($p < 0.01$), 60–80 min ($p < 0.01$), 80–100 min ($p < 0.05$), and 100–120 min ($p < 0.05$) time-points compared with their respective pretreatment baseline levels (Figure 2a). *Post hoc* tests indicated no significant difference in NAcc shell dopamine responses after experimenter-administered nicotine between saline- and LY379268- (1 and 3 mg/kg) pretreated animals at any time-point. Significant decreases in NAcc shell dopamine levels were found in animals pretreated with LY379268 (3 mg/kg) alone at the 0–20 min ($p < 0.05$) and 40–60 min ($p < 0.05$) time-points compared with their respective baseline levels (Figure 2a). *Post hoc* tests indicated no significant difference in NAcc shell dopamine levels between animals pretreated with LY379268 (1 mg/kg) and LY379268 (3 mg/kg) at any time-point.

Experiment 2: LY379268 Pretreatment did not Block the Increases in NAcc Shell Dopamine after an Experimenter-Administered Subcutaneous Injection of Nicotine in Nicotine-Experienced Rats in a Context Never Associated with Nicotine Self-Administration

No significant differences were observed in mean baseline NAcc shell dopamine levels between the saline + nicotine (22.51 \pm 0.98 fmol/30 μ l), LY379268 + nicotine (17.56 \pm 0.96 fmol/30 μ l), and LY379268 + saline (23.28 \pm 1.96 fmol/30 μ l) groups. An ANOVA revealed significant main effects of Treatment ($F_{2,19} = 5.718$, $p < 0.01$) and Time ($F_{12,228} = 5.401$, $p < 0.001$) and a significant Treatment \times Time interaction ($F_{24,228} =$

3.48, $p < 0.001$). The Newman–Keuls *post hoc* tests indicated significant increases in nicotine-stimulated NAcc shell dopamine responses in saline-pretreated animals at the 20–40 min ($p < 0.05$), 40–60 min ($p < 0.05$), 80–100 min ($p < 0.05$), and 100–120 min ($p < 0.05$) time-points compared with their respective pretreatment baseline levels (Figure 2b). Similarly, *post hoc* tests indicated significant increases in nicotine-stimulated NAcc shell dopamine responses in animals pretreated with LY379268 (1 mg/kg) at the 20–40 min ($p < 0.001$), 40–60 min ($p < 0.05$), 60–80 min ($p < 0.01$), and 80–100 min ($p < 0.01$) time-points compared with their respective pretreatment baseline levels (Figure 2b). No significant differences in NAcc dopamine responses were found after experimenter-administered nicotine between the saline- and LY379268-pretreated animals at any time-point. In the animals treated with LY379268 (1 mg/kg) alone, no significant change in NAcc shell dopamine levels was found compared with their respective baseline levels.

Experiment 3a: LY379268 Pretreatment Blocked the Increases in NAcc Shell Dopamine after a Single Self-Administered Intravenous Infusion of Nicotine in Nicotine-Experienced Rats in a Context Associated with Nicotine Self-Administration

No significant difference in mean baseline NAcc shell dopamine levels was observed between the LY379268- (8.27 \pm 1.96 fmol/15 μ l) and saline- (8.68 \pm 1.70 fmol/15 μ l)

Table 2 DA, DOPAC, HVA, and 5-HIAA Levels in all Experimental Groups^a

| Experiment | History of nicotine self-administration | Dialysis context | Dose and mode of nicotine delivery during the dialysis session | Pretreatment | Mean ± SEM after nicotine administration (values expressed as percentage of baseline) | | | |
|-----------------|--|--|---|---------------------------------|---|------------------------------|-----------------------------|-----------------------------|
| | | | | | DA (%) | DOPAC (%) | HVA (%) | 5-HIAA (%) |
| 1 ^b | None | Neutral | Single experimenter-administered subcutaneous injection (0.4 mg/kg) | LY379268 Saline ^c | 145 ± 10.7 155 ± 18.5 | 108 ± 2.13 96.2 ± 4.6 | 113 ± 4.2 115 ± 7.8 | 109 ± 4.7 104 ± 0.7 |
| 2 ^b | 0.03 mg/kg per infusion (3 weeks) | Never associated with nicotine self-administration | Single experimenter-administered subcutaneous injection (0.4 mg/kg) | LY379268 Saline | 190 ± 18.9 175 ± 21.3 | 125 ± 7.0 102 ± 6.7 | 123 ± 15.3 118 ± 9.3 | 117 ± 3.79 102 ± 6.54 |
| 3a ^d | 0.03 mg/kg per infusion (1 week)+0.06 mg/kg per infusion (3 weeks) | Associated with nicotine self-administration | Single self-administered intravenous nicotine infusion (0.06 mg/kg) | LY379268 Saline | 101 ± 11.7 254 ± 14.1 | 118.85 ± 7.1 141 ± 21.2 | 107 ± 7.1 135 ± 17.8 | 132.7 ± 16.9 113.7 ± 5.4 |
| 3b ^d | 0.03 mg/kg per infusion (1 week)+0.06 mg/kg per infusion (3 weeks) | Associated with nicotine self-administration | Single experimenter-administered intravenous nicotine infusion (0.06 mg/kg) | LY379268 Saline | 101 ± 11.7 207.4 ± 14.1 | 103 ± 9.2 88.90 ± 20.6 | 110 ± 11.2 93.4 ± 44.0 | 102 ± 8.9 103 ± 17.4 |
| 3c ^d | 0.03 mg/kg per infusion (1 week)+0.06 mg/kg per infusion (3 weeks) | Never associated with nicotine self-administration | Single experimenter-administered intravenous nicotine infusion (0.06 mg/kg) | LY379268 Saline | 217.6 ± 34.0 203 ± 33.1 | 111.2 ± 19.3 107.5 ± 13.3 | 103.7 ± 22.8 96.3 ± 15.1 | 106.7 ± 9.9 103.2 ± 15.2 |

Abbreviations: DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindole acetic acid.

^aValues expressed as percentage of baseline.

^bValues reflect DA, DOPAC, HVA, and 5-HIAA levels 40 min after subcutaneous nicotine injection.

^cValues reflect DA, DOPAC, HVA, and 5-HIAA levels 10 min after a single intravenous infusion of nicotine.

^dValues reported only for 1 mg/kg dose of LY379268.

pretreated groups. An ANOVA revealed significant main effects of Treatment ($F_{1,13} = 25.2$, $p < 0.001$) and Time ($F_{11,143} = 7.31$, $p < 0.001$) and a significant Treatment \times Time interaction ($F_{11,143} = 6.68$, $p < 0.001$). The Newman-Keuls *post hoc* test indicated significant increases in NAcc dopamine responses after a single self-administered infusion of nicotine in saline-pretreated animals at the 0–10 min ($p < 0.001$), 10–20 min ($p < 0.05$), and 30–40 min ($p < 0.05$) time-points compared with their respective baseline levels. LY379268 pretreatment blocked the increases in NAcc shell dopamine levels induced by self-administration of a single nicotine infusion, revealed by the Newman-Keuls *post hoc* test that indicated significant differences in NAcc dopamine responses between the saline- and LY379268-pretreated animals at the 0–10 min ($p < 0.001$), 10–20 min ($p < 0.001$), 20–30 min ($p < 0.001$), 30–40 min ($p < 0.05$), and 50–60 min ($p < 0.001$) time-points after nicotine self-administration (Figure 3). Animals pretreated with LY379268 exhibited a significant increase in the latency (16.12 ± 4 min) to complete the FR5 schedule to receive the nicotine infusion compared with saline-pretreated rats (5.33 ± 1.3 min; $t_{1,12} = 2.37$, $p < 0.01$). Because sampling was initiated after the animals had received an infusion of nicotine, the difference in nicotine-induced dopamine responses between the saline- and LY379268-pretreated groups was not likely attributable to this difference in latency between the two groups.

Experiment 3b: LY379268 Pretreatment Blocked the Increases in NAcc Shell Dopamine after a Single Experimenter-Administered Infusion of Nicotine in Nicotine-Experienced Rats in a Context Associated with Nicotine Self-Administration

No significant difference in mean baseline NAcc shell dopamine levels was observed between the LY379268- (7.83 ± 0.98 fmol/15 μ l) and saline- (5.37 ± 0.77 fmol/15 μ l) pretreated groups. An ANOVA revealed significant main effects of Treatment ($F_{1,7} = 15.26$, $p < 0.01$) and Time ($F_{11,77} = 1.93$, $p < 0.05$) and a significant Treatment \times Time interaction ($F_{11,77} = 1.98$, $p < 0.05$). The Newman-Keuls *post hoc* test indicated a significant increase in NAcc dopamine levels in saline-pretreated animals after a single experimenter-administered infusion of nicotine at the 0–10 min ($p < 0.01$) time-point compared with their respective baseline levels. Pretreatment with LY379268 (1 mg/kg) blocked the increase in NAcc shell dopamine levels induced by a single experimenter-administered infusion of nicotine in a context associated with nicotine self-administration in nicotine-experienced animals, revealed by *post hoc* tests that indicated significant differences in NAcc dopamine responses between the saline- and LY379268-pretreated animals at the 0–10 min ($p < 0.01$) time-point immediately after nicotine administration (Figure 4). Both

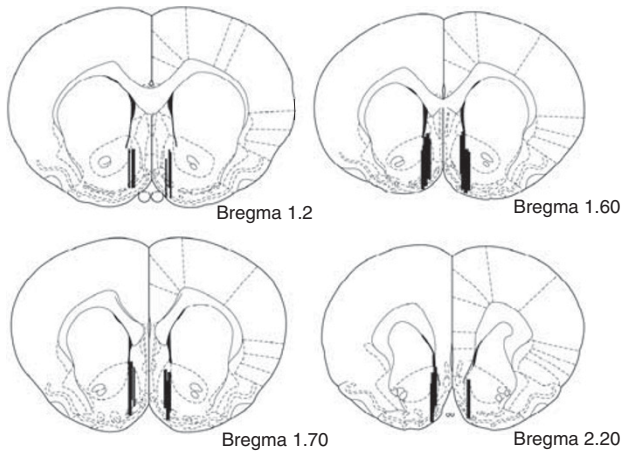


Figure 1 Diagrams of coronal sections from the rat brain atlas (Paxinos and Watson, 1998). Marked lines represent histologically confirmed locations of microdialysis probe tips (active membrane) in the nucleus accumbens (NAcc) shell from animals that were included in the statistical analyses for all experiments. Numbers next to the coronal section indicate the distance from bregma in millimeters.

the active and inactive levers were extended into the chambers just before the passive infusion and retracted immediately after the nicotine infusion. None of the animals pressed either the active or inactive lever either before or during the lever extension despite their previous experience with nicotine self-administration.

Experiment 3c: LY379268 Pretreatment did not Block the Increases in NAcc Shell Dopamine after a Single Experimenter-Administered Infusion of Intravenous Nicotine in Nicotine-Experienced Rats in a Context Never Associated with Nicotine Self-Administration

No significant difference in mean baseline NAcc shell dopamine levels was observed between the LY379268- (4.48 ± 0.44 fmol/15 μ l) and saline- (3.58 ± 0.78 fmol/15 μ l) pretreated groups. An ANOVA revealed a significant main effect of Time ($F_{11,99} = 2.29$, $p < 0.05$) but no main effect of Treatment ($F_{1,9} = 0.01$, $p > 0.05$) and no Treatment \times Time interaction ($F_{11,99} = 0.65$, $p > 0.05$). This pattern of results and subsequent *post hoc* tests (see below) indicated that pretreatment with LY379268 did not block the increased NAcc shell dopamine levels after a single passive infusion of nicotine in a context never associated with nicotine self-administration in nicotine-experienced animals (Figure 5). Specifically, the Newman-Keuls *post hoc* test revealed a significant increase in NAcc dopamine levels in both saline- and LY379268-pretreated animals after a single experimenter-administered infusion of nicotine in nicotine-experienced animals in the absence of a nicotine-associated context at the 0–10 min time-point compared with their respective baseline levels ($p < 0.05$). Importantly, no statistically significant differences in nicotine-induced dopamine responses were found between the saline- and LY379268-treated groups at any time-point.

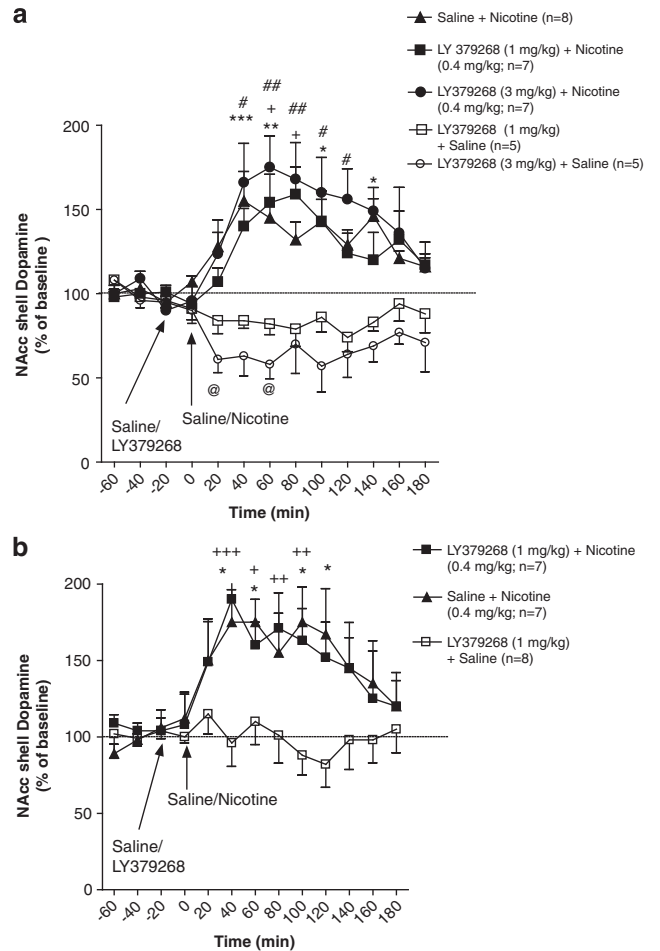


Figure 2 Effect of LY379268 ([–]-2-oxa-4-aminobicyclo [3.1.0] hexane-4,6-dicarboxylate; 1 and 3 mg/kg) on nucleus accumbens (NAcc) shell dopamine after experimenter-administered subcutaneous nicotine (0.4 mg/kg) administration in (a; Experiment 1) nicotine-naïve and (b; Experiment 2) nicotine-experienced rats in a context never associated with nicotine self-administration. Dopamine levels (mean \pm standard error of mean [SEM]) are expressed as a percentage of baseline values. (a) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, saline + nicotine compared with respective baseline levels; + $p < 0.05$, LY379268 (1 mg/kg) + nicotine compared with respective baseline levels; # $p < 0.05$, ## $p < 0.01$, LY379268 (3 mg/kg) + nicotine compared with respective baseline levels; and @ $p < 0.05$, LY379268 (3 mg/kg) + saline compared with respective baseline levels. (b) * $p < 0.05$, saline + nicotine compared with respective baseline levels; and + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$, LY379268 (1 mg/kg) + nicotine compared with respective baseline levels.

DISCUSSION

In saline-pretreated rats, nicotine increased NAcc shell dopamine regardless of whether the dialysis session occurred in a context associated with or never associated with nicotine self-administration. Pretreatment with the mGlu2/3 receptor agonist LY379268 completely blocked the nicotine-induced increases in NAcc shell dopamine in nicotine-experienced rats only in the presence of a context associated with nicotine self-administration (Figures 3 and 4). In contrast, pretreatment with LY379268 did not block the nicotine-induced increases in NAcc shell dopamine in nicotine-experienced rats tested in a context not associated with nicotine self-administration or nicotine-naïve rats

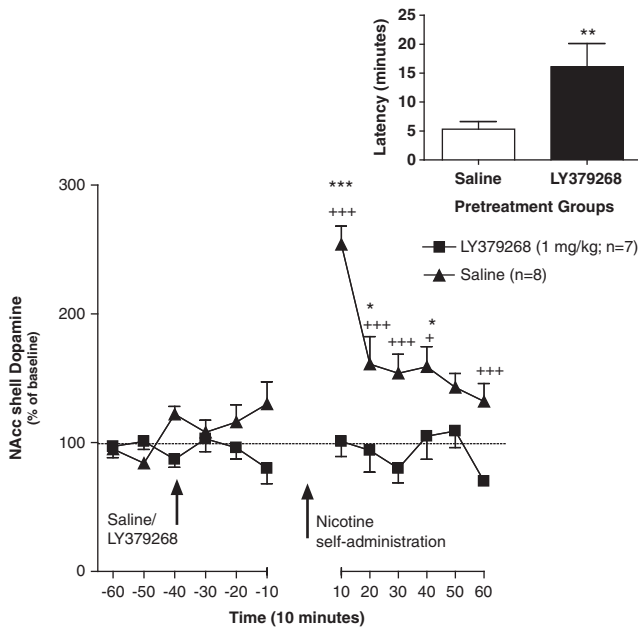


Figure 3 Effect of LY379268 ([−]-2-oxa-4-aminobicyclo [3.1.0] hexane-4,6-dicarboxylate; 1 mg/kg) on nucleus accumbens (NAcc) shell dopamine after self-administration of a single intravenous infusion of nicotine (0.06 mg/kg per infusion over 1 s) in nicotine-experienced animals in a context associated with nicotine self-administration (Experiment 3a). Dopamine levels (mean ± standard error of mean [SEM]) are expressed as a percentage of baseline values. * $p < 0.05$, *** $p < 0.001$, saline-pretreated compared with their own baseline levels; and + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$, saline-pretreated compared with LY379268-pretreated animals. (Inset) Latency (mean ± SEM) in minutes to self-administer nicotine in saline- and LY379268-pretreated animals. ** $p < 0.01$, LY379268-pretreated group compared with saline-pretreated group. The time period between −10 and 10 depicts the time taken by the animals to self-administer nicotine and the dead volume of the probe (no samples were collected during that period). The time taken to self-administer nicotine varied among LY379268- and saline-pretreated animals. On the basis of the dead volume of the tubing, sample collection was initiated 2 min after nicotine self-administration in all animals.

tested in a neutral context. Thus, the present data suggest that mGlu2/3 receptors are involved in regulating the combined effects of nicotine and contextual/discrete stimuli on NAcc shell dopamine in rats.

Contextual and discrete stimuli are two distinct entities that can have differential behavioral effects, possibly mediated by different subdivisions (core vs shell) of the NAcc (Chaudhri et al, 2010; Bossert et al, 2007; Fuchs et al, 2004, 2008). This study focused on providing a well-supported conclusion about the conditions that lead to the reversal of nicotine-induced increases in NAcc dopamine levels after the initial surprising lack of reversal of nicotine-induced dopamine increases by LY379268 in Experiment 1. Differentiating between the effects of the context and discrete environmental stimuli on the observed effects was not feasible in this study because of the nature of the data that were gradually emerging with each subsequent experiment. Each experiment built on the surprising and revealing results of the previous experiments, with the necessity of maintaining identical experimental conditions with the exception of varying one variable at a time in each subsequent experiment. Nevertheless, the generated results

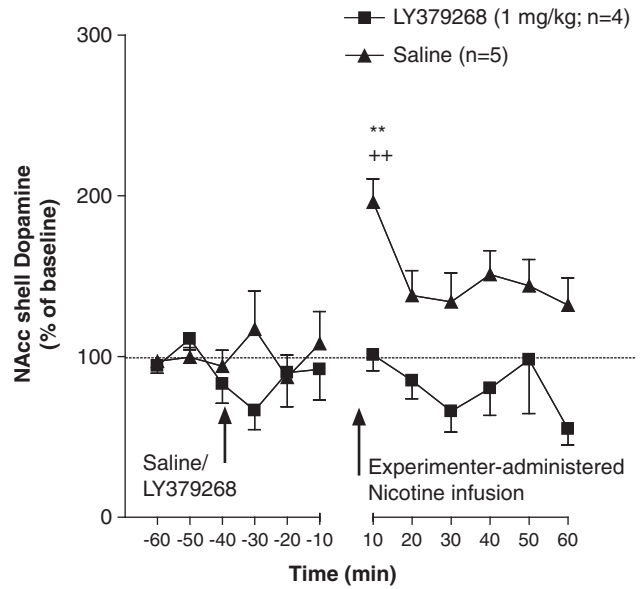


Figure 4 Effect of LY379268 ([−]-2-oxa-4-aminobicyclo [3.1.0] hexane-4,6-dicarboxylate; 1 mg/kg) on nucleus accumbens (NAcc) shell dopamine after a single experimenter-administered intravenous infusion of nicotine (0.06 mg/kg per infusion over 1 s) in nicotine-experienced animals in a context associated with nicotine self-administration (Experiment 3b). Dopamine levels (mean ± standard error of mean [SEM]) are expressed as a percentage of baseline values. ** $p < 0.01$, saline-pretreated compared with their own baseline levels; ++ $p < 0.01$, saline-pretreated compared with LY379268-pretreated animals. The time period between −10 and 10 depicts the dead volume of the probe, which was 2 min.

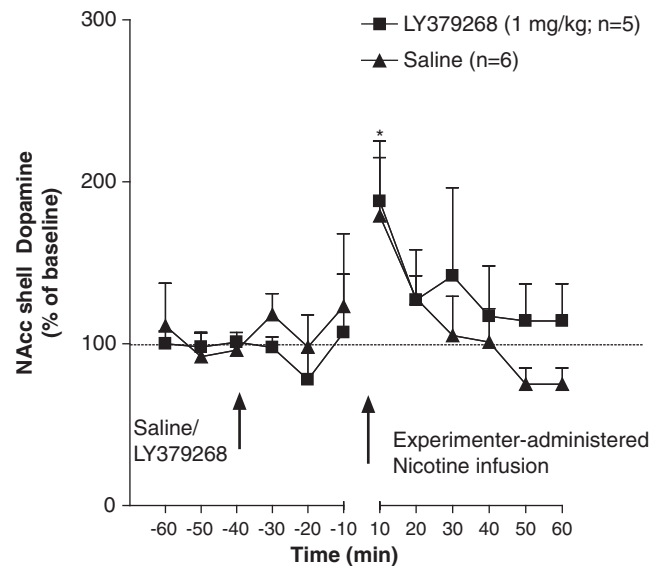


Figure 5 Effect of LY379268 ([−]-2-oxa-4-aminobicyclo [3.1.0] hexane-4,6-dicarboxylate; 1 mg/kg) on nucleus accumbens (NAcc) shell dopamine after a single experimenter-administered intravenous infusion of nicotine (0.06 mg/kg per infusion over 1 s) in nicotine-experienced animals in a context never associated with nicotine self-administration (Experiment 3c). Dopamine levels (mean ± standard error of mean [SEM]) are expressed as a percentage of baseline values. * $p < 0.05$, saline- and LY379268-pretreated compared with their respective baseline levels. The time period between −10 and 10 depicts the dead volume of the probe, which was 8 min.

provide clear evidence about the neurochemical mechanisms that contribute to the effects of agonist actions at mGlu2/3 receptors on nicotine self-administration and drug-seeking behavior. Both of these behaviors involve responses to context + stimuli previously associated with nicotine availability and self-administration. These findings are highly relevant to situations encountered by human beings, in which both contexts and stimuli associated with smoking behavior are encountered simultaneously, similar to the present experiments.

Nicotine-naïve animals had the highest basal dopamine levels (Experiment 1). In Experiment 2, animals self-administered nicotine 0.03 mg/kg per infusion for 3 weeks and had slightly lower basal dopamine levels compared with the nicotine-naïve animals. The highest exposure to nicotine was in animals from Experiments 3a–c who self-administered nicotine 0.03 mg/kg per infusion for 1 week and 0.06 mg/kg per infusion for 3 weeks. These animals also had the lowest basal dopamine levels compared with all other groups. The lower basal dopamine levels in nicotine-experienced rats (Experiments 2 and 3a–c) are consistent with previously published findings (Rahman *et al*, 2004). Using the no-net-flux technique, Rahman *et al* (2004) demonstrated that nicotine self-administration experience, similar to that used in our study, resulted in lower basal dopamine levels in nicotine-experienced animals compared with nicotine-naïve animals. These data suggest that nicotine exposure dose-dependently results in decreased NAcc shell dopamine levels. However, drawing firm conclusions about the basal levels of dopamine across experimental groups with different self-administration experiences based solely on the data presented here is difficult because the no-net-flux technique was not used in this study. The differences in basal dopamine levels between nicotine-naïve and -experienced rats, although interesting, are peripheral to the questions this study sought to answer. Because of our awareness of these differences in basal levels, quantitative comparisons about effect sizes among groups from different experiments groups were not made. Comparisons were only made between groups within the same experiment that exhibited no significant differences in basal dopamine levels. These comparisons addressed the experimental question of whether LY379268 could reverse or at least attenuate the effects of nicotine on NAcc dopamine.

The NAcc dopamine response (Experiment 1) after subcutaneous nicotine administration (0.4 mg/kg) in nicotine-naïve animals was similar to that reported previously (Bednar *et al*, 2004; Kosowski *et al*, 2004; Schilstrom *et al*, 1998). Similarly, increases in NAcc shell dopamine after subcutaneous nicotine administration (0.4 mg/kg) in nicotine-experienced animals (Experiment 2) were also consistent with previously published findings (Bednar *et al*, 2004; Benwell and Balfour, 1992; Cadoni and Di Chiara, 2000). Both a self- and experimenter-administered single infusion of nicotine (0.06 mg/kg over 1 s) significantly increased NAcc shell dopamine in animals with a history of chronic nicotine self-administration regardless of the context used during the dialysis session (ie, context associated with nicotine self-administration *vs* context never associated with nicotine self-administration). However, a previous study reported no change in NAcc dopamine levels after a very slow (over 60 s) intravenous infusion of the same

nicotine dose (0.06 mg/kg) in animals with chronic nicotine self-administration experience (Rahman *et al*, 2004). The discrepancy between the findings of Rahman *et al* (2004) and this study could be attributable to methodological differences between the two studies, particularly the rate at which nicotine was infused during the dialysis session (60 *vs* 1 s in our study) and location of the probes (core and shell *vs* shell in our study).

LY379268 (1 mg/kg) alone did not alter NAcc shell dopamine levels in nicotine-naïve or -experienced rats (Figure 2a and b). The higher LY379268 dose (3 mg/kg), when tested on its own in nicotine-naïve rats, decreased NAcc shell dopamine levels at some time-points post-administration compared with baseline dopamine levels (Figure 2a). This LY379268-induced dose-dependent decrease in basal NAcc shell dopamine levels in nicotine-naïve rats is consistent with previously published results (Green-slade and Mitchell, 2004; Hu *et al*, 1999; Kim *et al*, 2005; Xi *et al*, 2010a,b). Taken together, these findings reflect the fact that activation of mGlu2/3 receptors on presynaptic glutamate terminals decreases glutamate release, which in turn results in lower activation of dopamine neurons in areas such as the VTA and consequently decreases in dopamine release in terminal areas such as the NAcc. Because LY379268 administration (3 mg/kg) blocked both nicotine and food self-administration (Liechti *et al*, 2007), indicating a lack of reinforcer selectivity at this dose and impairment of locomotor activity at higher doses (6–10 mg/kg; Imre, 2007), we did not assess the effects of 3 mg/kg or higher doses of LY379268 beyond Experiment 1. Importantly, LY379268 (1 mg/kg) selectively attenuated nicotine, but not food, self-administration and had no effect on locomotor activity (Imre, 2007; Liechti *et al*, 2007); therefore, this dose was used in all subsequent experiments.

In the presence of a nicotine-associated context, pretreatment with LY379268 (1 mg/kg) completely blocked the increase in NAcc shell dopamine induced by a single intravenous infusion of nicotine, independent of whether the nicotine was self- or experimenter-administered (Figures 3 and 4). In contrast, in the absence of a nicotine-associated context, pretreatment with LY379268 did not attenuate intravenous nicotine-induced increases in NAcc shell dopamine (Figure 5).

In addition, LY379268-pretreated rats, which did not exhibit increases in NAcc shell dopamine after self-administration of nicotine in the presence of a nicotine-associated context, exhibited a significant delay in initiating nicotine seeking and nicotine self-administration compared with saline-pretreated rats tested under otherwise identical experimental conditions (see Figure 3, inset). Nicotine-associated contexts and cues can significantly impact behavioral responses to nicotine. Indeed, animals show enhanced nicotine self-administration in the presence of contexts and cues associated with nicotine (Caggiula *et al*, 2001, 2002; Chaudhri *et al*, 2006). Previous work from our laboratory has shown that LY379268-pretreated animals exhibit decreased nicotine self-administration in the presence of nicotine-associated contexts and cues (Liechti *et al*, 2007). In addition, the same study by Liechti and co-workers and work from other laboratories reported that LY379268 attenuates context/cue-induced motivated behavior, such as cue-induced reinstatement of nicotine-,

heroin-, and food-seeking behavior (Baptista *et al*, 2004; Bossert *et al*, 2005, 2006; Liechti *et al*, 2007). Therefore, the increase in latency to self-administer nicotine after treatment with LY379268 likely reflects a decrease in motivation to seek and self-administer nicotine and is consistent with the results of the aforementioned studies. Altogether, the present neurochemical and behavioral data (ie, increased latency to self-administer nicotine in LY379268-pretreated rats) suggest that a possible mechanism that contributes to the attenuation of nicotine self-administration by LY379268 (Liechti *et al*, 2007) may be inhibition of nicotine-induced dopamine release in the NAcc shell.

It must be emphasized, however, that nicotine increases the release of several neurotransmitters, including glutamate, by activating excitatory nicotinic heteroreceptors located on presynaptic terminals of neurons containing these neurotransmitters (Balfour, 2009; Fu *et al*, 2000; Reid *et al*, 2000; Wonnacott, 1997). In addition, drug-associated context and cues increase glutamatergic neurotransmission (Hotsenpiller *et al*, 2001; Bell *et al*, 2000). Therefore, blockade of nicotine-induced dopamine release in the NAcc shell may not be the only mechanism by which LY379268 increases the latency to self-administer nicotine and attenuates nicotine intake. As described in the Introduction, LY379268 decreases glutamate release by activating inhibitory presynaptic mGlu2/3 receptors (Cartmell and Schoepp, 2000; Xi *et al*, 2002a), and blockade of glutamatergic neurotransmission also attenuates the reinforcing effects of nicotine (Kenny *et al*, 2009, 2003; Liechti and Markou, 2007; Palmatier *et al*, 2008; Paterson and Markou, 2005; Paterson *et al*, 2003). Therefore, mGlu2/3-mediated attenuation of glutamatergic neurotransmission is likely to be another mechanism contributing to LY379268-induced increases in the latency to self-administer nicotine (this study) and attenuation of the reinforcing effects of nicotine (Liechti *et al*, 2007).

These data are consistent with a significant body of literature that demonstrated an attenuation of both basal and psychostimulant-induced increases in mesolimbic dopaminergic neurotransmission upon either direct or indirect activation of mGlu2/3 receptors (Greenslade and Mitchell, 2004; Hu *et al*, 1999; Kim *et al*, 2005; Uslaner *et al*, 2009; Xi *et al*, 2010a, b). Importantly, these results advance data from the aforementioned studies because they emphasize a role for nicotine-associated contexts and cues in the effects of LY379268 on nicotine-induced increases in the NAcc dopamine response.

Although the mechanism of action by which the nicotine-associated context influences the effects of LY379268 is not known, our findings can be explained on the basis of alterations in mGlu2/3 receptor function, some of which are reported in the literature. Animals tested in the presence of a nicotine-associated context were also exposed to this context during the habituation period (18 h). Drug-associated contexts and cues enhance glutamatergic transmission (Hotsenpiller *et al*, 2001; Bell *et al*, 2000). Because mGlu2/3 receptors negatively regulate glutamatergic transmission, we postulate that mGlu2/3 receptor function is rapidly upregulated in the presence of a nicotine-associated context to counteract the context-induced enhancement of glutamatergic transmission. Recent studies reported that upregulation in mGlu2/3 receptor function may be respon-

sible for the increased pharmacological efficacy of the mGlu2/3 agonist LY379268, resulting in a leftward shift of the dose–response curve (Aujla *et al*, 2008; Hao *et al*, 2010). Thus, based on the results from these studies and our findings, we hypothesize that the nicotine-associated context resulted in rapid upregulation of mGlu2/3 receptors, which in turn enhanced the pharmacological efficacy of LY379268, resulting in the attenuation of nicotine-induced increases in NAcc shell dopamine only in the presence of a nicotine-associated context.

By contrast, in the absence of a nicotine-associated context, repeated nicotine self-administration experience and subsequent early withdrawal (~24 h after nicotine self-administration) results in mGlu2/3 receptor function downregulation, reflected by decreased coupling of mGlu2/3 receptors to G proteins (Liechti *et al*, 2007). This downregulation possibly occurs as a counteradaptation to the hypothesized development of a hypoglutamatergic state during early withdrawal after a period of chronic nicotine self-administration (Kenny and Markou, 2004). Such downregulation in mGlu2/3 receptor function would result in a rightward shift of the dose–response curve for agonists that act at the mGlu2/3 receptor. Consistent with this hypothesis, a rightward shift of the dose–response curve for the mGlu2/3 agonist 2R,4R-4-aminopyrrolidine-2,4-dicarboxylate was seen after downregulation in mGlu2/3 receptor function in animals with chronic cocaine self-administration experience (Xi *et al*, 2002b). Thus, we hypothesize that in the absence of a nicotine-associated context, mGlu2/3 receptor function was downregulated in animals with chronic nicotine self-administration experience, resulting in a rightward shift of the dose–response curve, consequently resulting in the lack of efficacy of LY379268 on the nicotine-induced increases in the NAcc dopamine response.

The above hypothesis, however, does not explain why LY379268 is not effective in nicotine-naïve animals. In nicotine-naïve animals, mGlu2/3 receptors may not have a role in regulating nicotine-induced increases in NAcc shell dopamine (Fu *et al*, 2000), or very high doses may be required to attenuate nicotine-induced increases in NAcc shell dopamine. Another possible mechanism for the lack of effects of LY379268 in nicotine-naïve and -experienced animals tested in a context not associated with nicotine self-administration could be novelty-induced stress induced by exposure to a new environment (Chambers A) in these animals. However, novelty-induced stress is unlikely to be a factor because animals were housed in the dialysis chambers for at least 18 h before dialysate sample collection.

The precise concentration of LY379268 achieved in the synapse after subcutaneous LY379268 (1 mg/kg) administration is not known. LY379268, although relatively specific for mGlu2/3 receptors ($EC_{50} = 3\text{--}6\ \mu\text{M}$), can have weak agonist activity at mGlu4 ($EC_{50} = 20\ \mu\text{M}$), mGlu6 ($EC_{50} = 400\ \mu\text{M}$), and mGlu8 ($EC_{50} = 2\ \mu\text{M}$) receptors. Thus, actions at these receptors may potentially contribute to the effects of LY379268 at high doses. However, the present results are not likely to be attributable to the action of LY379268 on receptors other than mGlu2/3 receptors because of the low dose (1 mg/kg) of LY379268 used in this study compared doses (3–10 mg/kg) frequently used in the literature to observe reliable behavioral or neurochemical effects (Cartmell *et al*, 2000a–c; Liechti *et al*, 2007; Riegel *et al*, 2003).

Because LY379268 acts on both mGlu2 and mGlu3 receptors, determining which group of receptors played a more important role in attenuating the combined effects of nicotine and a nicotine-associated context seen in this study is difficult. Presynaptically located mGlu2 receptors are expressed at higher densities than mGlu3 receptors in several limbic nuclei, including the NAcc in the rat (Gu et al, 2008). Furthermore, mGlu2 receptor knockout mice exhibited an increase in cocaine-induced place preference compared with wild-type mice, suggesting that activation of mGlu2 receptors has a more important role than mGlu3 receptors in inhibiting the effects of drug-associated contexts and cues (Morishima et al, 2005). In addition, LY379268 blocked amphetamine-induced hyperactivity in wild-type and mGlu3 knockout mice, but not in mGlu2 knockout mice, indicating that mGlu2 receptors are required for the ability of LY379268 to attenuate the behavioral effects of psychostimulants (Woolley et al, 2008). Nevertheless, because knockout mice likely undergo compensatory changes, and LY379268 acts on both mGlu2 and mGlu3 receptors, conclusively determining which of the two mGlu receptors (mGlu2 or mGlu3) have a more important role in blocking the combined effects of nicotine and a nicotine-associated context on NAcc shell dopamine in these studies is difficult.

In summary, pretreatment with the mGlu2/3 receptor agonist LY379268 attenuated the nicotine-induced increases in NAcc shell dopamine only in the presence of a nicotine-associated context. These data therefore suggest that activation of mGlu2/3 receptors has a greater impact on the synergistic effects of nicotine and nicotine-associated contexts than on the pharmacological actions of nicotine alone on nicotine-induced increases in NAcc shell dopamine. These results advance our understanding of the role mGlu2/3 receptors in nicotine-induced neurochemical and behavioral responses. In conclusion, these findings have high clinical relevance because human tobacco smokers are more likely to relapse to tobacco smoking after a period of abstinence in an environment associated with smoking. Therefore, by blocking the combined effects of nicotine and nicotine-associated contexts, mGlu2/3 receptor agonists may be effective in preventing relapse to tobacco smoking in abstinent smokers.

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DISCLOSURE

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